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Research Article

Effects of Alcohol and Passive Smoking on Some Hematological Variables of Swiss Albino Mice*

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Abstract: In this study effects of alcohol and passive smoking on certain hematological variables were studied in 137 female and 85 male adult Swiss albino mice. The animals of both sexes were divided into four groups serving as controls, and alcohol (ALC), passive cigarette (PCS) and ALC+PCS exposure traits. Exposures were made with 10%, 20% or 30% v/v alcohol in drinking water, and 4, 8 or 12-lit cigarettes per cage and day for the 1st, 2nd and 3rd to 7th weeks of the experiment, respectively. Blood was collected at the end of the experiment from tail tip for determination of hematological variables. The data were analyzed via M-ANOVA. A p value of ≤ 0.05 was set as a significant level.

In general, leukocyte (WBC) and neutrophil (PMN) concentrations, packed cell volume (PCV) and erythrocyte fragility were the most but not necessarily consistently affected variables by ALC and PCS exposures. While the WBC of male mice exposed to ALC+PCS was significantly decreased when compared with the controls ($P < 0.05$), its decrease could not be confirmed statistically in females. Also PCV showed an increase only in male animals exposed to ALC+PCS ($P < 0.05$). However, MetHb concentrations were higher and PMN concentrations were lower in both female and male mice exposed to ALC+PCS than that of controls. Besides, there were multiple interactions between interventions (ALC, PCS and ALC+PCS exposures) and sex of animals. It was concluded that alcohol and passive smoking have partly time and sex-dependent deleterious effects on mice. Furthermore, they have synergetic effects when used together.

Key Words: Alcohol, passive smoking, blood cells, erythrocyte fragility, mouse

Alkol ve Pasif Sigara İçiciliğinin Swiss Albino Farelerinin Bazı Hematolojik Değişkenleri Üzerine Etkileri

Özet: Bu çalışmada alkol ve/veya sigaranın 137 dişi ve 85 erkek ergin Swiss albino faresinde bazı kan değişkenleri üzerine etkileri incelenmiştir. Erkek ve dişi fareler kontrol, alkol, pasif sigara ve alkol+pasif sigara gruplarına ayrılımışlardır. Alkol ve pasif sigara etkileşimi sırasıyla 1., 2. ve 3.-7. haftalarda %10, %20 ve %30 v/v alkol içme suyunda ve 4, 8, 12 sigara/kafes/gün olacak şekilde artırılarak gerçekleştirilmiştir. Hematolojik değişkenlerin belirlenmesi için hayvanlardan kan örnekleri deney sonunda kuyruk ucundan alınmıştır. Verilerin değerlendirilmesi M-ANOVA ile yapılmış ve $p \leq 0.05$ değeri anlamlı kabul edilmiştir.

Genel olarak alkol, pasif sigara ve alkol+pasif sigaradan en çok WBC, PMN ve PCV değerleri ile eritrosit frajilitesinin etkilendiği, ancak bu etkinin kararlı olmadığı görülmüştür. Kontrol grubuyla karşılaştırıldığında alkol ve sigara alan erkek farelerde WBC önemli ölçüde azalmış ($P < 0.05$), ancak dişilerde azalma istatistiksel olarak onaylanmamıştır. PCV değeri de sadece alkol ve sigara uygulanan erkek farelerde önemli bir artış göstermiştir ($P < 0.05$). Ayrıca, alkol ve sigara uygulanan dişi ve erkek farelerde MetHb değerleri önemli ölçüde artarken, PMN oranları azalma göstermiştir ($P < 0.05$). Tüm bu etkilerin kısmen zamana ve cinsiyete bağlı olduğu saptanmıştır. Bunun yanında etki faktörleri (ALC, PCS ve ALC+PCS) ile cinsiyet arasında önemli etkileşimler olmuştur. Ayrıca, alkol ve sigara kombinasyonunda sinerjetik etkiler dikkat çekicidir.

Anahtar Sözcükler: Alkol, pasif sigara içiciliği, kan hücreleri, eritrosit frajilitesi, fare

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Introduction

Alcohol is known to cause vacuolization in bone marrow cells and suppresses hematopoiesis (1-4). However, this effect has not been always reflected in peripheral blood picture because of changes in peripheral leukocytes, erythrocytes or other variables related to erythrocytes like packed cell volume (PCV) and hemoglobin (Hb) are very controversial and vary from anemia and leukopenia to erythrocytosis and leukocytosis (1,2,4,5).

Furthermore, little information is available for the effects of cigarette smoking on hematological variables. It may cause an elevation of mean corpuscular volume (MCV) due possibly to the decrease in vitamin B_{12} concentration (5). Heavy long-term smoking may cause erythrocytosis due to hypoxemia (6). The effects of cigarette smoking on leukocytes are very discrepant, varying from leukopenia to leukocytosis (7-10). However, different studies clearly indicate an impairment of leukocyte functions and immune system (11-13).

On the other hand, although the simultaneous use of alcohol and cigarettes is very widely distributed among different socio-demographic populations, our knowledge about their interactive effects is very sparse. In general, available studies are related to the effects of alcohol plus passive cigarette smoking (ALC+PCS) or nicotine application on the fetal and neonatal life periods (14-17). Only a few studies are concerned with the effects of alcohol plus passive cigarette smoking on the blood cells in humans and animals (7,10). It is not clear, however, whether the effects of these two agents are additive or one of them modify the effects of another. Solely, Leichter (14) suggested recently that the cigarette and alcohol exposure exerted a synergistic negative effect on fetal growth of rat pups.

Furthermore, there is also evidence that alcohol and smoking cause membrane deformity, and as a result a modification of the osmotic fragility in different cell types (18-21).

Actually, alcohol and cigarette abuse, alone or in combination, shows an increasing tendency, especially among women and adolescents of both sexes in many developing countries. Furthermore, many individuals are exposed to passive cigarette smoke. It is yet not forecastable exactly which kinds of effects are to be valued in a short or long term alcohol and/or sidestream cigarette exposure. Besides, it is not clear whether the effects of these two agents are additive or one of them modifies the effects of the other, as well.

This study aimed to investigate the effects of an acute but gradually increased alcohol and passive cigarette exposure alone or in combination on some hematological variables, and the possible interactions of alcohol and passive smoking in this respect in adult female and male Swiss albino mice.

Materials and Methods

Animals: A total of 222 ten-week-old Swiss albino mice, 137 females and 85 males, were used. At the beginning of experiment the body weights of female and male animals were 17-34 g and 21-39 g, respectively. Before and during the experiment all animals were fed with a standard commercial food for mice and rats (Best Yem, Gebze) and took tap water. Both food and water were available ad libitum.

Animals of both sexes were divided randomly into 4 groups consisting of 15 to 45 animals per group (Table 1), and were held in polypropylene cages in small groups of 4 to 5 mice per cage during the experiment. Animals of experimental groups were put in an isolated smoke chamber within their cages, which had a 0.90 cm^3 volume capacity and placed in a corner of the experimental room. The smoke chamber had a burning oven connected on one side, and on the opposite side an air exhaust leading the air to the outside of the room. A speed-control

Table 1. The general design of experimental groups.

♀	♂
Alcohol (ALC) (n = 42)	Alcohol (ALC) (n = 35)
Passive cigarette smoke (PCS) (n = 30)	Passive cigarette smoke (PCS) (n = 15)
Alcohol + passive cigarette smoke (ALC + PCS) (n = 45)	Alcohol + passive cigarette smoke (ALC + PCS) (n = 15)
Control (CON) (n = 20)	Control (CON) (n = 20)

ventilator was connected to the air outflow of the smoke chamber for controlling the inner air velocity. The speed of air exchange was determined before the experiment so that always a high enough smoke concentration within the smoke box was guaranteed. All exposures were carried out for a time period of 7 weeks. Alcohol and sidestream smoke exposure were increased gradually to allow adaptation of animals. For this reason alcohol was given in drinking water in a ratio of 10, 20 and 30% (v/v) and 4, 8 and 12 lit cigarettes (Short Samsun, Tekel®) per cage including 4 to 5 mice and day were burnt in the burning oven for the 1st, 2nd and 3rd to 7th weeks, respectively.

Sample collection: At the end of experiment blood samples for hematological analyses were collected from animals under ether anesthesia by tail-tip cutting into EDTA containing tubes. Erythrocyte (RBC), leukocyte (WBC), hemoglobin (Hb) and methemoglobin (MetHb) concentrations, leukocyte differentiation, packed cell volume (PCV) and the erythrocyte fragility were determined. RBC and WBC determined with standard methods in an improved Neubauer hemocytometry by mixing blood with a solution ad modum Hayem or Türk, respectively. Blood films were made immediately after blood withdrawal and processed by the panoptic method ad modum Pappenheim (22). On two separate blood films 200 leukocytes per animal were differentiated under light microscope, and concentrations of different leukocyte types per µl of blood were calculated by WBC per µl x % value of the given cell type / 100. The Hb and MetHb values were determined by photometry at 546 and 630 nm, respectively (Microlab 200, Merck®, Holland) (23). The erythrocyte fragility was determined as described by Jain (24).

Statistical Analysis: Data were analyzed with analyses of variance (M-ANOVA) in relation to within or between group differences and interactions by SPSS (Version 8.0 for Windows). Results were expressed as the mean ± SD, with their minima and maxima. Where the differences between groups were statistically significant, Least Significant Difference (LSD) was used to determine from which group they originated. The interactions between groups and sex as affecting factors were tested with two-way analyses of variance (M-ANOVA) (25).

Results

While the usage of parametrical test methods requires under others that data were usually assumed to come from populations with a Gaussian distribution, all data were controlled for group homogeneity by using the Levene test before carrying out the variance analyses. It was seen that the variances for levels of MetHb were unequal. In general, variance differences were smallest when data underwent to logarithmic transformation. Thus, the necessity has arisen to transform the MetHb data logarithmically.

Peripheral Blood Cells: Blood cell values of the female and male mice are presented in Table 2 to 7. Statistical analyses revealed that all interventions influenced certain hematological variables in both sexes. But there were some sex-dependent differences. For example, in female mice exposed to ALC the percentage of neutrophils was increased while it was decreased in the group exposed to ALC+PCS. As a result, the difference between the ALC and ALC+PCS groups for neutrophil percentages was statistically significant ($P < 0.05$). In general, the mean MetHb values of mice exposed to ALC, PCS or both ALC+PCS were higher than that of controls; however, only the difference between the control and ALC+PCS group was statistically confirmed ($P < 0.05$). In male mice, on the other hand, the mean WBC and neutrophil concentrations of the control group were significantly higher than that of animals exposed to ALC+PCS ($P < 0.05$). However, MetHb concentration was increased in the group exposed to ALC+PCS when compared to the controls ($P < 0.05$). Besides, the difference between the control and ALC+PCS groups for PCV values was confirmed, as well ($p = 0.05$). For this respect, the results also suggest that there were some sex-related differences (Tables 2 to 7). Furthermore, some interactions between affecting factors (treatments and sex) were also detected (Table 8). Analyses of post hoc results indicate that the differences among groups arose mainly from ALC+PCS groups.

Fragility: The calculated mean values of fragility and the fragility curves are presented in Figure. The results indicate that all interventions had deleterious effects on the membrane properties of RBCs, as well. Briefly, ALC, PCS and ALC+PCS enhanced the erythrocyte fragility, in general. Statistical evaluation of data showed that there were some sex-dependent characteristics in relation to

Table 2. The values of red blood cells (RBC), packed cell volume (PCV), hemoglobin (Hb) and methemoglobin (MetHb) in blood of the female mice exposed to alcohol (ALC), passive cigarette smoke (PCS) and their combination (ALC + PCS).

Variables	Groups			
	ALC X ± S.D. (Min-Max)	PCS X ± S.D. (Min-Max)	ALC + PCS X ± S.D. (Min-Max)	Control X ± S.D. (Min-Max)
RBC [x 10^6/µl]	10.00 ± 1.90 (6.10-12.80)	9.50 ± 1.20 (7.00-10.50)	10.30 ± 0.90 (9.10-11.40)	9.70 ± 0.60 (4.00-10.60)
PCV [%]	50.15 ± 3.58 (43.00-55.00)	47.50 ± 8.67 (30.00-52.00)	49.92 ± 2.23 (47.00-54.00)	50.38 ± 2.77 (46.00-54.00)
Hb [g/dl]	15.96 ± 2.61 (10.51-19.15)	14.98 ± 2.18 (12.86 –17.46)	14.85 ± 2.24 (12.09-17.42)	15.05 ± 1.43 (13.23 -17.24)
Met-Hb [%]	2.81 ± 2.49 (0.14-8.60)	1.45 ± 2.03 (0.14-5.70)	2.93 ± 2.61* (0.30-10.00)	0.92 ± 0.47* (0.18-1.48)

* $P < 0.05$

Table 3. The concentrations of leukocytes in blood of female Swiss albino mice exposed to alcohol (ALC), passive cigarette smoke (PCS) and their combination (ALC + PCS) [x 10^3/µl].

Variables	Groups			
	ALC X ± S.D. (Min-Max)	PCS X ± S.D. (Min-Max)	ALC + PCS X ± S.D. (Min-Max)	Control X ± S.D. (Min-Max)
WBC	7.26 ± 2.81 (4.25-11.29)	6.45 ± 1.90 (3.76-8.33)	6.37 ± 1.78 (3.11-8.93)	7.63 ± 3.12 (4.21-11.36)
Lymphocytes	5.55 ± 2.66 (3.10-9.88)	5.41 ± 1.63 (3.27-7.12)	5.84 ± 1.05 (4.80-7.45)	6.55 ± 2.57 (3.58-6.55)
Neutrophils	1.29 ± 0.69* (0.90-2.32)	0.85 ± 0.36* (0.40-1.24)	0.58 ± 0.43 (0.14-1.34)	0.86 ± 0.39 (0.59-1.14)
Monocytes	0.13 ± 0.14 (0.04-0.34)	0.13 ± 0.07 (0.06-0.21)	0.06 ± 0.04 (0.03-0.06)	0.05 ± 0.00 (0.04-0.05)
Eosinophils	0.29 ± 0.15 (0.07-0.41)	0.17 ± 0.12 (0.06-0.28)	0.02 ± 0.04 (0.08-0.20)	0.22 ± 0.22 (0.03-0.06

*, + $P < 0.05$

Table 4. The percentages of leukocytes in blood of female Swiss albino mice exposed to alcohol (ALC), passive cigarette smoke (PCS) and their combination (ALC + PCS).

Variables	Groups			
	ALC X ± S.D. (Min-Max)	PCS X ± S.D. (Min-Max)	ALC + PCS X ± S.D. (Min-Max)	Control X ± S.D. (Min-Max)
Lymphocytes	77.49 ± 6.09*+ (69.50-89.50)	80.20 ± 9.96 (60.00-89.50)	88.25 ± 5.34+ (82.50-96.00)	86.90 ± 6.98* (80.00-98.00)
Neutrophils	16.83 ± 10.04 (9.00-30.50)	15.70 ± 7.17 (10.50-28.00)	8.33 ± 4.84 (2.00-15.00)	9.75 ± 4.60 (1.50-12.50)
Monocytes	1.88 ± 1.75 (1.00-4.50)	1.90 ± 0.42 (1.50-2.50)	1.00 ± 0.71 (0.50-2.00)	0.75 ± 0.35 (0.50-1.00)
Eosinophils	3.80 ± 1.44 (1.50-5.00)	2.20 ± 1.72 (0.50-4.50)	2.42 ± 0.86 (1.50-3.50)	2.60 ± 1.85 (0.50-5.00)

*, + $P < 0.05$

Table 5. The values of red blood cells and certain related variables in blood of male Swiss albino mice exposed to alcohol (ALC), passive cigarette smoke (PCS) and their combination (ALC + PCS).

Variables	Groups			
	ALC X ± S.D. (Min-Max)	PCS X ± S.D. (Min-Max)	ALC + PCS X ± S.D. (Min-Max)	Control X ± S.D. (Min-Max)
RBC [x 10^6/µl]	9.50 ± 0.90 (8.10-10.40)	9.70 ± 2.00 (7.70-13.20)	9.70 ± 0.60 (8.70-10.40)	7.80 ± 1.60 (6.10-9.30)
PCV [%]	48.50 ± 1.69 (47.00-52.00)	47.17 ± 5.19 (39.00-53.00)	50.50 ± 3.73# (46.00-55.00)	45.00 ± 4.36# (42.00-50.00)
Hb [g/dl]	16.62 ± 1.85 (14.08-18.86)	16.24 ± 3.07 (10.07-17.98)	16.38 ± 2.65 (12.94-19.63)	13.76 ± 1.13 (12.72-14.96)
Met-Hb [%]	1.81 ± 1.85 (0.15-5.60)	3.78 ± 4.86 (0.30-12.25)	0.99 ± 0.72* (0.29-2.00)	0.26 ± 0.11* (0.12-0.40)

* $P < 0.05$, # $P = 0.05$

Table 6. The concentrations of leukocytes in blood of male Swiss albino mice exposed to alcohol (ALC), passive cigarette smoke (PCS) and their combination (ALC + PCS) [x 10^3/µl].

Variables	Groups			
	ALC X ± S.D. (Min-Max)	PCS X ± S.D. (Min-Max)	ALC + PCS X ± S.D. (Min-Max)	Control X ± S.D. (Min-Max)
WBC	6.06 ± 2.00 (3.70-8.60)	6.16 ± 3.20 (3.80-12.10)	3.40 ± 1.30* (1.20 – 4.80)	8.92 ± 7.30* (1.44-18.93)
Lymphocytes	4.02 ± 0.95 (2.77-5.45)	4.79 ± 1.91 (3.07-6.59)	2.31 ± 1.03 (0.76-2.88)	5.15 ± 4.07 (1.06-10.79)
Neutrophils	1.46 ± 1.74 (0.32-3.47)	1.30 ± 0.87 (0.83-1.96)	0.60 ± 0.24 (0.42-0.77)	3.23 ± 3.15 (0.22-7.67)
Monocytes	0.20 ± 0.20 (0.02-0.47)	0.14 ± 0.07 (0.08-0.22)	0.02 ± 0.00 (0.02-0.02	0.12 ± 0.06 (0.04-0.16)
Eosinophils	0.25 ± 0.20 (0.09-0.60)	0.17 ± 0.08 (0.08-0.24)	0.11 ± 0.14 (0.00-0.31)	0.25 ± 0.16 (0.11-0.47)

* $P < 0.05$

Table 7. The percentages of leukocytes in blood of male Swiss albino mice exposed to alcohol (ALC), passive cigarette smoke (PCS) and their combination (ALC + PCS).

Variables	Groups			
	ALC X ± S.D. (Min-Max)	PCS X ± S.D. (Min-Max)	ALC + PCS X ± S.D. (Min-Max)	Control X ± S.D. (Min-Max)
Lymphocytes	74.70 ± 16.75 (47.00-88.00)	77.75 ± 7.15 (70.50-84.50)	75.79 ± 11.64 (63.00-91.50)	68.63 ± 11.46 (54.50-80.50)
Neutrophils	18.12 ± 12.02 (9.51-39.65)	15.75 ± 3.00 (13.04-19.20)	20.25 ± 11.76 (8.50-33.08)	26.00 ± 7.98 (14.00-37.21)
Monocytes	2.88 ± 2.14 (0.50-5.50)	2.83 ± 2.02 (1.00-5.00)	0.83 ± 0.58 (0.50-1.50)	2.20 ± 0.57 (1.50-3.00)
Eosinophils	4.30 ± 1.99 (2.00-7.00)	3.67 ± 2.36 (1.00-5.50)	3.13 ± 3.68 (0.50-8.50)	3.17 ± 2.18 (1.50-7.50)

Table 8. The effects of alcohol (ALC), passive cigarette smoke (PCS) and alcohol plus passive cigarette smoke (ALC + PCS) (groups) and sex of the animals on certain hematological variables of Swiss albino mice (p values of two-way analyses of variance; n.s. = not significant) [Only these variables with any significance were considered].

Dependent Variables	Independent Variables		Interactions
	Groups	Sex	Groups / Sex
RBC	n.s.	< 0.05	< 0.05
PCV	< 0.05	n.s.	n.s.
Hb	< 0.05	n.s.	n.s.
WBC [x 10^3/µl]	< 0.05	< 0.05	< 0.05
Lymphocytes [x 10^3/µl]	n.s.	< 0.05	n.s.
Eosinophils [x 10^3/µl]	n.s.	< 0.01	< 0.05
Lymphocytes [%]	n.s.	< 0.05	n.s.
Neutrophils [%]	n.s.	0.01	n.s.
Monocytes [%]	< 0.05	n.s.	< 0.01

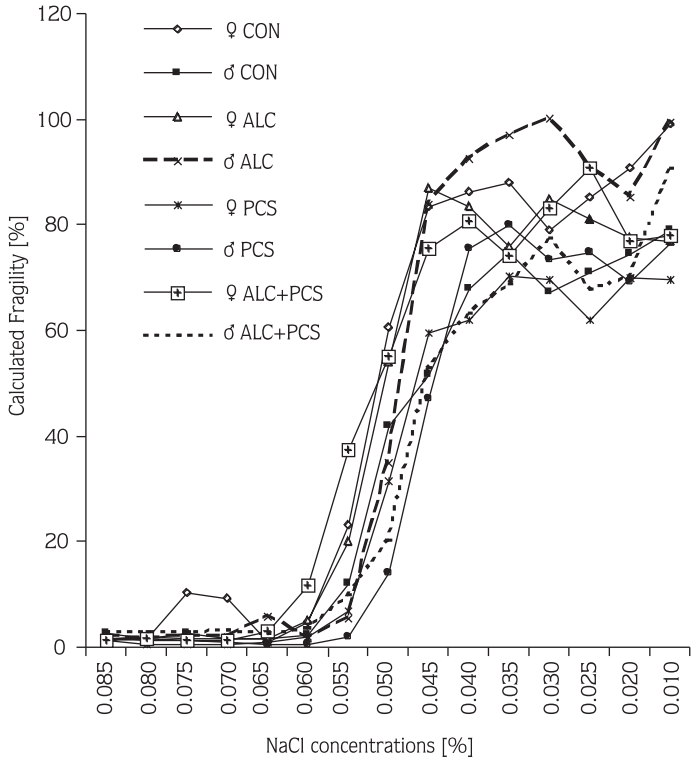

Figure. The fragility curves of the erythrocytes from female and male Swiss albino mice exposed to the alcohol, passive smoking or a combination of alcohol passive smoking (Lines indicate the group means).

the osmotic behavior of erythrocyte membranes. For example, no group differences among the females could be observed while in males significant differences were observed at dilution rates of .70%, .65%, .55%, .50% and .45% ($P < 0.05$, $P < 0.05$, $P < 0.001$, $P < 0.001$ and $P < 0.05$, respectively). Furthermore, two-way analyses of variance showed that there were multiple interactions between the affecting factors on the fragility properties of erythrocytes at different NaCl concentrations, which were also partly different in both sexes (Table 9).

Discussions

The results revealed that ALC and/or PCS exposure affected the leukocyte, neutrophil, PCV and met-Hb values as well as the red blood cell fragility in female and male Swiss albino mice. But these effects had definite sex-dependent characteristics. Furthermore, there were some interactions between affecting factors as alcohol, sidestream smoking, alcohol plus sidestream smoking and sex of animals as well.

The effects of ALC, PC smoking and ALC+PCS on the blood cells are the substance of many discussions because of the discrepant nature in clinical cases. Indeed, the changes in the levels of WBC, RBC, PCV, Hb etc. are very controversial. This is due partly to the difficulty in interpreting medical history data on drinking or smoking habits in respect to level of exposure or length of exposure time quantitatively as well as very sparse and incomplete evidence from experimental research. However, it was as early as the 1960s when the first evidence was observed that alcohol causes vacuolization in bone marrow cells (1), and suppresses hematopoiesis (2,4). Following studies revealed that acute and chronic alcohol consumption causes the impairment of hematopoietic system (3,26,27) and the function of immune system (11-13,28). The suppression of the hematopoiesis by the alcohol results also in remarkable changes in peripheral blood picture (3,4). Among the acute effects the decrease in PCV value and thrombocytopenia are noticeable (3). The most notable chronic effects include macrocytosis and microvolemia of RBCs and thrombocytopenia (4). The increase in RBC and

Table 9. The effects of alcohol, cigarette and alcohol plus cigarette (groups) and sex on the erythrocyte fragility in female and male Swiss albino mice (C= control, A= alcohol, S = passive smoke, and AS = alcohol plus smoke exposed groups; p values of analyses of variance; n.s. = not significant).

% NaCl [w/v]	f						m					
	P values						P values					
	C—A	C—S	C—AS	A—S	A—AS	S—AS	C—A	C—S	C—AS	A—S	A—AS	S—S
0.85	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.063	0.085	n.s.	n.s.	n.s.
0.80	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.067	< 0.01	n.s.	n.s.	n.s.	0.059
0.75	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.062	< 0.05	0.062	n.s.	n.s.	n.s.
0.70	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	< 0.01	< 0.01	< 0.05	n.s.	n.s.	n.s.
0.65	n.s.	0.054	n.s.	n.s.	n.s.	n.s.	0.074	0.054	n.s.	n.s.	< 0.01	< 0.01
0.60	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	< 0.05	< 0.05
0.55	n.s.	0.086	n.s.	n.s.	n.s.	n.s.	n.s.	0.086	< 0.01	n.s.	< 0.001	< 0.001
0.50	n.s.	< 0.05	n.s.	n.s.	n.s.	n.s.	n.s.	< 0.05	< 0.01	< 0.05	< 0.001	< 0.01
0.45	n.s.	n.s.	n.s.	0.086	0.091	n.s.	n.s.	n.s.	n.s.	n.s.	< 0.05	n.s.
0.40	0.063	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.082
0.35	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
0.30	0.051	0.097	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
0.25	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	< 0.05
0.20	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
0.10	n.s.	n.s.	n.s.	n.s.	n.s.	0.070	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

%0.00 NaCl [Blank control]

Hb levels due to the long-term smoking depends on the increase in carbon monoxide concentration (7).

ALC, PC smoking and ALC+PC smoking had also some remarkable effects on the erythrocyte fragility, which was characterized by a tendency to the enhanced osmotic hemolysis in this study. Several studies suggested that alcohol and cigarette smoke impair the antioxidant defense mechanisms of the cell membrane (20,21,29), especially by changing the lipid fluidity in the middle zone of the bilayer (18,19). It was also demonstrated that the effects of different agents causing membrane deformation on the rate of hemolysis might vary greatly (19). Thus, the results of this study might be the reflection of the oxidative stress effects of these two popular substances on the cell membrane.

In general, the results of this study support the discrepant nature of reactions to the alcohol and smoking observed in humans and animals. Although sex-dependent effects of ethanol on the liver were previously reported (30), different reactions of certain blood variables between the two sexes could not be explained with the current knowledge. However, the results of this study and available literature reveal that different species and sexes might respond differently to the same substance. Also, some effects attributed to smoking, for example, could be a result of the contamination of tobacco by environmental toxicants, such as lead (30). The synergism of alcohol and cigarette smoking could be the results of interactions of these two substances within the body, as well. Recently, Ericson et al. (31) suggested that nicotine causes approximately 80% increase in ethanol intake in rats. Lastly, endocrine statue of the female and male animals could play a definite role in their reactions to alcohol and cigarette exposure. There is evidence that the metabolic interactions of nicotine were mediated through insulinergic effects (32). Thus, these factors and methodical approaches should be considered when comparing the results of studies.

In conclusion, the results of this study suggest that alcohol and sidestream smoking affect various blood variables in female and male mice. In addition, it can be concluded from the post hoc analyses that ALC and PCS exposure have a more severe effect when combined than alone. However, there are certain controversial results for the same variable between the two sexes. This could be a reflection of the effects of endocrine system. But even this aspect, as many others, needs to be clarified through further detailed studies.

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