Research Article

The effect of ethanol on platelet count, bleeding time, and white blood cells in rats

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ABSTRACT

Introduction: Alcohol consumption is suggested as a major cause of many health impairments and diseases worldwide which contributes in 15–30% of admission cases in different counties. **Objective:** The aim of this study is to assess the effect of alcohol on platelet counts, bleeding time, and white blood cell (WBC) count in rats. **Materials and Methods:** Rats were divided into five groups, each group with five rats in separate cages. Three cages were gastric lavage fed with different ethanol concentrations (15%, 20%, and 25%) continuously for 21 days, while in the rest two groups, one was control fed with water only and the other were given aspirin solution to compare with alcohol effect on bleeding. **Results:** The findings of the present study showed that there is significant change in bleeding time ($P^* > 0.05$), but there is no significant change in platelet and WBC counts. **Conclusion:** In conclusion, alcohol may be used with caution to patients taking antiplatelet and anticoagulant drugs as alcohol is shown increasing in bleeding time; in addition, patients with immune-compromised risk should consider alcohol taking which may affect WBC mobilization rather than its count.

KEY WORDS: Bleeding time and white blood cells, Ethanol, Platelet

INTRODUCTION

Alcohol consumption is a major cause of many health impairments and diseases worldwide which contributes in 15–30% of admission cases in different counties.[1,2] Moreover, many cardiovascular diseases (CVDs) cause mortality and morbidity particularly in the western world, and epidemiology studies show that moderate consumption of alcohol can reduce this mortality due to CVD.[3,4] In the same point, alcohol may also contribute in many cancer diseases such as liver, mouth, and lynx.[5-7] However, the mechanism by which alcohol may reduce the heart attack diseases by 20–60% and overall death by 10% is not understood well.[8,9] Moreover, long time-consuming alcohol can induce neuroendocrine changes which may associated with increased stresses, and a result stimulates hypothalamic-pituitary-adrenocortical axis and the sympathetic nervous system.[10-12] Platelets have a major role in the incidence of stroke and myocardial infarction, and several studies have reported that alcohol can inhibit *in vitro* platelet aggregation.[13,14] Inhibition of arachidonic acid release and metabolism apparently has a role in this effect of alcohol on aggregation.[15,16]

Furthermore, the effect of ethanol on immune system is not clear, but there is some evidence on change of different WBC types such as lymphocyte, neutrophils, and eosinophils. Moreover, many studies show that ethanol has an effect on the distribution of various WBC types.[17-19] Therefore, there is a high association of cancer risk and immunity attenuation with the alcohol consumption, but the mechanism of this risk not yet determined; however, some evidence are attributed this cancer effect due to the oxidative metabolite of acetaldehyde.[20-22]

Many studies indicate that ethanol or its metabolite impairs platelet function due to both platelet injury and extracorpuscular factors.[18,23,24] More in this point, the findings of several researches have been suggested that acute dose of ethanol can affect blood fibrinolysis activity; as a result, whole blood clotting time may increase by 26±29%.[25] Furthermore, evidences are suggesting that alcohol can increase the bleeding risk of gastric and duodenal in non-predisposed subjects.[26,27] In the present study, it has been tried to determine the effect of ethanol on WBCs types and to

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determine if there is any effect of ethanol on platelet count which may lead to increase clotting time.

MATERIALS AND METHODS

Materials

EDTA tubes, plain tubes, micro hematology tubes, diethyl ether as esthetic agent for rats, 25 rats, diluted ethanol (15%, 20%, an 25%), aspirin solution of 100 mg, different syringe size, and Genex count 60 device to analyze the blood samples were used.

Methods

Experiment protocol and animal care

A total of 25 rats with the same weight (250 g/rat) were divided into five groups which include five rats in each cage. The rats were housed in stainless steel cages in an animal house with a 25°C to keep the adaptation of the rats in the same environment. The humidity was between 50% and 60% and all the animals care procedures were guided according to the committee of animal house in the university.

Ethanol and Aspirin Administration for 21 days

Ethanol solutions of different concentration were prepared (15%, 20%, and 25%). Aspirin (AVONCHEM Co.) solution of 100 mg in 100 ml was prepared in water freshly and daily to avoid decomposition to compare with ethanol effect on blood clotting time and platelet count. Aspirin and different ethanol concentrations were used to feed the rats which were fed for 21 days as continuous feeding by gastric lavage with daily observation to ensure normal and proper feeding. Moreover, one of the five groups were fed with water (control group).

Blood Samplings

Before feeding, blood samples were drawn from all rats to check the normal values of WBC and platelet count, and blood samples were collected from the tail vein of rats after using diethyl ether as esthetic. After 21 days, blood samples were collected again to evaluate the changes in WBC and platelet count.

Count Analyses of WBCs and Platelets

Samples were analyzed in laboratory using Genex count 60 device to count the values of WBC and platelet count. Furthermore, clotting time was measured using manual micro hematology tubes breaking pieces before and after feeding. All samples were collected in EDTA tubes to prevent clotting until the required tests were done.

Statistical Analyses

The experimental data were presented by means ± standard error. Moreover, Minitab software was used to analyze the data and *t*-test was used to evaluate the data before and after ethanol administration. The differences were considered when $P > 0.05$.

RESULT

WBC Counts

WBCs were counted using Genex count device, and different types of WBCs also measured lymphocyte, monocyte, and neutrophil. The findings are shown that no significant change is appeared after ethanol feeding for 21 days for any of WBCs types. Interestingly, there is a tendency toward increasing of WBCs, especially with ethanol concentration 15%, as show in the Figure 1.

Clotting Time

The clotting time was calculated by manual process of breaking the microtube hematology until clotting was appeared so the time was recorded as shown in Table 1. The results are demonstrated that clotting time of the rats which are fed with ethanol of different concentrations is significantly increased ($P^* < 0.05$).

Platelet Count

Platelets were counted using Genex count device before and after rats were fed with different concentrations of ethanol and aspirin solution as well. The findings are that no significant changes in platelet count after ethanol administration for rats ($P > 0.05$). Table 2 illustrates the results after and before the ethanol feeding and the platelet count for each group of the rats.

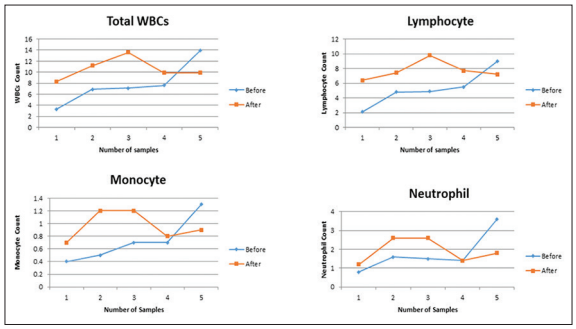

Figure 1: The total white blood cell, lymphocyte, monocyte, and neutrophil counts before and after 21 days of ethanol 15% continuous feeding. There are no significant changes after feeding with 20% ethanol ($P > 0.05$)

Table 1: The clotting time before and after ethanol feeding for 21 days

Before ethanol feeding (min)	After ethanol feeding (min)
02:15	03:30
02:30	04:30
03:30	04:00
02:30	03:00
03:00	04:30

Table 2: The platelet count reading for each group before and after ethanol and aspirin feeding for 21 days

Control		Ethanol 15%		Ethanol 20%		Ethanol 25%		Aspirin	
After	Before	After	Before	After	Before	After	Before	After	Before
557	353	424	45	415	669	372	586	499	522
537	525	448	471	401	1559	40	242	429	456
488	22	435	413	365	464	50	22	682	412
541	334	509	116	378	390	308	279	476	425
365	471	450	533	361	615	176	127	568	417

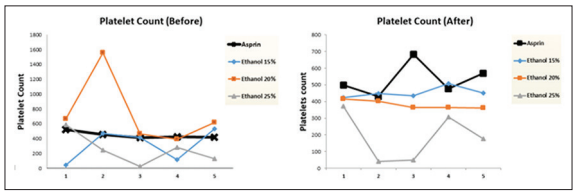

Figure 2: The effect of ethanol in comparision with aspirin solution of 100 mg which shows that platelet count after administration of ethanol for 21 days are reduced significantly (ethanol 20% and 25%, $P^* < 0.05$) in correlation with ethanol concentration

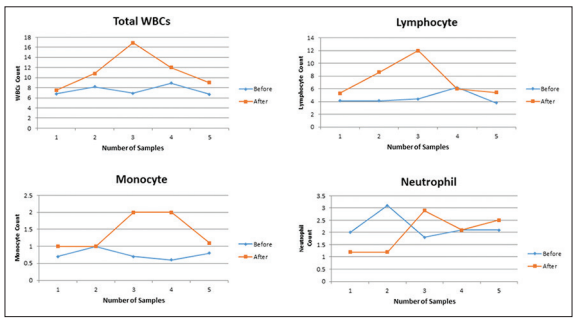

Figure 3: The total white blood cell, lymphocyte, monocyte, and neutrophil counts before and after 21 days of ethanol 20% continuous feeding. There are no significant changes after feeding with 20% ethanol ($P > 0.05$)

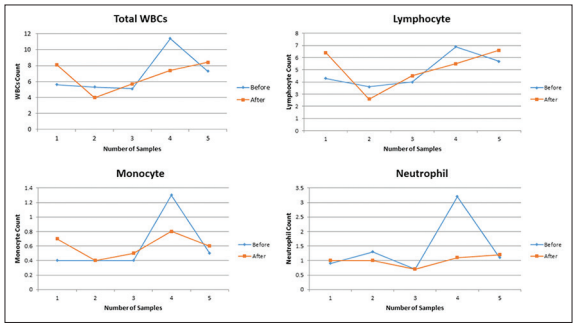

Figure 4: The total white blood cell, lymphocyte, monocyte, and neutrophil counts before and after 21 days of ethanol 25% continuous feeding. There are no significant changes after feeding with 25% ethanol ($P > 0.05$)

Aspirin Comparison

Aspirin solution of 100 mg was used to compare with ethanol to evaluate the effect of ethanol on platelet count. The results show that ethanol concentrations 20% and 25% are significantly changed the platelet count in comparision with aspirin solution ($P^* = 0.04$ and $0.02<0.05$), respectively. Therefore, the previous result may suggest that platelet count may change and decrease with the ethanol concentration as illustrate in Figure 2.

DISCUSSION

As alcohol consumption is increased all over the world and according to the WHO was stated that about 3.3 million people die annually from alcohol consumption, alcohol effect on different organs should be studied regularly.[28] Although there are no profound recent studies regarding the alcohol effect on hematology of human and particularly on leukocyte, erythrocyte, and platelet count, the present study is focused on the possible changes of leukocytes and bleeding risk in rats and compared it with aspirin.

Interestingly, as many researches are reported that alcohol has no significant changes on leukocyte, this study is shown no significant alteration in different WBC types as shown in Figures 1, 3 and 4 with $P > 0.05$. However, several studies are discussed that mobilization of leukocytes is diminished with alcohol consumption, and this may lead to increase the infection in alcoholic patients.[29-31]

Regarding the platelet count, there are no significant changes in platelet count ($P > 0.05$) which is assessed with different ethanol concentration, but importantly, the clotting time is increased. Moreover, the time needs to form a clot is increased significantly ($P^* < 0.05$) and this may be linked to other factors rather than platelets number. Furthermore, many articles are suggested that alcohol may affect blood fibrinolysis process which may increase the bleeding time.[13,32,33]

In the present study, it can be argued that ethanol has no or little effects on platelet count. Accordingly, several studies have been concluded that ethanol has an effect on platelets function rather than platelets count.[34] Moreover, several studies are identified that moderate consumption of wine can reduce platelet responsiveness due to alcohol antioxidant activity.[35] Importantly, researchers are found that accumulation of platelet cGMP can inhibit its aggregation and interestingly ethanol can induce cGMP accumulation and as a result inhibit platelets aggregation.[36,37]

CONCLUSION

Alcohol is a wide drink worldwide, so caution should be considered during use it to avoid unwanted complication in the long term. Therefore, it is better to avoid using of alcohol with antiplatelet and anticoagulant drugs as alcohol shows increasing bleeding time in the present study. Although alcohol is demonstrated no significant effect on WBC count, a patient with immune-comprised risk should use alcohol with caution to prevent any negative consequences as alcohol may reduce WBC mobilization to the injured site.

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