The Effect of Concomitant Ethanol and Opium Consumption on Lipid Profiles and Atherosclerosis in Golden Syrian Hamster's Aorta

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Original Article

Abstract

Background: Cardiovascular disease (CVD) is the main cause of mortality in the world and is normally argued as the third cause of all mortalities. Opium and alcohol every day consumption can cause people to have many health problems. The present study aimed to assess the effect of ethanol and opium consumption on lipid profiles and atherosclerosis in aorta.

Methods: Twenty four male golden Syrian hamsters were randomly divided into four treatment groups (n = 6): Control, addicted (40 mg/kg), alcohol (6.0 g/kg) and combination of opium and alcohol. All of the hamsters were scarified and their livers were removed immediately and fixed in formalin solution 10%. The plasma levels of the lipid profiles were measured enzymatically. Aorta sections were examined by a pathologist.

Findings: The amount of the total cholesterol significantly increased in ethanol ($P < 0.05$) and combination ($P < 0.05$) groups, while it had a non-significant decrease in opium group. Serum triglyceride significantly increased in ethanol ($P < 0.05$) and combination ($P < 0.001$) groups, as well as this parameter increased in opium group but it was not significant. Low-density lipoprotein cholesterol (LDL-C) markedly increased in the combination group ($P < 0.05$). No significant difference was observed in serum LDL-C among other treatment groups. Levels of high-density lipoprotein cholesterol had a significant rise only in ethanol group. Change in aorta histology was not significant.

Conclusion: The results showed that consumption of opium plus alcohol has harmful effects on lipid profile; however, it had no effect on aorta histology that was maybe due to the short period of the treatment.

Keywords: Opium, Ethanol, Cholesterol, Atherosclerosis, Aorta

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Introduction

Reviewing the history of using opium indicates that it goes back to the earliest times of recorded history. Almost 3000 years BC, the Sumerians were the first people who used opium for religious purposes.[1] Opium has been known and used as an analgesic since ancient times. This substance is extracted from poppy plant called Papaver Somniferum. Opium contains eighty different alkaloid compounds the most important of which is morphine.[2,3]

Besides, alcoholic drinks which are contained different percentages of ethanol are used orally.[4] Ethanol has always been considered as one of the most well-known risk factors for various diseases.[5] Fortunately, in Iran alcohol consumption is much less common due to religious, cultural and society beliefs. Alcohol is quickly absorbed by liver after oral or intravenous consumption; moreover, some of it would be unchanged excreted through urine, sweat and breath.[5-7] Approximately 90% of alcohol in liver is metabolized to estolide and then to acetate. Ethanol is commonly the cause of the primary etiologic factor or one among many factors associated with esophageal dysfunction.[6] Indiscriminate use of alcohol may break down the gastric mucosal barrier as well as causing acute and chronic gastritis.[5] It may cause chronic diarrhea and malabsorption in the small intestine.[5] Ethanol alters fat and cirrhotic hepatitis in the liver. Alcohol is a primary oppressive for the central nervous system (CNS) which has negative effects on the cardiovascular system and consequently causes heart failure such as arrhythmia, cardiomyopathy, hemorrhagic stroke, and increased systolic and diastolic blood pressure. In addition, it can decrease skeletal muscle strength and irreversible damages and also can cause feeling warm and increase sweating. Ethanol also inhibits the vasopressin release from the posterior pituitary and may cause increased dieresis. Furthermore, alcohol can cause gradual loss of proteins, vitamins and minerals, and body might be susceptible to complications due to nutritional deficiencies.[7-10] It might negatively impact on fetus, endocrine, and immune system.[8,9,11] The present study aimed to identify the effect of concomitant ethanol and opium consumption on lipid profiles and atherosclerosis in golden Syrian hamster's aorta.

Methods

Animal Preparation

First, 24 male Syrian golden hamsters were prepared by the Animal's House of School of Medicine. They were randomly divided into four groups each contained six hamsters. Each hamster was marked by a special number. The animals were kept under the following conditions: Temperature 20°C ± 1, humidity 50% to 55%, 12 hours light (set by a timer), 12 hours darkness (set by a timer). All of them were kept with the above conditions for a month and their cage and place was cleaned twice a week.

Study Population

Syrian golden hamsters were divided into the following groups (6 in each group): 1. Control (including 6 hamsters received their normal diet, and were not alcoholic and addicts), 2. Addicted (including 6 hamsters received opium), 3. Alcoholic (6 hamsters received alcohol), 4. Combined alcohol and drug (including 6 hamsters that received simultaneous opium and alcohol).

Method of Opium Addicting

In order to develop oral addiction, hamsters were gavaged with oral opium for four 48-hours periods within two days. Thus, 10 mg at the first 48 hours, 20 mg at the second 48 hours, 30 mg at the third 48 hours, and finally 40 mg of opium were solved in hot water at the end of the day 30 and then after cooling it was gavaged to the hamsters 0.5 ml in 7 a.m. and 0.5 ml in 7 p.m.[12] After the forth night, in order to make sure they are addicted, 4 mg/kg naloxone injection intravenously was injected to the head of two randomly selected animals and morphine withdrawal symptoms were examined.[13,14]

Method of Alcohol Addicting

In the ethanol group, 6 g/kg ethanol was gavaged.[15] Thus, 0.3 g ethanol in 7 a.m. and 0.3 g in 7 p.m. was gavaged to the hamsters.

Sampling Method

First, the hamsters were anesthetized through intraperitoneal injecting with sodium thiopental (50 mg/kg) and then were placed in side. Their

head was pulled back using tip of the thumb and forefinger and then the needle of blood sampling tool was entered into the orbital sinus.[16] Three milliliters of blood was taken and the samples were placed in numbered tubes and then were centrifuged in 30 minutes and their serum was eliminated; the serum was kept in -20°C until sending the samples to the laboratory. The serums were sent to the Razi Laboratory of Kerman and their lipid profiles were measured.

Assessment of Aortic Samples

For aorta separations, first animals were anesthetized through intraperitoneal injecting with sodium thiopental (50 mg/kg) and then were sacrificed through cutting the neck vessels. Then, they were placed in supine position and their chest was opened using mead substernal incision and their heart was extracted with their aorta. Thereafter, the aortic arch was removed and fixed in 10% formalin solution for at least 24 hours.

In the next step, the paraffin blocks were prepared from the animal aorta and were converted to rotary microtome to 5 micrometer sections. The cutoffs were stained with hematoxylin eosin. For assessing the macrophage marker, marker CD68 was used to evaluate the staining using heat induce Epitop retrival (HIER). For IHC through HIER, first 7-micron tissue sections were transferred to pre-prepared Poly-L-lysine slides. Then, after the removal of paraffin and hydration with alcohol, rinse was done by neutralizing the endogenous peroxidase by hydrogen peroxide. Epitope retrieval was then conducted by a microwave and antibody CD68 was carefully added after the washing steps. Incubation was performed after rewashing and detection of antibodies was performed by adding envision reagent and 3, 3' diaminobenzidine (DBA). Thereafter, the slides were washed and staining the slide's background was performed by hematoxylin and they were ready to be reviewed microscopically. Histological slides were examined by light microscope by a blinded pathologist in terms of presence or absence of fatty streak, fibrous plaque formation and calcification of the media and changes intensity were surveyed and then grading was performed as below:[17]

Grade 1: absence, Grade 2: mild, Grade 3: moderate, and Grade 4: intense

Data Analysis for Study Objectives

The results gained by the effect of opium on aortic pathology of opium and ethanol addicted, and normal hamsters was done using the SPSS for Windows 15.0 (SPSS Inc., Chicago, IL, USA) and Kruskal-Wallis test, and while $P < 0.05$, the differences were statistically significant. SPSS and analysis of variance (ANOVA) were used for analyzing tests such as total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), and while $P < 0.05$, the differences were statistically significant.

Results

Table 1 illustrates the mean and SD of lipid parameters in the four studied groups. In opium group, the triglyceride (TG) and LDL-C level increased compared to the control group ($P < 0.05$). In this group, HDL level decreased ($P < 0.05$). In ethanol group, the cholesterol, TG and HDL level increased compared to the control group ($P < 0.05$). In the opium-ethanol group, cholesterol, TG and LDL-C level increased compared to the control group ($P < 0.05$). In the opium-ethanol group, cholesterol, TG, HDL-C and LDL-C level increased compared to the opium group ($P < 0.05$). In the opium-ethanol group, TG, HDL-C and LDL-C level increased compared to the ethanol group ($P < 0.05$).

Histological Findings

No change was observed in the opium group compared to the control group. No change was observed in ethanol group compared to the control group. Besides, no change was observed in the opium-ethanol group compared to the control group (Figures 1-4).

Discussion

In the present study, the level of TG and LDL increased and HDL decreased in the opium group compared to the control group. The study of Mohammadi et al. showed that opium decreased cholesterol, LDL and HDL.[12] In addition, the study of Najafipour et al. showed that cholesterol, LDL and TG were unchanged in addicted groups than non-addicted groups; however, the HDL level decreased in the addicted groups than non-addicted groups.[18] Furthermore, Kouros et al. suggested that the serum cholesterol level was

Table 1. The level of lipid parameters in the groups

Groups	Cholesterol (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Control	78.2 ± 5.1	88.4 ± 4.4	34.4 ± 3.4	27.2 ± 4.2
Opium	71.1 ± 4.6	101.2 ± 7.1	24.1 ± 2.0	55.7 ± 4.7
Ethanol	128.4 ± 7.0	187.2 ± 6.3	71.8 ± 5.5	22.7 ± 2.3
Opium-Ethanol	135.4 ± 6.2	230.2 ± 9.2	39.5 ± 3.2	121.6 ± 6.5

The numbers are as mean ± SD (6 hamsters in each group); TG: Triglycerides
HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol

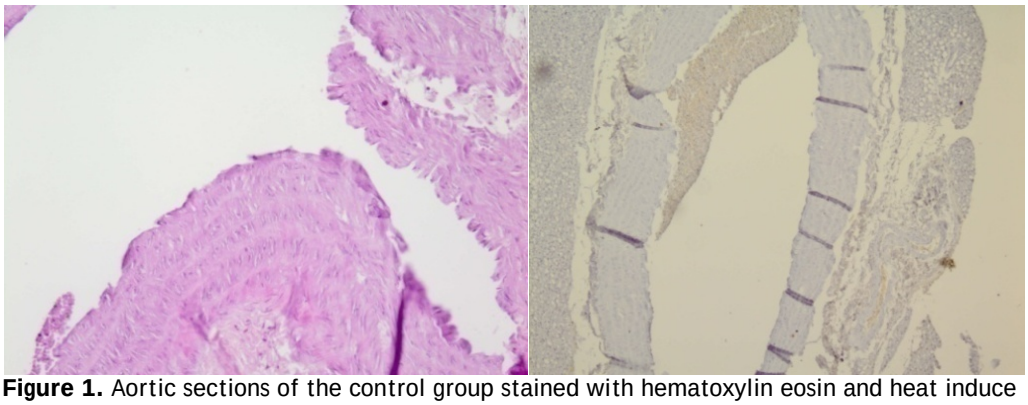

Figure 1. Aortic sections of the control group stained with hematoxylin eosin and heat induce epitope retrieval
No certain changes were observed in the test groups compared to the control group

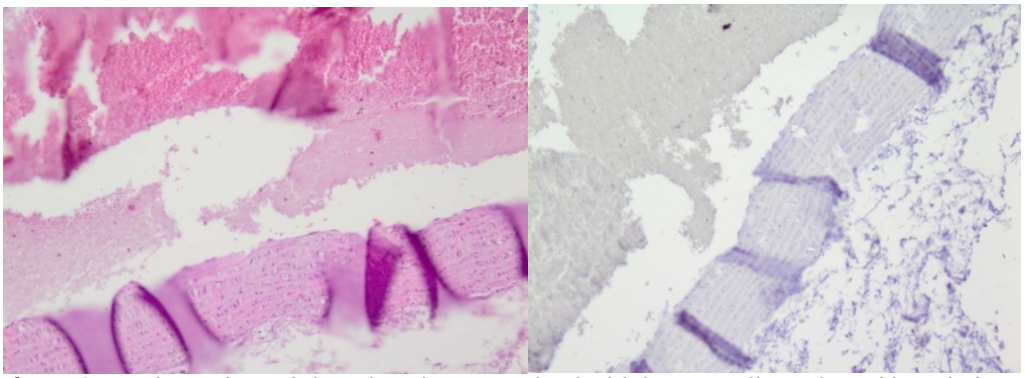

Figure 2. Aortic sections of the ethanol group stained with hematoxylin eosin and heat induce epitope retrieval
No certain changes were observed in the test groups compared to the control group

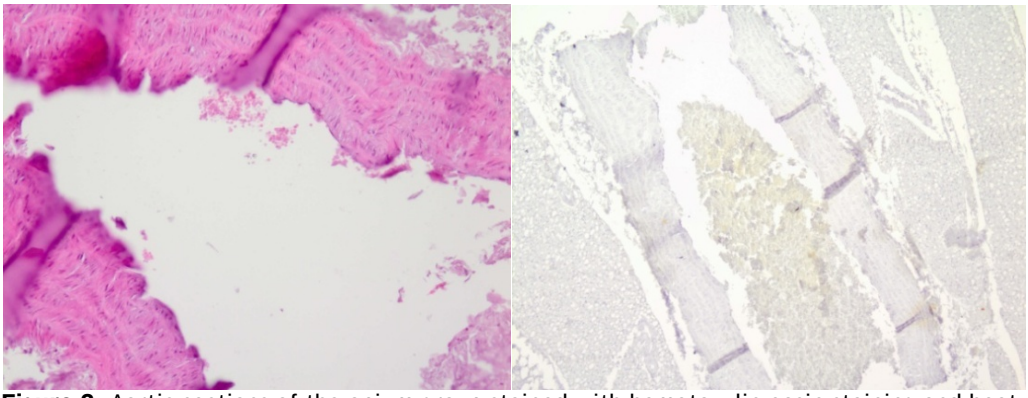

Figure 3. Aortic sections of the opium group stained with hematoxylin eosin staining and heat induce epitope retrieval
No certain changes were observed in the test groups compared to the control group

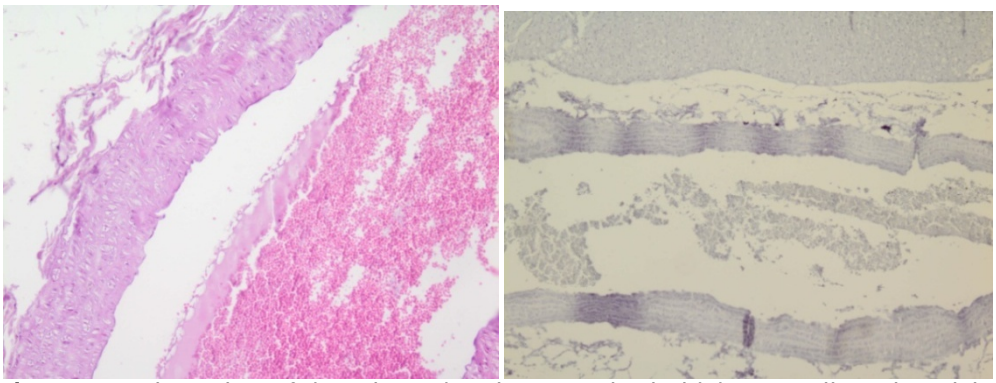

Figure 4. Aortic sections of the opium-ethanol group stained with hematoxylin eosin staining and heat induce epitope retrieval
No certain changes were observed in the test groups compared to the control group

decreased in the addicted groups than non-addicted groups while TG level of addicted groups than non-addicted groups showed no significant difference.[19]

The study of Karam et al. also showed that HDL level was lower in addicted diabetes individuals with non-insulin dependency than non-addicted diabetes individuals with non-insulin dependency.[2] Since the HDL level is an independent predictor of atherosclerosis,[15] it seems that opium dependency can increase the atherosclerosis through decreasing the HDL.[12] As shown in the study of Sadeghian et al., the major cause of premature coronary artery disease in males in Iran is due to opium addiction.[20] The study of Foody et al. also introduced HDL as a major and independent predictor of survival after the coronary artery bypass graft surgery.[21]

In the present study, cholesterol, TG and HDL level increased in the ethanol group compared to the control group. In the study of Baraona et al. consumption of ethanol increased the HDL level in rats.[22] Moreover, another study showed that alcohol can increase the HDL level.[23,24]

Gottrand et al. showed that ethanol may increase HDL and is associated with increased concentration of apolipoprotein A-I and A-II.[25] Besides, Hojnacki et al. showed that ethanol consumption can increase HDL and concentration of apolipoprotein AI in monkeys.[26]

Out of the mentioned studies, it can be resulted that alcohol can increase HDL level through increasing the transport of apolipoprotein A-I and A-II.

In the present study, given the increase of cholesterol, TG and LDLD level in the ethanol and opium groups than the control group, it can be concluded that concomitant consumption of ethanol and opium has much more harmful effects than the groups that use none of the substances.

In this study, none of the treatment groups had any histological changes compared to the control group. The results of the present study were completely in accordance with the study results of Shirpoor et al. In that study, atherosclerotic plaques were observed in hematoxylin eosin staining of the ethanol group, besides, +CD68 active macrophages has been reported using the IHC staining.[27] The difference of the present study with Shirpoor's study was in the animals used and the length of the treatment regimen.

Unlike the study results of Shirpoor et al., the study of Thun et al. showed that ethanol consumption has decreased the risk of myocardial infarction (MI).[28] Besides, the study of Rehm et al. confirmed the protective effects of ethanol on heart.[29]

Conflict of Interests

The Authors have no conflict of interest.

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تعیین اثر مصرف هم‌زمان تریاک خوراکی و اتانول بر شاخص‌های چربی سرم و آترواسکلروز آئورت در همستر سوری طلایی

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مقاله پژوهشی

چکیده

مقدمه: اعتیاد یکی از پدیده‌های شوم قرن حاضر می‌باشد که شیوع آن در چند دهه اخیر، به ویژه در کشورهای جهان سوم افزایش یافته است. با توجه به مصرف بالای مواد مخدر و الکل توسط برخی افراد، ضرورت دارد که اثرات این دو ترکیب به صورت جداگانه و نیز به صورت مصرف ترکیبی بر روی بافت‌شناسی آئورت، به علت اهمیت زیاد این بافت مورد بررسی قرار گیرد.

روش‌ها: در مطالعه حاضر از ۳۲ همستر سوری طلایی نر که بین ۹۰ تا ۱۱۰ گرم وزن داشتند، استفاده شد. همسترها به چهار گروه با رژیم درمانی متفاوت تقسیم شدند: ۱- گروه شاهد (غذای ساده)، ۲- گروه تریاک (غذای ساده + ۴۰ میلی‌گرم تریاک در روز به صورت خوراکی)، ۳- گروه الکل (غذای ساده + الکل خوراکی به میزان ۶ گرم به ازای هر کیلوگرم) و ۴- گروه ترکیبی (غذای ساده + تریاک خوراکی + الکل خوراکی). بعد از یک ماه همسترها بیهوش شده و آئورت آن‌ها جدا و از نظر پاتولوژی مورد بررسی قرار گرفت. همچنین خون حیوانات سانتریفیوژ شده و سرم آن‌ها جدا و تا زمان ارسال به آزمایشگاه در دمای ۲۰- درجه سانتی‌گراد نگهداری گردید.

یافته‌ها: در گروه استفاده کننده از تریاک میزان تری‌گلیسیرید و LDL (Low-density lipoprotein) نسبت به گروه شاهد افزایش و میزان HDL (High-density lipoprotein) کاهش یافت ($P < ۰/۰۵$). در گروه استفاده کننده از اتانول میزان کلسترول، تری‌گلیسیرید و HDL نسبت به گروه شاهد افزایش داشت ($P < ۰/۰۵$). در گروهی که هم‌زمان از اتانول و تریاک استفاده کرده بودند، میزان کلسترول، تری‌گلیسیرید و LDL نسبت به گروه شاهد افزایش نشان داد ($P < ۰/۰۵$). در گروهی که هم‌زمان از اتانول و تریاک استفاده می‌کرد، میزان کلسترول، تری‌گلیسیرید، HDL و LDL نسبت به گروه استفاده کننده از تریاک افزایش داشت ($P < ۰/۰۵$) و در گروهی که هم‌زمان از اتانول و تریاک استفاده می‌کرد، میزان تری‌گلیسیرید، HDL و LDL نسبت به گروه استفاده کننده از اتانول افزایش نشان داد ($P < ۰/۰۵$). از لحاظ تغییرات بافت‌شناسی در گروه‌های درمانی در مقایسه با گروه شاهد تغییر خاصی مشاهده نشد که ممکن است به علت زمان کم مطالعه بوده باشد.

نتیجه‌گیری: این آزمایش نشان داد که مصرف تریاک به همراه الکل اثرات مضری بر روی شاخص‌های چربی دارد؛ در حالی که اثری روی بافت‌شناسی آئورت نداشت و ممکن است در نتیجه کوتاه بودن دوره مصرف باشد.

واژگان کلیدی: تریاک، الکل، کلسترول، آترواسکلروز، آئورت

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