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Research Article

THE EFFECT OF THE CIGARETTE SMOKE ON ACTIVITIES OF SOME SERUM ENZYMES IN DIABETIC RATS

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ABSTRACT
Although Nicotine has been known as the most toxic component of cigarette, many of the effects of smoking, such as cardiovascular disease, respiratory disease, cancers, especially lung, throat and mouth cancer are not related to the nicotine, but are related to the other toxins and carcinogens in the smoke or extract of tobacco. Cigarette smoke contains the main and lateral (active and passive) smokes. Lateral smoke is the smoke from a burning cigarette, which is inhaled by people around the smoker and the smoke is much more toxic than mainstream cigarette smoke. According to studies, it has been shown that cigarette smoke has a great influence on the mandatory or voluntary companions of the smokers. In this study the effects of passive smoke inhalation on the activity of serum enzymes in diabetic rats were evaluated.
In this intervention study, 24 male Wistar rats weighing 200 to 220 g randomly divided into 4 groups of 6 animals: healthy control, diabetic control, smoker treatment and smoker-diabetic treatment groups. In the diabetic control and treatment groups the diabetes was induced with a single dose (65mg / kg) injection of streptozotocin in the IP form. The blood sugar levels of rats were measured using a glucometer after 24 hours; so, the rats with higher glucose levels than 250 mg / dl were considered as diabetic. In healthy control group no intervention was applied. The healthy treatment and diabetic treatment groups were exposed to the inhalation of passive smoke of a cigarette burned during 1 to 2 minutes for 30 days routinely. After one month, the rats were decapitated and the blood samples were obtained. The samples were centrifuged and the serum was separated. The activity of serum AST, ALT, ALP, LDH and CPK were measured using diagnostic kits and spectrophotometric method and the obtained results were analyzed by ANOVA method. During the study, all groups were exposed to the same environmental and light conditions as well as unlimited access to food and water. Statistical comparison of the results obtained in this study showed that there is no significant difference in understudied factors of the groups.

Keywords: *Cigarette, Passive Smoke, Serum Enzymes, Diabetic Rats*

INTRODUCTION
Very volatile alkaloid, nicotine is as a major component of cigarette smoke and can cause many detrimental effects on the body. A cigarette has from 2 to 24% nicotine. (VijayammalP1999). According to published papers it has been demonstrated that cigarette smoke has s significant effect on mandatory or voluntary companions of a smoker besides him/her (Zenzes, 2000). According to forecasts, in the next 30 years, 10 million people in every year will be added to the addicted people to smoking that 70% of the victims will be from developing countries (Zenzes, 2000).
Nicotine is an alkaloid drug in tobacco leaves (Nicotiana tabacum). Nicotine has the visual characteristics such as white to pale yellow, which becomes brown in color on exposure to light or air. It is decomposed at 247°C also has the Solubility in water at 60°C. It should be stored in nitrogen away from light and in sealed containers at temperatures below 25°C. Its chemical formula is N14H10C2. The half-life of nicotine is a half to 2 hours (Vijayammal, 1999). When nicotine enters the body, it is transmitted rapidly to blood flow and cross the BBB-brain barrier within 7 seconds. Nicotine is metabolized in the liver by the cytochrome enzyme P450 (CYP2A6) and (CYP2B6). Cotinine is the major metabolite of nicotine.
Cotinine is the byproduct of nicotine metabolism that remains in the blood more than 48 hours, which is the index of smoking pleasure. Nicotine has an influence on nicotine acetylcholine receptors, especially

the ganglion and the central nerve system (CNS). By binding nicotine to ganglion receptors, the Epinephrine, a stimulating hormone, stream will be increased which causes in turn to cell depolarization and calcium current from the voltage-dependent calcium channels. This causes the epinephrine and norepinephrine release to blood circulation. The Release of adrenaline causes an increase in blood pressure, increased heart rate and respiratory rate. With nicotine binding to nicotinic receptors (CNS), the dopamine level increases in the brain, causing the pleasure of smoking (Miceli, 2005). Nicotine also increases saliva, gastric motility and acid secretion. Nicotine is a stimulant of the nervous system that increases awareness, concentration, and decreased appetite. Frequent consumption of nicotine will cause tolerance and dependence (Kavitharaj, 1999). Although nicotine is known as the most toxic component of cigarette, a lot of complications, such as cardiovascular disease, respiratory disease, cancers, especially lung, throat and the mouth cancers are not related to nicotine, but other toxins and carcinogens in smoke or extract of tobacco (Ahmadi, 2001) and (Benowitz, 1988). Two types of smoke produced by burning tobacco:

1) Main Smoke (active smoke): the smoke inhaled by the smoker.
2) Lateral smoke (passive smoke): smoke from burning tobacco, which is inhaled by the people around smokers (these kind of non-smoker inhalers is called passive smokers) and very toxic than the main smoke. The mandatory exposition to cigarette smoke for 30 minutes can harm the non-smoker's heart and increase the risk of heart disease by 30 percent. Lateral cigarette smoke contains 5 times more carbon monoxide and 6 times more nicotine than that smokers inhale because cigarette has a protective filter. The cigarette smoke has over 4000 toxic and mutagenic components such as carbon monoxide, aromatic hydrocarbons, and nicotine (Carvalho, 2006).

Cigarette smoke contains a range of oxidants and free radicals that can increase lipid peroxidation. About 10^{14} free radicals enter the lungs in every inhalation of smoke. Free radicals can directly or indirectly induce oxidative stress in the body (Hecht, 2002). Considering the metabolism of nicotine in liver as well as its influence on the nervous system, the present study concentrated on the effect of passive cigarette smoke on some serum enzyme activity (AST, ALT, ALP, LDH, CPK) in diabetic rats.

Cigarette smoke is one of the factors in oxidant production and free radicals. It must be noted that adding some aromatic substances in cigarettes has an important role in increased damages caused by free radicals (Dasgupta, 2009).

MATERIALS AND METHODS

In this intervention study, 24 male Wistar rats weighing 200 to 220 g randomly divided into 4 groups of 6 animals: healthy control, diabetic control, smoker treatment and smoker-diabetic treatment groups. The rats were kept in boxes made of polypropylene with dimensions 30 x 30 x 60 cm. In the diabetic control and treatment groups the diabetes was induced with a single dose (65mg / kg) injection of streptozotocin in the IP form. The blood sugar levels of rats were measured using a glucometer after 24 hours; so, the rats with higher glucose levels than 250 mg / dl were considered as diabetic. The healthy treatment and diabetic treatment groups were exposed to the inhalation of passive smoke of a cigarette burned during 1 to 2 minutes for 30 days routinely. After one month, the rats were decapitated and the blood samples were obtained. The samples were centrifuged and the serum was separated. The activity of serum AST, ALT, ALP, LDH and CPK were measured using diagnostic kits and spectrophotometric method and the obtained results were analyzed by ANOVA test. During the study, all groups were exposed to the same environmental and light conditions as well as unlimited access to food and water. In the control group, no intervention was applied.

RESULTS AND DISCUSSION

Results

The Mean Values of AST (u/l) in the Groups: Average levels of AST were measured in different groups, and the results were compared using ANOVA test at a possibility level of 95% and a significant level of 0.05 were compared and the results are shown in Table 1

Table 1: Comparison of AST (u/l) in understudied groups

Group	Mean±SE	SD	Sig (P value)
Smoker-diabetic treatment	180.38 ± 9.18	25.97	
Smoker treatment	144.50 ± 9.59	23.50	
Diabetic control	232.00 ± 44.68	109.45	0.13
Healthy control	175.00 ± 6.00	8.48	

According to the obtained results from this study, there was no significant statistical difference in AST among the groups (P>0.05).

The Mean Values of ALT (u/l) in the Groups: Average levels of ALT were measured in different groups, and the results were compared using ANOVA test at a possibility level of 95% and a significant level of 0.05 were compared and the results are shown in Table 2.

Table 2: Comparison of ALT (u/l) in understudied groups

Group	Mean±SE	SD	Sig (P value)
Smoker-diabetic treatment	113.00 ± 8.62	24.40	
Smoker treatment	42.67 ± 7.24	17.73	
Diabetic control	152.17 ± 40.52	99.26	0.10
Healthy control	73.5 ± 9.50	13.43	

According to the obtained results from this study, there was no significant statistical difference in ALT among the groups (P>0.05).

The Mean Values of ALP (u/l) in the Groups: Average levels of ALP were measured in different groups, and the results were compared using ANOVA test at a possibility level of 95% and a significant level of 0.05 were compared and the results are shown in Table 3

Table 3: Comparison of ALP (u/l) in understudied groups

Group	Mean±SE	SD	Sig (P value)
Smoker-diabetic treatment	593.38 ± 53.42	151.11	
Smoker treatment	523.67 ± 95.58	234.12	
Diabetic control	702.00 ± 62.51	153.12	0.06
Healthy control	299.00 ± 60.00	84.85	

According to the obtained results from this study, there was no significant statistical difference in ALP among the groups (P>0.05).

The Mean Values of LDH (u/l) in the Groups: Average levels of LDH were measured in different groups, and the results were compared using ANOVA test at a possibility level of 95% and a significant level of 0.05 were compared and the results are shown in Table 4

Table 4: Comparison of LDH (u/l) in understudied groups

Group	Mean±SE	SD	Sig (P value)
Smoker-diabetic treatment	1527.00 ± 130.91	370.27	
Smoker treatment	1464.17 ± 98.36	240.94	
Diabetic control	1635.67 ± 59.58	145.94	0.33
Healthy control	1859.00 ± 25.00	35.35	

According to the obtained results from this study, there was no significant statistical difference in LDH among the groups (P>0.05).

The Mean Values of CPK (u/l) in the Groups: Average levels of CPK were measured in different groups, and the results were compared using ANOVA test at a possibility level of 95% and a significant level of 0.05 were compared and the results are shown in Table 5

Table 5: Comparison of CPK (u/l) in understudied groups

Group	Mean±SE	SD	Sig (P value)
Smoker-diabetic treatment	3316.13 ± 307.81	870.61	
Smoker treatment	3550.50 ± 220.81	540.88	
Diabetic control	3604.67 ± 300.91	737.08	0.84
Healthy control	3767.00 ± 154.36	1016.82	

According to the obtained results from this study, there was no significant statistical difference in CPK among the groups (P>0.05).

Discussion

Serum ALP activity is a suitable index to measure the biliary tract injuries. Furthermore, the activity of serum AST and ALT enzymes is an indicator of liver cell damage (Raja, 2011). Nowadays, the effects of smoking on liver enzyme activity are discussed. Some of studies found that smoking causes no liver damage and the serum activity of liver enzymes, which is consistent with our results (Harris, 2000). In a study it was found that smoking caused a significant increase in serum activity of ALP enzyme, which is in contradiction with our results (Mohammed, 2013). In another study, serum ALT and AST activity in smokers showed not significant difference compared with non-smokers, which is consistent completely with our findings (Mohammed, 2013). Very few studies have been conducted on the effect of concomitant diabetes and smoking on serum enzyme activity. This study showed that passive cigarette smoke has a slight and non-significant effect on liver cells and the activity of serum enzymes. It is recommended that the high-dose long-term effects of tobacco smoking on serum biochemical parameters be investigated in future studies.

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