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Research Article

Effects of Alcohol, Passive Smoking and Alcohol Plus Passive Smoking on Some Serum Biochemical Variables in Mice

Mustafa ALTINIŞIK
Adnan Menderes University, Faculty of Medicine, Department of Biochemistry, Aydın - TURKEY
Muharrem BALKAYA
Adnan Menderes University, Faculty of Veterinary Medicine Department of Physiology, Aydın - TURKEY
Funda KARGIN
Adnan Menderes University, Faculty of Veterinary Medicine Department of Biochemistry, Aydın - TURKEY
Hümeyra ÇELER, Cengiz ÜNSAL
Adnan Menderes University, Faculty of Veterinary Medicine Department of Physiology, Aydın - TURKEY

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Abstract: The effects of alcohol, passive smoking and alcohol+passive smoking on certain biochemical variables were investigated in mice serum. For this purpose, 72 female and 49 male Swiss albino mice were used. The animals were approximately 10 weeks old at the beginning of the experiment and their initial body weights ranged from 22 to 31 g. All animals were divided randomly into four groups; the control, alcohol, passive smoking and alcohol+passive smoking groups. Alcohol was given in drinking water at a v/v ratio of 10% during the first week, 20% during the second week and 30% during the following four weeks. Side-stream smoking was carried out over a two-hour period per day. A total of 4, 8 or 12 lit cigarettes per cage containing 5 mice were used for the first, second and the following four weeks, respectively. Water and food were given to the animals *ad libitum*.

Blood samples were collected at the end of experimental period from the *Aorta abdominalis* into serum tubes under ether anaesthesia. The activities of enzymes ALT, AST and alkaline phosphatase, and the concentrations of glucose, total protein, total bilirubin, total cholesterol, triglycerides, chloride, potassium and sodium were determined in serum.

The data was analysed with a t-test and analyses of variance (M-ANOVA) in relation to within or between group differences with significance assessed at the level of $p<0.05$.

In the control group, serum ALT activity and glucose concentration were higher in female animals than in males ($p<0.05$). Furthermore, serum ALT activities as well as serum glucose and total bilirubin concentrations of both female and male animals in the alcohol drinking group were significantly lower when compared with those of the control group ($p<0.05$, $p<0.05$ and $p<0.01$, respectively). In the group exposed to smoke, only serum sodium levels in females were significantly higher, whereas those levels in males were significantly lower when compared to the controls ($p<0.001$). In the alcohol+passive cigarette smoking group, only the chloride levels of males were significantly higher in comparison to the control group ($p<0.001$).

Key Words: Mice, male and female, alcohol, passive smoking, serum biochemistry

Alkol, Pasif Sigara İçiciliği ve Alkol + Pasif Sigara İçiciliğinin Farelerde Bazı Serum Biyokimyasal Değişkenlerine Etkileri

Özet: Bu çalışmada alkol, pasif sigara ve alkol+pasif sigara içilmesinin farelerde bazı biyokimyasal parametreler üzerine etkileri araştırıldı. Bu amaçla ortalama 28 g canlı ağırlığında, yaklaşık 10 haftalık yaşta 72 dişi ve 49 erkek, toplam 121 adet Swiss albino fare kullanıldı. Fareler kontrol, alkol, sigara ve alkol+sigara grubu olmak üzere dört gruba ayrıldı ve deney süresince 5 fare/kafes olacak şekilde barındırıldı. Alkol, içme suyu içinde 1. hafta %10, 2. hafta %20 ve 3-7. haftalarda %30 (v/v) oranlarında olacak şekilde uygulandı. Sigara etkileşimi ise kafes başına 1. hafta 4, 2. hafta 8 ve 3-7. haftalarda 12 sigara olacak şekilde günde iki saat süreyle pasif içim şeklinde gerçekleştirildi. Deney sürecinde su ve yem *ad libitum* uygulandı. Kan, eter anestezisi altında abdominal aortadan direkt olarak serum tüplerine alındı. Elde edilen serumlarda Alanin ALT, AST ve ALP aktiviteleri ile glikoz, total protein, total bilirubin, total kolesterol, trigliserid ve elektrolitlerden klor, potasyum ve sodyum konsantrasyonları belirlendi. Sonuçlar t-testi ve variyanz analizi (M-ANOVA) ile değerlendirildi.

Kontrol gruplarında serum ALT aktivitesi ve glikoz düzeylerinin dişilerde erkeklerden daha yüksek olduğu görüldü ($p<0.05$). Kontrol grubu ile karşılaştırıldığında, alkol grubunda erkek ve dişi farelerde serum ALT aktivitesi ($p<0.05$) ile glikoz ($p<0.05$) ve total bilirubin ($p<0.01$) konsantrasyonlarının önemli ölçüde daha düşük olduğu belirlendi. Diğer değişkenler açısından önemli bir farklılık bulunmadı. Pasif sigara grubunda incelenen değişkenler arasında sadece serum sodyum değerlerinde dişilerde kontrol grubuna göre anlamlı bir azalma, buna karşın erkeklerde anlamlı bir artış olduğu saptandı ($p<0.001$). Alkol+pasif sigara grubunda incelenen değişkenlerden sadece serum klor düzeyinin erkeklerde kontrol grubuna göre önemli düzeyde yüksek olduğu belirlendi ($p<0.001$).

Anahtar Sözcükler: Fare, erkek ve dişi, alkol, pasif sigara içimi, serum, biyokimya

Introduction

There is an increasing interest in the potential effects of alcohol and cigarettes, alone or in combination, on human health worldwide. Studies indicate many negative effects of alcohol and cigarettes in all age groups (1-6). For example, acute or chronic alcohol consumption causes degeneration in different internal organs and systems of adults (7-12). Similarly, maternal alcohol consumption affects different organs and systems of the developing fetus as well (5,6,13). As a result of these negative effects, important functional disorders of these organs and systems occur frequently. High alcohol consumption is known to cause diarrhoea, other gastro-intestinal symptoms and, in advanced states, decreases in body weight (8). In general, it is accepted that, in individuals with high alcohol consumption, malnutrition develops depending on the possible changes in intestinal absorption mechanisms and dysfunction of some organs such as the liver and the pancreas (2,3,8,9). Furthermore, maternal alcohol consumption during gestation is known to cause fetal growth retardation in humans and laboratory animals (14,15), an effect persisting for a long period after parturition (16,17). The results of experimental studies conducted on rats demonstrate that alcohol negatively affects the body weight and the weight and length of bones (14,15). Although the exact mechanism by which alcohol causes growth retardation is not known, evidences indicate that ethanol interacts with nutrients (2,18,19-23).

Similarly, it is well known that cigarette smoking impairs food consumption (20), and affects the hemodynamic mechanisms (24,25) as well as the placental transport of nutrients and their metabolites between the mother and the fetus (14,22). These, in turn, result in disabilities of fetal development and cause births with low neonate weights (15,26) or morphologic and functional impairments in adults (27,28). It is accepted that the metabolic interactions of nicotine is mediated, at least in part, by insulinergic effects (29), but there is no sufficient information about the effects of long-term cigarette smoking.

The studies on the effects of cigarette smoking in humans are assessed on the basis of "smoked cigarettes per day" stated by individuals enrolled in such studies (30). Consequently, the data gathered from such studies on the basis of this information could cause major misconclusions (31). This problem is most significant when studying the effects of passive cigarette smoking in humans. This is why controlled long-term experimental studies with passive smoke exposure under defined conditions must be carried out to understand the effects exactly.

Furthermore, although the concomitant use of alcohol and cigarettes is very common, there are few studies about their interactive effects on different biochemical variables. Whether the effects of these two agents are additive or one of them modifies the effects of the other is not clear. Leichter (15) stated that cigarettes affect fetal growth independently of food consumption, and the effects of alcohol are synergetic to this effect of smoke.

There has been an increase in alcohol and cigarette use in our society in recent years, especially among women and adolescents of both sexes. It is highly possible that the first evidence of alcohol and cigarette use could cause changes in different variables, primarily in the blood, which as a fluid tissue is subjected to acute and profound changes continuously, depending on diverse internal and external factors. Thus, the aim of this study was to investigate the effects of long-term alcohol consumption and side-stream cigarette smoke as well as alcohol+passive cigarette smoke on certain serum biochemical variables in male and female mice.

Materials and Methods

This study was conducted on 49 male and 72 female Swiss albino mice. At the beginning of the experiment the animals were ca. 10 weeks old and their mean body weight was ca. 28 g with a range of 22.60 to 31.08 g. The animals were divided randomly in four groups: one group served as the control group, and the others were subjected to either alcohol, passive smoking or alcohol+passive smoking. Each group consisted of 9 to 25 animals.

The animals were placed in a semi-controlled room at 23ºC ± 3ºC and a light/dark cycle of 14:10 h two weeks prior to the initiation of the experiment. They were held in polypropylene cages in groups of 5 animals per cage, and given a standard diet for mice and rats (Best Yem, Gebze), and tap water. Food and water were given ad libitum to all groups.

The exposure methods of alcohol and cigarettes were the same as those described elsewhere (32). To avoid

anorexia and weight loss resulting from the abrupt introduction of high alcohol doses to the mice, the ethanol concentration in drinking water was increased gradually. A period of adaptation was also necessary for smoking. In brief, alcohol was given in the drinking water at a v/v ratio of 10% during the first week, 20% during the second week and 30% during the following four weeks. Side-stream smoking was carried out over a two-hour period per day. For this purpose, a total of 4, 8 or 12 lit cigarettes per cage containing 5 mice were used for the first, second and following four weeks, respectively. Short Samsun cigarettes (Tekel®) were chosen, because they are one of the most popular in Turkey. The ethanol and cigarettes were products of Tekel® and were obtained from a commercial source.

Sample collection

The water intake per cage and the body weights of all animals were recorded weekly after a food withdrawal period of ca. 14 hours. At the end of the experiment the animals were weighed, then a ventral midline incision was made under ether anesthesia, the Aorta abdominalis was cut and freely flowing blood was withdrawn into serum tubes immediately. Thereafter, blood samples were centrifuged, and serums were separated, collected and analysed. All sample collections were carried out within two consequent days between $09.^{00}$-$16.^{00}$.

In serum samples, the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) and the concentrations of total bilirubin, glucose, total protein, total cholesterol and triglycerides were analysed by autoanalyser (ILab 900) photometrically. The concentrations of chloride, potassium and sodium were also determined by ion-selective electrolyte apparatus (EasyLyte Plus Na/K/Cl Analyser, Medica).

Statistical Analyses

The data were analysed by t-test and analyses of variance (M-ANOVA). Results for each group were given as the mathematical means ± 1 standard deviation, with their minima and maxima. Before the statistical analyses of the data were carried out, the data were checked for group homogeneity by using the Levene Test, and, if necessary, transformations were carried out, according to the recommendations of Sachs (33). When differences between groups were statistically significant, least significant difference (LSD), Tukey's method and Scheffé intervals were used to determine from which group the differences originated (34,35). The interactions were tested with two way analyses of variance (M-ANOVA).

Results

Since parametrical test methods require the data which usually assumed to come from populations with a Gaussian distribution, before carrying out of variance analysis, all data was checked for group homogeneity by the Levene test, according to the recommendations of Sachs (33). In doing so it was seen that the variances of ALP activities and concentrations of total bilirubin and triglycerides were unequal. In general, the differences in variances between groups were smallest when the data underwent a logarithmic transformation. Thus the necessity arose to transform the data of these parameters logarithmically.

The activities of ALT, AST, and ALP as well as the concentrations of glucose, total protein, total bilirubin, total cholesterol, triglycerides, chloride, potassium and sodium are given in Tables 1 and 2 and in the Figure 1.

When the data gathered from male and female control groups were compared, it was seen that serum ALT activity and glucose concentrations were higher in females than in males ($p<0.05$). No sex-dependent difference was confirmed for other variables.

Furthermore, it was found that alcohol, passive cigarette smoke and alcohol+passive cigarette smoke had important effects on certain biochemical variables. These effects were also sex-dependent (Tables 1 and 2). For example, in the alcohol group, the ALT activity ($p<0.05$), concentrations of glucose ($p<0.05$) and total bilirubin ($p<0.01$) were significantly lower when compared with those of the control group. No significant difference was found for other variables.

In the cigarette smoking group, only the sodium concentration decreased significantly in female animals. In contrast, in male animals the sodium concentration increased markedly ($p<0.001$). In the alcohol+cigarette group only the serum chloride concentration increased significantly in male mice ($p<0.001$).

It is noteworthy that the effects of alcohol, cigarette and alcohol+cigarette exposure on certain variables show different characteristics in male and female animals. In female mice the differences in ALP activity between the

Table 1. The effects of alcohol and/or cigarettes on some serum biochemical variables.

Groups		♀			
		Alcohol (n = 15)	Passive cigarette smoke (n = 9)	Alcohol + passive cigarette smoke (n = 25)	Control (n = 23)
Variables		X ± S.D.	X ± S.D.	X ± S.D.	X ± S.D.
ALP (U/L)	♀	95.47 ± 73.04^{+}	58.00 ± 27.07	52.81 ± 29.60^{+}	74.81 ± 15.91
	♂	54.18 ± 18.70*	79.00 ± 18.68*$^{\#}$	88.43 ± 25.92$^{\#}$	73.70 ± 30.97
AST (U/L)	♀	279.25 ± 156.13	235.17 ± 112.14	352.72 ± 139.18	267.53 ± 134.21
	♂	277.62 ± 110.77$^{\#}$*	136.55 ± 83.28$^{\#+}$	134.00 ± 53.92*	241.50 ± 156.67^{+}
ALT (U/L)	♀	62.06 ± 21.41$^{\#}$	56.50 ± 13.97*$^{\#}$	80.90 ± 29.35	100.32 ± 56.82*
	♂	70.69 ± 41.07	64.78 ± 28.70	40.29 ± 12.39*	95.70 ± 85.54*
CHOL (mg/dl)	♀	70.25 ± 13.95*	89.78 ± 24.49*$^{+}$	82.62 ± 13.66	70.05 ± 31.38^{+}
	♂	85.00 ± 14.23*	91.33 ± 18.23$^{\#}$	89.38 ± 17.68‡	67.60 ± 18.08$^{\#‡}$*
T-BIL (U/L)	♀	0.10 ± 0.00	0.12 ± 0.04	0.11 ± 0.05	0.12 ± 0.04
	♂	0.11 ± 0.03$^{\#}$	0.12 ± 0.04*	0.19 ± 0.07$^{\#‡}$*	0.12 ± 0.04‡
TRGLY (mg/dl)	♀	130.50 ± 46.79	135.22 ± 69.51	149.52 ± 63.72	143.05 ± 85.24
	♂	151.20 ± 72.76	135.67 ± 61.21	139.75 ± 46.99	131.60 ± 52.44
Glucose (mg/dl)	♀	167.75 ± 70.55^{+}	157.00 ± 60.79‡	203.67 ± 67.70	229.60 ± 60.49$^{+‡}$
	♂	173.67 ± 61.02	187.00 ± 77.76	135.43 ± 82.69	181.70 ± 65.23
T-PROT (g/dl)	♀	6.17 ± 0.59	6.49 ± 2.02	6.07 ± 0.56	6.25 ± 1.00
	♂	6.07 ± 0.71$^{\#}$	5.54 ± 0.55	5.99 ± 0.83*	5.29 ± 0.58$^{\#}$*

The means with the same symbols in a row differ significantly at the level of p<0.05 (* and $^{+}$), or p<0.01 ($^{\#}$ and ‡).

Table 2. The effects of alcohol and/or cigarettes on main serum electrolyte concentrations.

Groups		♀			
		Alcohol (n = 15)	Passive cigarette smoke (n = 9)	Alcohol + passive cigarette smoke (n = 25)	Control (n = 23)
Variables		X ± S.D.	X ± S.D.	X ± S.D.	X ± S.D.
Na (mEq/L)	♀	148.93 ± 1.00	146.82 ± 2.37	149.54 ± 2.03	147.55 ± 1.21
	♂	157.15 ± 2.77$^{¥Ø\#}$	149.20 ± 1.35¥	152.22 ± 3.36$^{\#}$*	147.90 ± 1.88^{Ø}*
K (mEq/L)	♀	5.61 ± 0.79	5.97 ± 1.62	6.81 ± 2.45	6.17 ± 0.78
	♂	6.24 ± 1.13	6.24 ± 1.19	5.27 ± 0.68	5.90 ± 0.93
Cl (mEq/L)	♀	115.33 ± 2.35	115.32 ± 2.80	117.64 ± 1.65	117.65 ± 0.79
	♂	121.95 ± 2.27$^{\#‡}$	114.85 ± 3.91$^{\#}$*	119.32 ± 3.85*$^{+}$	114.83 ± 2.07$^{‡+}$

The means with the same symbols in a row differ significantly at the level of p<0.05 (* and $^{+}$), p<0.01 ($^{\#}$ and ‡), or p<0.001 ($^{¥}$ and ^{Ø}).

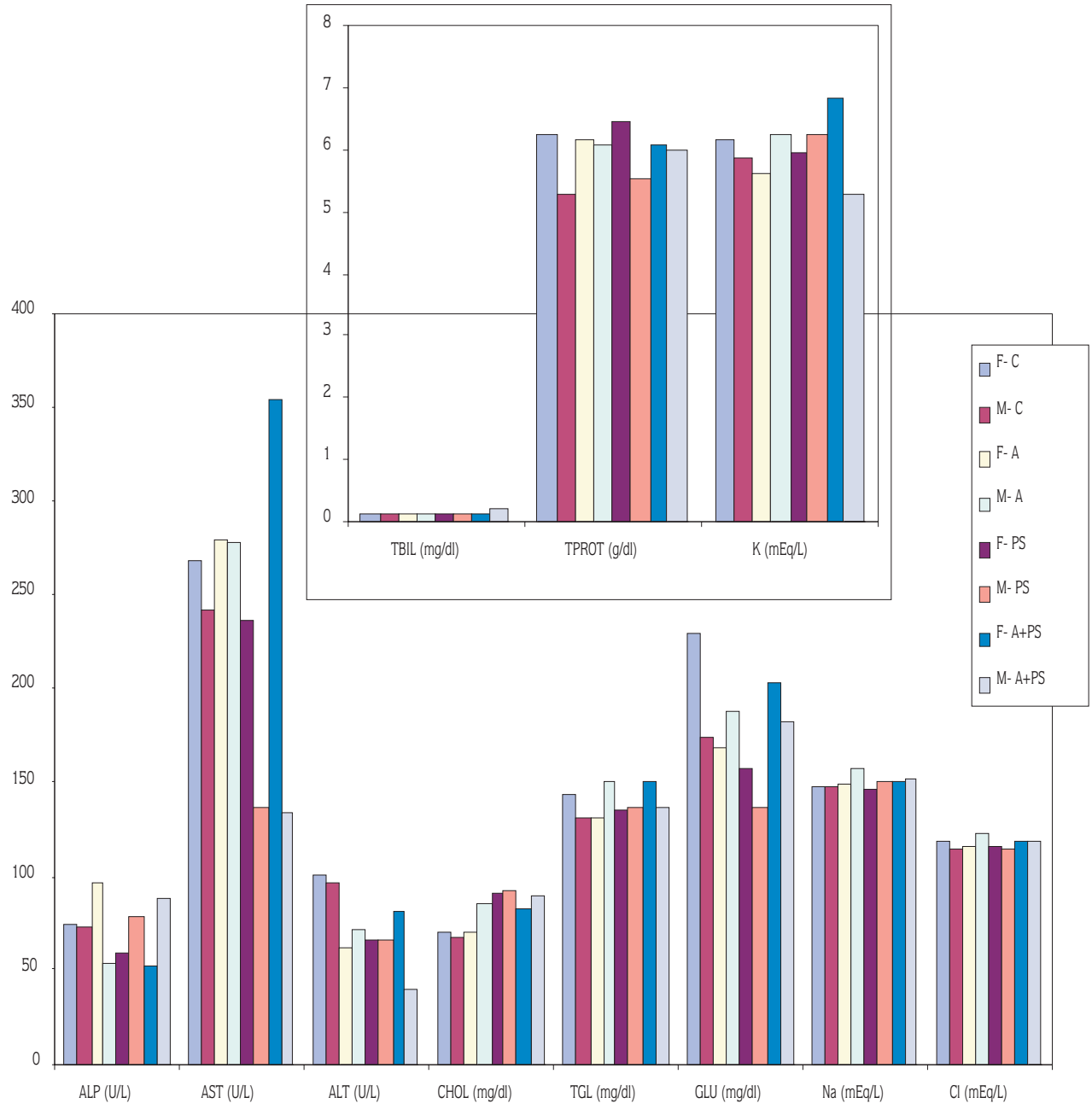

Figure. Some serum biochemical values in mice exposed to alcohol, passive smoking or both.

cigarette and alcohol+cigarette groups were significant ($p<0.05$). In contrast, ALP activities significantly differed between the alcohol and cigarette groups and the cigarette and alcohol+cigarette groups in male animals ($p<0.05$). For AST activity, only in male animals there were significant differences between the alcohol and cigarette groups and the cigarette and alcohol+cigarette groups ($p<0.05$). With regard to chloride values, there was a significant difference between the cigarette group and the alcohol+cigarette group in male animals ($p<0.01$). The sodium values were also significantly different between the alcohol and alcohol+cigarette groups in females, while in males significant differences occurred between the alcohol and cigarette, alcohol and

Table 3. The effects of the alcohol, cigarettes and alcohol+cigarettes on some serum biochemical variables in mice (p values of a two-way analysis of variance; n.s. = not significant).

Dependent Variables	Independent Variables		Interactions
	groups	sex	Groups / Sex
ALP	n.s.	n.s.	< 0.01
AST	n.s.	< 0.05	< 0.05
ALT	< 0.05	n.s.	n.s.
Glucose	n.s.	n.s.	n.s.
Cholesterol	< 0.05	n.s.	n.s.
Total bilirubin	< 0.05	< 0.05	< 0.05
Triglycerids	n.s.	n.s.	n.s.
Total protein	n.s.	< 0.05	n.s.
Cl-	< 0.05	n.s.	< 0.01
K+	n.s.	n.s.	n.s.
Na+	< 0.01	< 0.01	< 0.01

alcohol+cigarette and cigarette and alcohol+cigarette groups ($p<0.001$). However, ALT activities and concentrations of total protein and potassium did not vary significantly in female and male animals. Cholesterol values in the three experimental groups were higher than those of controls in male mice, but in females only the smoking group was significantly different from the controls (Table 1, Figure 1).

Furthermore, the results of a two-factorial analysis of variance considering the groups and sex of animals as the affecting factors showed that there were some important bi-directional interactions between affecting factors (Table 3). None of these factors alone had any effect on the ALP activity, but there was an interaction between these factors ($p<0.01$). The effects of sex and groups on the serum AST and ALT activity, respectively, were significant ($p<0.05$). Furthermore, a significant interaction between these factors was found in relation to the effect on the AST activity ($p<0.05$). It was notable that the effect of sex on the total protein concentration was significant ($p<0.05$), but the groups had no effect nor there was any interaction between the two factors in this respect. With regard to the cholesterol concentration, only the groups had a significant effect ($p<0.05$). The effect of sex on the total bilirubin concentration was found to be significant, and there was also an important interaction between the two factors ($p<0.05$). Similarly, the effects of these two factors on the serum sodium concentrations were significant ($p<0.001$). A significant interaction of the factors in this respect was also established ($p<0.01$). The effects of groups on the serum chloride concentrations were significant; there were also an important interaction between these two factors ($p<0.05$ and $p<0.01$, respectively). In contrast, for the serum glucose, triglyceride and potassium concentrations there was neither significant effect nor interaction.

Discussion

Mitruka and Rawnsley (36) stated that in mice the serum biochemical variables were not sex-dependent. The results of the control groups in the present study were in accordance with the results of these authors except those concerning ALT activity and glucose concentration.

It is obvious that profound changes could occur in the function of the liver when the amount of alcohol consumed by mice in this study is considered. It is well known that alcohol affects primarily the liver in humans (37-39). In humans, it was determined that acute alcohol caused a significant reduction in the concentrations of plasma total protein, glucose and sodium, while it caused a significant increase in the serum AST activity. Studies on rats drinking alcohol indicated that the reduction in total protein concentration was due to the reduction of albumin fraction (40).

In the present study, the effects of chronic alcohol consumption on the serum total protein of female mice were not significant, but in male mice there were significant differences between the control group and the groups exposed to alcohol and alcohol plus passive smoke. No information about the effects of alcohol and cigarettes on serum ALP, ALT and AST activities nor the concentrations of total protein, cholesterol, total bilirubin, triglycerides, glucose, Na^+, K^+ and Cl^- in mice was found. In a study conducted with hamsters, Dontenwill et al. (41) stated that long-term exposure to cigarette smoke did not affect serum enzyme activities and concentrations of serum total protein and electrolytes. In contrast, an elevation in serum AST, γ-GPT, total bilirubin and LDH values in rats due to smoke have recently been reported (27). In addition, Chan-Yeung et al. (42) suggested that smoking was associated with lower total bilirubin, AST, total protein and blood urea nitrogen values in human serum. The evidences from our study suggests that alcohol and cigarettes in the passive smoking mode exerted

significant effects primarily on the liver enzyme activities and on some other organs and systems, as well. Thus our study did not confirm the findings of Dontenwille et al. (41), but had some similarities with results from rats (27,42). Therefore, these results support the well accepted views related to the multidirectional effects of alcohol or cigarettes on certain organs and systems (4,37,38,43-45). However, it must be highlighted that for many variables there is no consistency when the effects of alcohol, passive smoking or alcohol+passive smoking are considered in both sexes. Similarly, there is also no consistency in interactions between affecting factors under these circumstances.

Using smokeless tobacco increased sodium excretion in the urine of humans, whereas potassium excretion did not increase significantly (46). However, there was no information about how cigarettes could affect electrolyte concentrations in the blood serum in smokers. Furthermore, it is possible that in the case of active smokers electrolytes could be taken from tobacco, but for passive smoking no information is available. Thus, the discussion of the results from this study was strongly limited. However, the diverse behaviour of sodium in the male and female animals is interesting.

The results of certain studies indicated that alcohol and cigarettes affect the endocrinium and the nervous system (10,11,47-49). These effects are sex-dependent. The diverse reactions seen in the sodium values of male and female animals in this study could, at last partly, be due to the effects of cigarettes on regulatory systems and the possible sex-dependent characteristic of these effects.

Leichter (15) determined that alcohol and cigarettes exerted additive effects on the development of the fetus during gestation. The results of this study also indicate multi-directed interactions between alcohol and cigarettes, and suggest that these factors have additive effects on some of the biochemical variables analysed. In doing so, the results support the statement *ad modum* Leichter.

In conclusion, the results of this study support the statement that alcohol and passive cigarette exposure could affect the haematological and serum biochemical values in humans and animals. However, the effects of alcohol and cigarettes in mice, which are considered an important animal model of humans in many aspects, and in other animal species, must be documented in detail. In particular, research is needed to assess the differences that depend on environmental factors including the diet as well as the sex and age of animals, and genetic variations (line, race), and in a wider aspect the species dependent differences should be explained primarily.

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