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Biochemical factors, MDA levels and Antioxidant activity in Opium addicted Hamsters

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Abstract

In this experiment, 12 male golden Syrian hamsters were divided into two groups containing six animalseach: Group 1 received chow diet (control); group 2 received opium (addicted). All hamsters were sacrificed after 24 hours of the final treatment (after 4four weeks), and the blood of animals were collected. Lipid profiles and liver enzymes were measured enzymatically. Atherogenic Index (AI) and LDL-C were calculated. Also, the LDL-C to HDL-C ratiowas determined. Serum super oxide dismutase (SOD) activity was measured by the method Misra and Fridovich method. The level of malondialdehyde (MDA) was evaluated by Ohkawa et al method. Catalase (CAT) and reduced glutathione (GSH) activity were measured by Aebi and Beutler E, et al. methods respectively. Amount of total cholesterol had a non-significant decreased in opium group. Serum TG and VLDL-C increased in opium group but it was not significant. Change in liver enzymes, were not significant in opium group compared to control hamster. The plasma concentration of MDA markedly increased in opium ($p < 0.01$) group compared to control. SOD, GSH and catalase levels also markedly reduced in opium ($p < 0.05$) group compared to healthy hamster. In conclusion, oxidative stress is increased in opium treated animals.

Keyword: Opium, SOD, MDA, GSH, Cholesterol.

Introduction

Cardiovascular disease is the major reason of mortality and morbidity in the world. In addition to abnormal levels of LDL-C, cholesterol, the oxidative stress has been believed to be one of the major risk factors for cardiovascular disease. Consequently, improving the antioxidant ability may help to avoid various diseases, particularly cardiovascular disease[1,2].

Traditional theories without exact source in some country may create incorrect behavior. For example, in some countries in Asia older people believe that opium reduces triglyceride, cholesterol and glucose levels[3]. Opium has more than 40 alkaloids. The main alkaloids include morphine, codeine and the baine. On the other hand, opium has over than 70 components, such as various sugars and organic acids. The abuse of drug especially opium is a most important problem in several countries especially in the Middle East region[3].

This drug has been traditionally used in Iran for happiness or as a healing for many diseases such as diarrhea, pain and insomnia[4,5]. There are not plenty of correlated experiments in the accessible medicinal literature; however numerous investigators have studied the effects of opium on lipid and glucose levels[3,4,5]. The aim of the present experiment was assess the effect of opium on lipid profiles, lipid peroxidation, antioxidant activity and liver enzymes.

Material and Methods

In this study, 12 male golden Syrian hamsters were used. Hamsters maintained on a 12h light/12h dark cycle in an ambient temperature of 22 ± 1°C. After acclimatizing for one week, hamsters were randomly[6] into two groups containing six animals each: Group 1 received chow diet (control); group 2received 40 mg/ kg body weight/day opium (Court of Justice, Kerman city, Iran) (addicted). For induction of addiction, 10mg of opium was dissolved in 1ml of warm water, after that cooled at room temperature and was administered by gavage initially with two times per day, within a period of 8 days. The dose was slowly raised to 40mg per day and kept through the rest of the experiment. Animals were checked daily and their body weights were recorded every 48 hours. All the hamsters were sacrificed after 24 hours of the final treatment (after four weeks), and the

blood of animals were collected and centrifuged at 3000 rpm for 15 minutes[7-10]. Procedure of this study was approved by the ethics committee of the Kerman University of Medical Sciences (Kerman, Iran).

Plasma biochemical measurements: The serum levels of lipid profiles, γ-glutamyltransferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) were measure denzymatically with using of commercial kits instructions (Pars Azmoon Co., Ltd., Tehran, Iran). The Atherogenic Index (AI) and LDL-C levels were calculated according of the Rosenfeld and Freidwald formula respectively. Also, the LDL-C to HDL-C ratio was determined[11].

Antioxidant enzyme assays and Lipid peroxidation: The SOD activity was determined according the Misra and Fridovichmethod[12]. The test absorbance was determined at 480 nm for four minutes, and the enzyme activity was stated as the enzyme quantity which reserved the oxidation of epinephrine by 50 percent, that is equal to one unit. Catalase (CAT) activity was determined by Aebi methods with Triton X-100 by assess of the absorbance at 240 nm for one minute on a UV-Spectrophotometer (UV/VIS, PG Instrumental, America). Reduced glutathione (GSH) was analyzed by the manner of Beutler E et al. The dyed product was examined immediately at 412 nm in a spectrophotometer[9,13,9]. The MDA levels was evaluated by the manner which described by Ohkawa et al[9].

Statistical analysis: Data of this experiment were tested by ANOVA (version 16, SPSS, Inc) and *p*-values of less than 0.05 were considered to be significant. Data expressed as mean ± SEM.

Results and Discussion

Table-1 and table-2 show lipid profiles, atherogenic index (AI), LDL/HDL ratio and non-HDL-C in opium and control groups. Amount of total cholesterol had a non-significant decreased in opium group (addicted animal). Serum TG and VLDL-C increased in opium group but it was not significant. Change in serum ALT, AST, and GG, were not significant in addicted group compared to healthy animals. The plasma concentration of MDA markedly increased in addicted animal ($p < 0.01$) compared to healthy animal. SOD, GSH and catalase levels also markedly reduced in addicted animal ($p < 0.05$) compared to healthy animal (table-3).

Atherosclerosis is one of the chief reasons of death in the world. In this respect, high levels of LDL-C, total cholesterol, on the other hand low levels of HDL-C and low antioxidant capacity has long been associated with atherosclerosis. Consequently, controlling of lipid profiles are established for prevent of atherosclerosis. Many studies have shown that high HDL-C levels have a protecting effect on cardiovascular disease (CVD), whereas LDL-C elevation is associated with atherosclerosis risk[14]. In this experiment LDL-C increased in opium group. Raise of LDL or oxidation of LDL is the initial event in atherosclerosis. Oxidation of LDL makes chemical modification which increase atherogenic properties of this particle. The presence of oxidized LDL-C in the atherosclerotic lesion has been known for many years. Oxidized LDL can irritatesmooth muscle cell migration and provoke apoptosis in many cells[15]. Oxidized LDL can also, increased formation of foam cell which make it very potential atherogenic[16].

Table-1
Effects of opium on lipid profiles

Groups/parameters(mg/dl)	TC	TG	HDL-C	VLDL-C	LDL-C
Control	78. 22 ± 5.1	88.45 ± 4.4	34.44 ± 3.4	17. 13 ± 2.1	27.24 ± 4.0
Opium	71.11 ± 4.6	101. 22 ± 7.0	24.10 ± 2.0	22.48 ± 3.0	49.71 ± 4.7$^{\neq}$

Results are expressed as mean ± SEM. $^{\neq}p < 0.05$ compared to control. TC: Total cholesterol, TG: Triglyceride, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol.

Table-2
Effects of opium onatherogenic index, LDL/HDL ratio and non-HDL-Cand liver enzymes

AI	LDL/HDL	Rationon	HDL-	CAST(u/l)	ALT (u/l)	GGT(u/l)
Control	1.27 ± 0.15	0.79± 0.13	43.56 ± 4.61	45.65 ± 5.20	43.73 ± 4.10	36.51 ± 4.26
Opium	1.95± 0.21[a]	2.04± 0.17[c]	47.1± 5.52	50.23 ± 6.01	45.55 ± 5.24	44.30 ± 5.62

Results are expressed as mean ± SEM. [a]p< 0.05 compared to control. [b]p< 0.01 compared to control.[c]p< 0.001 compared to control. ALT: alanineaminotransferase, AST: aspartate aminotransferase, GGT: Gammaglutamyltransferase

Table-3
Antioxidant enzyme activities in the plasma of addict animals

	SOD(units/ml)	MDA (nmol/l)	GSH(µmol/ ml)	Catalase(units/ml)
Control	131.1 ± 9.2	2.3 ± 0.26	6.12 ± 0.58	67.5 ± 6.1
Opium	109.8 ± 7.4[a]	3.6 ± 0.20[b]	4.68 ± 0.22[a]	48.7 ± 4.5[a]

Data are expressed as mean ± SEM. [a]p< 0.05 compared to control. [b]p< 0.01 compared to control.Serum super oxide dismutase (SOD), malondialdehyde (MDA), *glutathione* (*GSH*).

The results of recent studies have showed that atherogenic index is powerful predictors of CVD than LDL-Calone. Atherogenic index significantly increased in addicted animals. In this experiment the LDL-C/HDL-C ratio markedly increased in opium-treated animals compared to control hamsters. Many experiments have statement that LDL-C/HDL-C ratio is a excellent predictor of CHD risk[17]. Liver injury generally evaluated by measurements of serum GGT, ALT and AST. Damage of hepatocytes changed liver function and membrane permeability, which lead to leakof enzymes from their cells to plasma. Serum ALT is an enzyme mostly produced in hepatic cells; we checked these enzymes as a biomarker to evaluate the damage of hepatocellular. In this experimental though liver enzymes increased in opium-treated group, but this change was not significant when compared tocontrol[10].

Reactive oxygen species (ROS) are poisonous oxygen-derived yields which produced in every aerobic cell. These products consist of hydrogen peroxide, superoxide and hydroxyl radical and disturb function of biological systems[18] ros. Since aerobic cells have antioxidant systems to catch and/or inactivate ROS, therefore an imbalance between inactivation by the defensive systems and ROS production lead to oxidative stress[19].

A mixture of external[20-25] and internal antioxidants (including; catalase, superoxide dismutase, peroxidase and glutathione per oxidase), protected cell from oxidative stress attack[26].Maintenance of health and normal cellular action is depending on antioxidants. They are capable to disable and stabilize of free radicals earlier than they attack to cells[26]. In our experiment the levels of MDA increased markedly in opium group compared to controls (56.52%). High MDA levels could be due to high construction of ROS due to the extreme oxidative damage produced in addicted animals. These oxygen species oxidized many other vital biomolecules such as membrane lipids. Therefore, high MDA levels have recognized as a positive control for lipid peroxidation. Lipid peroxidation is one of the preliminary procedures which occurred for the period of *in vitro* LDL oxidation (oxLDL), and that it is motivated by the many oxidants[27]. In the vessel wall oxLDL leads to cellular dysfunctions[28].

In our study, the levels of glutathione (GSH) significantly declined in opium-treated group (22.54%) when compared to healthy hamsters. The reduction of GSH may be due to the improved turnover, for avoiding oxidative harm in addicted proposing raise protection against oxidant damage. Glutathione act as a free radical scavenger and has vital task in the repairmen of biological injure due to free radical[29].

In the present experiment, catalase activity significantly decreased in the in opium-treated group compared to controls (27.85%, reduction). Catalase is an enzyme which mostly found in peroxisomes, that converts hydrogen peroxide to water and oxygen. Many experiment reported that hydrogen peroxideis involved in the atherosclerosis pathogenesis by inducing peroxidation of lipid. In the oxidative stress situation, catalase activity will be declined[29].

We observed a significant reduction in SOD in opium treated groups (16.24%) when compared to control group. SOD is a metalloprote in enzyme which known as a main defense against Superoxide anion (O_2^-), catalyzed of this free radical to H_2O_2 and O_2. . Consequently, SOD and catalase maintain cells against oxygen toxicity by catalyzing the dismutation of O_2 to H_2O_2 and the disintegration of hydrogen peroxide to oxygen and water[29].

Conclusion

In conclusion, oxidative stress is increased in opium treated animals. The data of our experiment have shown high levels of oxygen free radical production and decreased antioxidant activity such as catalase and SOD activity, maintain to oxidative stress in addict animals. Increased atherogenic index, LDL/HDL ratio, MDA and decreased antioxidant ability may contribute to the increased risk cardiovascular disease risk. Results of this study obviously showed that opium is capable to provoke the oxidative stress and also, has harmful effect on lipid profile and antioxidant enzyme.

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