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

Jahanbanoo Shahryari MD, Moein Poormorteza MD, Arash Noori-Sorkhani MD, Kouros Divsalar MSc, Ebrahim Abbasi-Oshaghi

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

Citation: Shahryari J, Poormorteza M, Noori-Sorkhani A, Divsalar K, Abbasi-Oshaghi E. The Effect of Concomitant Ethanol and Opium Consumption on Lipid Profiles and Atherosclerosis in Golden Syrian Hamster’s Aorta. Addict Health 2013; 5(3-4): 83-9.

Received: 23.9.2012 Accepted: 28.12.2012

1- Assistant Professor, Department of Pathology, Neuroscience Research Center, Institute of Neuropharmacology, Afzalipoor Medical College, Kerman University of Medical Sciences, Kerman, Iran
2- General Practitioner, Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran
3- Senior Researcher, Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran
4- Department of Biochemistry, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
Correspondence to: Ebrahim Abbasi-Oshaghi, Email: 7abbasi@gmail.com
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. 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.

Besides, alcoholic drinks which are contained different percentages of ethanol are used orally. Ethanol has always been considered as one of the most well-known risk factors for various diseases. 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. 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.

Indiscriminate use of alcohol may break down the gastric mucosal barrier as well as causing acute and chronic gastritis. 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 diuresis. Furthermore, alcohol can cause gradual loss of proteins, vitamins and minerals, and body might be susceptible to complications due to nutritional deficiencies. It might negatively impact on fetus, endocrine, and immune system. 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. 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.

Method of Alcohol Addicting
In the ethanol group, 6 g/kg ethanol was gavaged. In the ethanol group, 6 g/kg ethanol was gavaged. 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. 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 sera 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 retrieval (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.\textsuperscript{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.\textsuperscript{18} Furthermore, Kouros et al. suggested that the serum cholesterol level was
Table 1. The level of lipid parameters in the 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
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. 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). Besides, the study of Rehm et al. confirmed the protective effects of ethanol on heart.

**Conflict of Interests**

The Authors have no conflict of interest.

**References**

1. Katzung B, Masters S, Trevo Ar. Basic and Clinical Pharmacology. 11th ed. New York, NY: McGraw-Hill; 2009.
2. Karam GA, Reisi M, Kaseb AA, Khaksari M, Mohammadi A, Mahmoodi M. Effects of opium addiction on some serum factors in addicts with non-insulin-dependent diabetes mellitus. Addict Biol 2004; 9(1): 53-8.
3. Bird DA, Franceschi VR, Facchinini PJ. A tale of three cell types: alkaloid biosynthesis is localized to sieve elements in opium poppy. Plant Cell 2003; 15(11): 2626-35.
4. Alcohol consumption and ethyl carbamate. IARC monographs on the evaluation of carcinogenic risks to humans. Lyon France; 2010. p. 1-1440.
5. Cotran RS, Kumar V, Collins T, Robbins SL. Robbins pathologic basis of disease. 6th ed. Maryland, MO: Saunders; 1999.
6. Bode JC, Bode C. Alcohol, the gastrointestinal tract and pancreas. Ther Umsch 2000; 57(4): 212-9. [In German].
7. Marks V. Clinical pathology of alcohol. J Clin Pathol 1983; 36(4): 365-78.
8. Hardman JG, Limbird LE. Goodman & Gilman's the pharmacological basis of therapeutics. 10th ed. New York, NY: McGraw-Hill; 2001.
9. Harper JC, Littleton JM. Development of tolerance to ethanol in cultured adrenal chromaffin cells. Alcohol Clin Exp Res 1990; 14(4): 508-12.
10. Pohorecky LA, Jaffe LS, Berkeley HA. Effects of ethanol on the adrenal medulla of the rat. Pharmacology 1974; 12(6): 340-6.
11. Milovanovic T, Budec M, Balint-Peric L, Koko V, Todorovic V. Effects of acute administration of ethanol on the adrenal cortex. J Stud Alcohol 1990; 51(5): 387-94.
12. Mohammadi A, Darabi M, Nasry M, Saabet-Jahromi MJ, Malek-Pour-Afshar R, Sheibani H. Effect of opium addiction on lipid profile and atherosclerosis formation in hypercholesterolemic rabbits. Exp Toxicol Pathol 2009; 61(2): 145-9.
13. Badawy AA, Evans CM, Evans M. Production of tolerance and physical dependence in the rat by simple administration of morphine in drinking water. Br J Pharmacol 1982; 75(3): 485-91.
14. Punch LJ, Self DW, Nestler EJ, Taylor JR. Opposite modulation of opiate withdrawal behaviors on microinfusion of a protein kinase A inhibitor into the locus coeruleus or periaqueductal gray. J Neurosci 1997; 17(21): 8520-7.
15. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002; 106(25): 3143-421.
16. Hoff J. Methods of blood collection in the mouse. Technique J 2000; 29(10): 47-53.
17. Murakami S, Kondo Y, Sakurai T, Kitajima H, Nagate T. Taurine suppresses development of atherosclerosis in Watanabe heritable hyperlipidemic (WHHL) rabbits. Atherosclerosis 2002; 163(1): 79-87.
18. Najafipour H, Joukar S, Malekpour-Afshar R, Mirzaeipour F, Nasr HR. Passive opium smoking does not have beneficial effect on plasma lipids and cardiovascular indices in hypercholesterolemic rabbits with ischemic and non-ischemic hearts. J Ethnopharmacol 2010; 127(2): 257-63.
19. Kouros D, Tahereh H, Mohamadreza A, Minoo MZ. Opium and heroin alter biochemical parameters of human’s serum. Am J Drug Alcohol Abuse 2010; 36(3): 135-9.
20. Sadeghian S, Graeli P, Salarifar M, Karimi AA, Darvish S, Abbasi SH. Opium consumption in men and diabetes mellitus in women are the most important risk factors of premature coronary artery disease in Iran. Int J Cardiol 2010; 141(1): 116-8.
21. Foody JM, Ferdinand FD, Pearce GL, Lytle BW, Cosgrove DM, Sprecher DL. HDL cholesterol level predicts survival in men after coronary artery bypass graft surgery: 20-year experience from The Cleveland Clinic Foundation. Circulation 2000; 102(19 Suppl 3): III90-III94.
22. Baraona E, Lieber CS. Effects of chronic ethanol feeding on serum lipoprotein metabolism in the rat. J Clin Invest 1970; 49(4): 769-78.
23. Manttari M, Tenkanen L, Alikoski T, Manninen V. Alcohol and coronary heart disease: the roles of HDL-cholesterol and smoking. J Intern Med 1997; 241(2): 157-63.
24. Savolainen MJ, Kesaniemi YA. Effects of alcohol on lipoproteins in relation to coronary heart disease. Curr Opin Lipidol 1995; 6(4): 243-50.
25. Gottrand F, Beghin L, Duhal N, Lacroix B, Bonte JP, Fruchtart JC, et al. Moderate red wine consumption in healthy volunteers reduced plasma clearance of apolipoprotein AII. Eur J Clin Invest 1999; 29(5): 387-94.
26. Hojnacki JL, Cluette-Brown JE, Dawson M, Deschenes RN, Mulligan JJ. Alcohol delays clearance of lipoproteins from the circulation. Metabolism 1992; 41(11): 1151-3.
27. Shirpoor A, Salami S, Khadem-Ansari MH, Heshmatian B, Ilkhanizadeh B. Long-term ethanol consumption initiates atherosclerosis in rat aorta through inflammatory stress and endothelial dysfunction. Vascul Pharmacol 2012; 57(2-4): 72-7.
28. Thun MJ, Peto R, Monaco JH, Henley SJ, Heath CW, Jr., et al. Alcohol consumption and mortality among middle-aged and elderly U.S. adults. N Engl J Med 1997; 337(24): 1705-14.
29. Rehm J, Gmel G, Sempos CT, Trevisan M. Alcohol-related morbidity and mortality. Alcohol Res Health 2003; 27(1): 39-51.
تیمین اثر مصرف هیپزمان تریاک خوراکی و انعک ابری سرم و آترواسکلروز آنورت در همسپ سوري طلایی

دکتر جهانپناه شهربازی، دکتر ممنی پورمرتکی، دکتر آرش نوری سرخیانی، کورس دیوسالار، ایرانی عباسی عاشقی

مقاله بروزه‌ای

چکیده

مقدمه: ایجاد یکی از پدیده‌های شویه، قرن حاضر می‌باشد که شبیه آن در جدیده‌ای باشد. به‌ویژه در کشورهای جهان سوم افزایش یافته است. با توجه به شرایط بالایی جراحی سرم افتراقی باید است. با روی‌نگاهی نشانه‌ای از انسداد جاذبه و نیز بصرف صورت ترکیبی بر روی پایش‌نامه آنورت و علت اهمیت زیاد این بافت مورد بررسی قرار گرفت.

روش‌ها: در مطالعه حاضر از ۳۲ همسپ سوري طلایی نر که بین ۱۰۰ تا ۱۱۰ گرم وزن داشتند. استفاده شد. همسپره با بهره ورزی درمانی متغیر تیمین شدند. ۱- گروه شاهد (غذای ساده)، ۲- گروه تریاک (غذای ساده + ۴۰ میلی‌گرم تریاک در روز به شورت خوراکی)، ۳- گروه خواص (غذای ساده + ۴۰ میلی‌گرم تریاک + کلک خواکی). بعد از یک ماه همسپره یبهوش شده و آن‌ها جا و نظر پاتولوژی مورد بررسی قرار گرفت. همین‌طور خون حیات‌سنجی سانتریفیوز شده و سرم آن‌ها جا و نتاژ زمان از مشاهگاه دردید ۱۵ درجه سانتی‌گراد تهیه گردید.

یافته‌ها: در گروه استفاده کننده از تریاک میزان تریاک گلیسرید (Low-density lipoprotein) LDL و تریاک گلیسرید (High-density lipoprotein) HDL به گروه شاهد افزایش داشت (P<0.05). در گروه استفاده کننده از تریاک میزان تریاک گلیسرید و تریاک گلیسرید و تریاک خواص (P<0.05). در گروهی که هیپزمان از اثر انواع و تریاک استفاده می‌کرد، میزان کلسترول، تریاک گلیسرید و تریاک گلیسرید و تریاک خواص (P<0.05). در گروهی که هیپزمان از اثر انواع و تریاک استفاده می‌کرد.

نتیجه‌گیری: این ازمایش نشان داد که مصرف تریاک به همراه الکات اثر مضری پی بروی شاخص‌های جربی درد به حالی که آن را از تریاک گلیسرید، کلسترول، آترواسکلروز آنورت وازگان کلیدی: تریاک، کلسترول، آترواسکلروز، آنورت

ارجاع‌های: ۱) شهربازی جهانپناه، پورمرتکی ممنی، نوری سرخیانی، کورس، سیسالار، ایرانی عباسی عاشقی. تیمین اثر مصرف هیپزمان تریاک خوراکی و انعک ابری سرم و آترواسکلروز آنورت در همسپ سوري طلایی. تاثیرات ناشنده و محدود در نتیجه کوتاهی شدن در مصرف باشد.

تاریخ پذیرش: ۹۱/۱۰/۲۰۰۸

بحران حیاتی‌اصلی و راه حل‌های امکان‌پذیر. مجله ایالات و سلامت ۱۳۸۸/۸۰-۸۳-۸۰-۸۲:۳۸-۳۲

Email: 7abbasi@gmail.com

Addict Health, Summer & Autumn 2013; Vol 5, No 3-4

http://ahj.kmu.ac.ir, 7 October

89