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Alterations in Some Blood Parameters After High Level Ethanol Intake

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Summary: The effects of high level ethanol intake on some blood parameters over a 50 days period were examined in 36 Sprague Dawley rats divided into 3 treatment groups: Ad libitum fed control group (n=12), sucrose group (n=12) fed with solution of sucrose so calorie intake equated with the ethanol group, and ethanol group (n=12) added ethanol (15 % v/v) in drinking water. Significant decreases ($p<0.05$) were noted in ethanol group's white- and red-blood cell counts, haemoglobin concentration, erythrocyte diameter, erythrocyte sodium and potassium levels, while significant ($p<0.05$) increases were observed in their mean corpuscular volume and mean corpuscular haemoglobin levels. No changes were observed in hematocrit level. Results of this study suggest that high level ethanol intake in rats causes alterations in blood cells count and in erythrocyte diameter and in erythrocyte element composition and this negative effect of ethanol on blood parameters did not stem from the energy of ethanol.

Key Words: Ethanol, blood parameters, energy of ethanol.

Yüksek Seviyede Etanol Alımından Sonra Bazı Kan Parametrelerindeki Değişiklikler

Özet: Yüksek seviyede etanol alımının bazı kan parametreleri üzerine etkisi, 50 günlük bir periyotta 3 gruba bölünmüş 36 Sprague Dawley ırkı sıçanda incelendi. Çalışmadaki gruplar; ad libitum beslenen kontrol grubu, etanol grubundaki sıçanlar ile eşit kalori alması için sükroz solüsyonu verilen sükroz grubu ve içme sularına etanol eklenen (% 15 v/v) etanol grubuydu. Etanol grubundaki sıçanların akyuvar ve alyuvar sayılarında, hemoglobin konsantrasyonlarında eritrosit çaplarında, eritrosit sodyum ve potasyum seviyelerinde istatistiksel açıdan önemli ($p<0.05$) düşüşler dikkati çekerken, ortalama alyuvar hacmi ve ortalama alyuvar hemoglobininde istatistiksel açıdan önemli ($p<0.05$) artışlar gözlendi. Bununla birlikte hematokrit değerde önemli farklılık gözlenmedi. Bu çalışmanın sonuçları, sıçanlarda yüksek seviyede etanol alımının kan hücreleri sayısında, eritrosit çapında ve eritrosit element yapısında değişikliklere neden olduğunu ve etanolun bu olumsuz etkilerinin etanolun sahip olduğu enerjiden kaynaklanmadığını göstermektedir.

Anahtar Kelimeler: Etanol, kan parametreleri, etanolun enerjisi.

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Introduction

Ethanol is widely consumed as a sedative-hypnotic drug throughout the world. It has been shown that ethanol intake may lead to damage in several tissues such as brain, stomach, liver or erythrocytes[1-4] and ethanol administration to experimental animals or ethanol abuse in humans has a profound effect on total blood cellularity[4,5]. It has been stated that in rats drinking 5 % ethanol for a period of 4 weeks, there was a rise of the leukocyte count particularly of lymphocytes and a decrease in the erythrocyte count and haemoglobin level in the peripheral blood[2,6]. In another study, in which ethanol was administered via inhalation, it has been expressed that there was not any significant difference with regard to the red blood cell count, white blood cell count, and haemoglobin concentration between the ethanol administered and control groups, but ethanol altered relative proportion of lymphocytes and polymorphonuclear leukocytes in peripheral blood[1,7-9]. In another study, following oral alcohol ingestion (2 ml per animal per day), a significant decrease in total blood celularity has been detected. Furthermore, a fall in the erythrocyte count per was accompanied by decreased hematocrit values and haemoglobin levels in the alcohol treated animals[4]. On the other hand, in the same study, the mean corpuscular volume (MCV) and mean corpuscular haemoglobi concentration (MCHC) values were significantly elevated after alcohol ingestion and despite overall leucopenia observed after alcohol ingestion, there was a significant increase in the absolute neutrophil count[4]. The absolute lymphocyte count, however, fell significantly in the alcohol treated animals[10-12]. Eosinophils and basophils were not significantly altered by alcohol toxicity. Moreover, chronic ethanol consumption decreased white blood cells count[13], hematocrit values and haemoglobin concentration[2,14] and changed the proportion of lymphocytes and polymorphonuclear leukocytes[1]. It has also been stated that ethanol consumption not only reduces red blood cell count but also alters erythrocyte sodium and potassium levels[15-19].

According to the studies performed so far, the effect of ethanol on blood parameters is contradictory. Therefore, the aim of the present study was to examine the chronic effect of high level ethanol intake on some blood parameters in rats.

Materials and Methods

Animals: In the study, 36 Sprague Dawley rats were used. Approximate body weight of rats was 48 ± 2 g at the beginning of the study. The rats were taken from Experimental Animals Breeding and Research Center, Uludag University, Faculty of Medicine, Bursa, Turkey. The rats were kept in a room with a temperature between 20-24°C. The animals were housed under a 12:12 h light/dark (from 08:00 to 20:00 light) cycle with free access to food and water.

The experimental protocols were approved by the Animal Care and Use Committee of Uludag University and are in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals.

Experimental Procedure: When the rats were 20 days old, they were taken away from their mothers and were placed as three animals per cage. The animals were cared during 5 days with free access to food and water. When the rats were 25 days old, they were assigned into three groups as an ethanol group (n=12, animals were given 15% v/v ethanol in drinking water), a sucrose group (n=12, animals given a sucrose solution, so calorie intake equated with ethanol group), and a control group (n=12).

The animals in the ethanol group were given 15 % (v/v) ethanol in their water and fed ad libitum. The animals in the sucrose group were given water with sucrose, but calorie intake of the animals in the sucrose group was restricted to equate with that of the animals in the ethanol group for equal feeding. For the calculation, it was accepted that concentration of pure ethanol was 0.79 g/ml, energy level of sucrose was 4.0 Cal/g, energy level of ethanol was 7.1 Cal/g and energy level of food given was 2,697 Cal/g. In equal feeding, the amount of food and water that was consumed by the rats during the study was calculated by subtracting the quantity of the rest of food and water for the next day from the quantity of food and water given every day and also mean value was recorded once every five days (Figure 1, 2). The animals of the control group had ad libitum access to food and water. The water given to animals was changed daily.

The blood samples were taken from the hearts of the rats into tubes without additives and tubes with lithium heparin (Greiner Labortechnik, Kremsmünster, Austria) on the 50th day of the study.

Evaluation of Blood Samples: The blood samples in no additive tubes were centrifuged at 3500 rpm for 5 min and serum samples were obtained and kept in a freezer at -20^0C.

The blood samples taken into heparinized tubes were examined in terms of hematocrit (PCV), white and red blood cells counts (WBC and RBC), amount of haemoglobin (Hb), MCV, MCH, MCHC, erythrocyte diameter (ED), erythrocyte sodium and potassium (E_{Na} and E_K) levels, and white blood cell differentiation.

The PCV was determined using a heparinized capillary tube by centrifuging the blood in a micro-hematocrit centrifuge for 6 min at 12000 rpm. Hb concentration was measured by adding 20 µl of well-mixed whole blood to 6 ml of Drabkin's reagent. After 4 min haemoglobin concentration was measured in spectrophotometer (UV-1200 series, Shimadzu Corporation, Japan) set at 540 nm[20]. The RBC and WBC were counted in a haemocytometer Thomas chamber[21]. The hematimetric indices, MCV, MCH and MCHC were calculated from the RBC, PCV and Hb concentration. The morphometric determination of average ED was measured using blood smears. These were fixed with methanol and stained with May-Grünwald and Giemsa solutions (Merck Co., Darmstadt, Germany). The cells were examined at 100 x (oil immersion objective) and their dimensions were estimated by means of a calibrated eyepiece (Nikon Co, Tokyo, Japan). Hundred erythrocytes were measured from each smear. White blood cell counts differentiations were determined from the same smears.

Sodium and potassium levels were analyzed by flame photometer (Flame Photometer PFP 7, Jenna LTD, and England)[22,23].

Serum Ethanol Level: Serum ethanol level was measured with dry system method in the Victors 950 Chemistry System apparatus by using kit obtained from Victors (Victors, catalogue no: 8839102).

Data And Statistical Analysis: Data are presented as mean ± S.E.M. Analysis of variance was performed for appropriate groups. Tukey test was performed as a posterior test if significant interactions were found. A P value of less than 0.05 was considered significant.

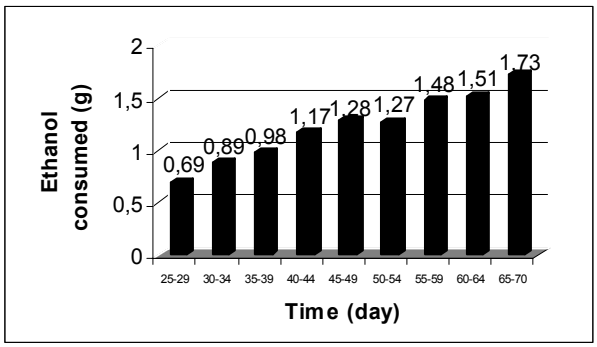

Fig. I:
The daily ethanol consumption of rats given ethanol 15 % (v/v).

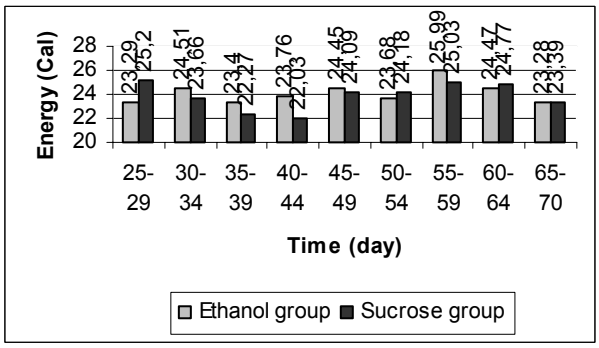

Fig. II:
The daily energy levels in the ethanol and sucrose groups.

Results

No significant difference were noted in the animal's weight (Control = 225 ± 5 g; ethanol = 220 ± 3 g; sucrose = 228 ± 7 g).

Serum ethanol levels in serum samples obtained from the animals in the ethanol group on the 50th day of the study was 53.75±19.89 ng/dl. Serum ethanol level varied according to the amount of water with ethanol that the animal consumed before the blood was collected.

As seen in Table I, significant ($p<0.05$) decrease was noted in RBC, WBC, Hb, ED, E_K and E_{Na} levels and MCHC, while significant increases were observed in MCV and MCH levels in ethanol group's rats. There was no difference in PCV levels of three groups.

White blood cell differentiations of the three groups are shown in Table II. Ethanol group had significantly ($p<0.05$) higher numbers of neutrophil and lower numbers of lymphocyte than other groups (Table II). There were no significant

difference in monocyte, lymphocyte, and basophil rates.

Table I. Hematologic values of blood samples collected from the three groups on the 25th and 50th days of the study (mean ± S.E.M.).

Parameters	Ethanol	Sucrose	Control
PCV (%)	46.1±0.9	45.6±0.3	47.1±0.6
RBC (10^6/mm^3)	4.28±0.2***	6.04±0.3	6.22±0.2
Hb (g/dl)	10.3±0.3***	12.1±0.2	12.0±0.2
MCV (μ^3)	107.7±2.6***	75.4±3.3	75.7±3.1
MCH (pgr)	24.1±0.8***	20.0±1.0	19.3±1.0
MCHC (%)	22.3±0.6***	26.5±0.6	25.5±0.7
ED (μm)	5.89±0.1***	6.59±0.1	6.68±0.1
WBC (10^3/mm^3)	5.1±0.4***	7.4±0.2	7.3±0.4
E_K (mEq/l)	71.1±0.9***	84.4±0.9	86.3±0.6
E_{Na} (mEq/l)	38.1±1.8***	56.1±2.3	60.7±3.1

* shows significance between ethanol and control groups.

** shows significance between ethanol and sucrose groups.

Table II. Differentiation white blood cell percent of blood samples collected from the three groups. (mean±S.E.M.)

Parameters	Ethanol	Sucrose	Control
Neutrophil (%)	27.2±1.7***	17.4±2.2	18.4±1.6
Lymphocyte (%)	70.4±1.7***	80.3±2.6	79.5±1.5
Eosinophil (%)	0.8±0.3	0.9±0.3	0.7±0.2
Basophil (%)	0.2±0.1	0.4±0.1	0.2±0.1
Monocyte (%)	1.4±0.5	1.0±0.3	1.2±0.5

* shows significance between ethanol and control groups.

** shows significance between ethanol and sucrose groups.

Discussion

Alcoholism, which has been continuously increasing, is a very important health problem and it is a reality that the cure methods performed are not always successful. The researchers, who state that alcoholism causes various health and social problems, also claim that alcohol consuming has a negative effect on many tissues and organs in the body. In the present study, 36 Sprague-Dawley albino rats were used in order to investigate the influence of ethanol on blood cells. The rats used in the study were assigned into 3 groups as control, ethanol, and sucrose, which took equal amount of energy with ethanol group rats. Ethanol itself is a source of energy. The reason of forming sucrose group was to find out whether the effect on blood parameters was due to direct ethanol or energy by ethanol. Therefore, ethanol intake of the animals and energy intake depending on this was calculated by a five-day mean and energy intake of animals in sucrose group was restricted considering this condition (Fig 1, 2). The obtained findings showed that negative effect of ethanol on blood parameters did not stem from the energy of ethanol because in the present study, there was no remarkable difference in terms of blood parameters between the sucrose and control groups.

The decrease in RBC count and Hb concentration, and increases in MCV and MCH in ethanol group animals is in agreement with previous reports on chronic alcoholics[24-26]. Similar results occur with folic acid and vitamin B_{12} deficiency[26,27]. In view of the association between chronic alcoholism, folic acid and vitamin B_{12} malabsorption, and macrocytic anaemia, further studies are necessary to ascertain if ethanol at the dietary doses used in this study also exerts an effect on metabolism of these substances. Ethanol intake caused increase erythrocyte membrane cholesterol[6,28]. Given the increased membrane cholesterol in the rats consumed ethanol an alternative explanation for elevated MCV and hence erythrocyte size may be a greater loading of membrane with cholesterol at the cell periphery[29] versus its centre. This could have occurred during formation of neocytes in the bone marrow and/or during the circulation of mature red blood cells through lipid exchange mechanisms.

The significant depression in the WBC count found in the ethanol group rats is in agreement with previous studies on alcoholism[26,30]. This may be due to alcohol-induced metabolic derangement and hormonal influences[31,32]. It may be of interest to determine if this decrease in circulating WBCs results in an attenuated immune response.

Ethanol caused decreases in the E_K and E_{Na} levels of erythrocyte. This finding is in agreement with previous studies[15,16,19]. Decreased E_K and E_{Na} levels may emanate direct effect of ethanol on erythrocyte membrane. Studies on red blood cell membrane of alcoholics have shown a decrease in sialic acid content and a disorganization of the outer membrane leaflet of erythrocytes. In addition, ageing seems to affect RBC survival in

circulating blood and modify the presence of sialic acid on the outer surface of RBC membrane[33,34]. Moreover, adult rats were more easily affected by ethanol treatment and their erythrocyte membranes prepared from alcoholic animals were more sensitive to lipid peroxidation than those prepared from control rats[35,36].

In the present study, an increase was observed in neutrophil ration and a decrease in lymphocyte ration but no alterations were found in monocyte, basophil and eosinophil rations in ethanol consuming rats. In previous studies, it was reported that eosinophils and basophils were not significantly altered by alcohol toxicity and chronic ethanol consumption changed the proportion of lymphocytes and increased polymorphonuclear leukocytes[1].

In conclusion, ethanol influenced hematologic parameters of rats negatively and this negative effect of ethanol on blood parameters did not stem from the energy of ethanol. Additional research is necessary to clarify effect of ethanol on blood parameters at molecular level.

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