The Effect of Acute and Chronic Morphine on Some Blood Biochemical Parameters in an Inflammatory Condition in Gonadectomized Male Rats

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Abstract

Background: Opiates affect blood factors as well as pain and inflammation in a gender-dependent manner. The aim of the present study was to evaluate the effects of morphine on serum glucose, cholesterol, triglycerides, and urea in gonadectomized and inflammation conditions.

Methods: Animals were divided as follows: control group, carrageenan and chronic morphine recipients, acute morphine recipients, chronic morphine recipients, carrageenan recipients, acute morphine and carrageenan recipients, gonadectomized group, gonadectomized recipients of carrageenan, gonadectomized recipients of morphine, gonadectomized recipients of chronic morphine, gonadectomized recipients of carrageenan and chronic morphine, gonadectomized recipients of acute morphine and carrageenan.

Findings: Our results have shown that acute and chronic morphine elevates blood glucose level in the acute and chronic morphine group. Cholesterol level has shown to be increasing in the morphine and carrageenan recipient group compared with a group which merely received morphine. Triglyceride has shown to be decreasing in acute and chronic morphine recipient group compared with control group. A significant increase in serum urea was observed in acute and chronic morphine recipients compared with the carrageenan recipient group.

Conclusion: Morphine alters the serum glucose, cholesterol, triglyceride, and urea in the normal and inflammatory conditions differently, hence, this finding should be considered in the patients who use morphine as a relief of pain, especially in an inflammatory condition.

Keywords: Inflammation, Carrageenan, Morphine, Gonadectomized rat, Biochemical parameter

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**Introduction**

Opiates are known as the most effective analgesics whose anti-inflammatory effects have been frequently reported in the recent years. Unwanted effects such as tolerance and dependence restricted their use as anti-pain drugs in clinics. Previous research has shown that opiates affect both pain and analgesic systems. They also alter the levels of some blood biochemical parameters. Using opiates by addicted individuals result in a fluctuation in the level of blood sugar and may result in hypoglycemia or hyperglycemia.

A study on opium-addicted men and women revealed that the amounts of K+, Fe, and hemoglobin A1c (HbA1c) were higher in men than the control group, the amounts of serum total protein, albumin, alanine transaminase, high-density lipoprotein cholesterol, and cholesterol was significantly lower than the control group, and the amount of uric acid was higher than the control group. In another study, the same group also exhibited significantly higher amounts of serum glucose in female rats after injection of opium compared with the control group, and this increase was observed in male rats which in comparison with the female rats was observably higher.

In addition to the effects of opiates on the serum glucose, their effects have also been observed on the lipids level. There are some reports about drug-induced effects on fat and the synthesis of hepatic triglyceride. It was shown that the injection of 75 mg morphine for 5 days to rats resulted in elevation of cholesterol and triglyceride levels, so it was concluded that both stress and chronic morphine increased the plasma cholesterol and triglyceride levels.

There are several reports with regard to gender-dependent differences in lipid metabolism of rats. For example, it was found that the female rats had higher plasma cholesterol, but lower liver cholesterol compared with the male rats. The gonadal regulation of synthesis and catabolism of cholesterol in the liver and its relationship with serum cholesterol level have been investigated in intact, gonadectomized and hormone-treated rats in the presence and absence of dietary fat as well as cholesterol.

The reports disclosed that methyltestosterone led to hypercholesterolemia in male rats. It was found that estradiol enhanced the cholesterol biosynthesis. Also, Coleman et al. showed hepatic cholesterol biosynthesis decreased in female rats after being gonadectomized. Some researchers also found that estrogen administration stimulated the cholesterol biosynthesis in male rats. Estrogen has expected effects on plasma cholesterol and hepatic cholesterol level in female rats. It seems that the effects of androgens and testosterone on the male rats mainly appear in plasma cholesterol.

Recent ex vivo findings indicate that morphine enhances dopamine and xanthine oxidative metabolism and ascorbic acid oxidation in the rat striatum. There are few reports on the effects of opiates on serum urea and uric acid concentrations. Based on a study on the effect of opium addiction on some serum parameters in rabbits, it was found that sex does not determine the effect of opiates on the serum factors of glucose, cholesterol, triglyceride, urea, and uric acid. However, in this study, blood urea and uric acid were not affected by addiction to opium.

Most studies investigated the effects of opiates on serum biochemical parameters in humans and experimental models in addicted or healthy animals, while many opiate consumers are patients suffering from inflammation and pain condition. This study was planned to investigate the effect of acute and chronic use of morphine on blood glucose, triglyceride, cholesterol, and urea in an inflammatory condition in gonadectomized male rats.

**Methods**

In this experimental study, 84 male Wistar rats (200-220 g) were selected and divided into 12 groups randomly with 7 in each as will be described in more details below. Animals were housed on a 12 hours light-dark cycle and freely accessed to food and water. The study was approved by the Ethical Committee of the Shahid Bahonar University of Kerman, Iran. All animals’ procedures were according to “guide for the care and use of laboratory animals (NIH US publication no. 85-23 revised 1985).”

The procedure of castration was according to Craft et al., method with minor modifications. The male rats were syncoped by ether and the hair of scrotal region was shaved and cleaned.
with betadine. Then, a one-centimeter wide cut was created in the scrotum zone and the blood vessel and sperm duct were looped by suture string after removal of the testis, epididim, and fatty layers. Cut zone was sutured continually by suture string. Animals were restrained to recovery for 3 weeks.\textsuperscript{12}

Addiction to the analgesic effect of morphine was induced by double dose (8 AM and 8 PM) of morphine (10 mg/kg of the body weight) for 8 consecutive days by plantar injection.\textsuperscript{13} Inflammation was induced by injection of 1.5\% carrageenan (lambda) into plantar surface of the rats’ paw.\textsuperscript{1}

Fourteen animals (7 gonadectomized and 7 normal animals) received an intraperitoneal injection of chronic morphine. 14 animals (7 gonadectomized and 7 normal animals) were treated with intraperitoneal injection of acute morphine (10 mg/kg of the body weight).\textsuperscript{13} 14 animals (7 gonadectomized and 7 normal animals) received carrageenan for making inflammation. 14 animals (7 gonadectomized and 7 normal animals) received chronic morphine and carrageenan 30 minutes after the last morphine injection. 14 animals (7 gonadectomized and 7 normal animals) were treated with acute morphine and then received carrageenan. The control group (7 gonadectomized and 7 normal animals) received merely normal saline as vehicle.

At the end of treatment protocol a single dose of 10 mg/kg morphine was injected and 6 hours afterward animals were anesthetized with ether and then blood was collected. Animals were humanely killed after blood collection. Serum was separated and glucose, cholesterol, triglyceride, and urea were determined using kits from Darman Kave Company (Iran), according to manufacturers’ protocol.

Data were analyzed using one-way analysis of variance and Tukey post-hoc test. Data were expressed as mean ± standard error. A probability level of $P \leq 0.050$ was considered as significant.

**Results**

According to figure 1, a comparative study of the effect of morphine on serum glucose level in the presence and absence of inflammation indicates that with the exception of recipients of acute morphine and carrageenan, in all gonadectomized groups serum glucose level was elevated in comparison to control groups but did not significantly.

![Figure 1. Comparison of chronic and acute effects of morphine on serum glucose in the presence and absence of carrageenan-induced inflammation in gonadectomized and normal male rats groups](http://ahj.kmu.ac.ir)

**P < 0.010**: Significant differences with intact control group; **P < 0.001**: Significant differences with intact group that acute morphine recipient; **\textsuperscript{##}P < 0.010, \textsuperscript{###}P < 0.001**: Significant differences with intact acute morphine and carrageenan recipient; **\textsuperscript{+++}P < 0.001**: Significant differences with intact chronic morphine and carrageenan recipient group; **\textsuperscript{##}P < 0.001, \textsuperscript{##}P < 0.010, \textsuperscript{###}P < 0.000**: Significant differences with intact group that received carrageenan; $P < 0.050$: Significant differences with intact chronic morphine recipient group; $P < 0.001$: Significant differences with gonadectomized recipient of chronic morphine group; $P < 0.001$: Significant differences with gonadectomized recipient of carrageenan group; $P < 0.001$: Significant differences with gonadectomized recipient of acute morphine and carrageenan group.
Among the healthy control group, gonadectomized group, healthy group, and gonadectomized recipients of acute morphine, glucose was elevated significantly too (P < 0.010 and P < 0.001 respectively). Also, it was observed that carrageenan causes significant reduction of serum glucose level in healthy and gonadectomized group. This reduction was significant (P < 0.001) in gonadectomized recipients of chronic and acute morphine and control group compared with gonadectomized recipients of carrageenan. There was a significant difference between the above-mentioned group and healthy recipients of carrageenan (P < 0.001) as well. Serum glucose level in healthy male rats that received acute and chronic morphine was significantly higher compared with the group treated with carrageenan. Compared with carrageenan group, elevation of glucose was found to be significant (P < 0.001 and P < 0.050, respectively) in the control group and recipients of chronic morphine too.

According to figure 2, a comparative study of the effect of morphine on serum cholesterol level in the presence and absence of inflammation in healthy male rats. A significant elevation was observed compared with healthy control group and carrageenan recipient group. In comparison to healthy control group, both morphine and carrageenan significantly increased the serum cholesterol level in healthy male rats. In chronic morphine and carrageenan recipient group a significant elevation was observed compared with healthy control group and chronic and acute morphine recipients (P < 0.001 and P < 0.050 respectively).

Figure 3 indicates the results of a comparative study of the effect of acute and chronic morphine on serum triglyceride in the presence and absence of inflammation in healthy male rats. As it reveals, except for the control group, triglyceride level was elevated in healthy groups compared with gonadectomized groups. This elevation in healthy recipients of acute morphine and carrageenan was noticeable (P < 0.001) in
comparison with gonadectomized recipients of acute and chronic morphine, and gonadectomized recipients of chronic morphine and carrageenan. The study of gonadectomized group also disclosed that both treatment with morphine and carrageenan reduced serum triglyceride level compared with control group. This decrement in acute morphine recipient group was significant compared with control group.

The results of the comparative study of the effect of morphine on serum urea level in the presence and absence of inflammation in healthy male rats and gonadectomized group are presented in figure 4. As the results reveal, gonadectomized control group exhibit a significant elevation in their serum urea level (P < 0.001) compared with healthy control group. In addition, among the gonadectomized groups, acute morphine group recipients of carrageenan had lower serum urea level compared with the other groups. Among healthy groups, the control group had the lowest serum urea level compared with acute and chronic morphine recipients (P < 0.001 and P < 0.010).

Discussion

In the present study, acute and chronic effects of morphine on some blood biochemical parameters (glucose, cholesterol, triglycerides and urea) were investigated in gonadectomized and inflammation conditions.

The level of serum glucose indicated that both acute and chronic morphine increased blood glucose levels, but this increase was not statically significant.

Previous studies have shown that the opiates play an important role in glucose homeostasis. Although some studies have shown that opiates simulate hyperglycemia, some others have indicated that the opiates may cause hypoglycemia. Although the exact mechanisms of opiates involved in blood glucose are still unclear, it has been proposed that alteration in the levels of hormones such as glucagon, adrenaline, and cortisol can change the blood sugar level as a result of morphine injection. However, the influence of these effects depends on the morphine dosage and time period in that, the low dose of morphine decreases the plasma epinephrine and leads to the hypoglycemia, while high doses result in an increase in the glucose production by the liver. Moreover, it has been proposed that the change in insulin secretion may affect adjustment of glucose by opiates.

Figure 3. Comparison of chronic and acute effects of morphine on serum triglyceride in the presence and absence of carrageenan-induced inflammation in gonadectomized and normal male rats groups

***P < 0.001, **P < 0.010, *P < 0.050: Significant differences with intact recipient of acute morphine and carrageenan group; P < 0.050: Significant differences with intact recipient of acute morphine group; P < 0.050: Significant differences with gonadectomized recipient of chronic morphine and carrageenan group; P < 0.050: Significant differences with intact recipient of carrageenan group; P < 0.050: Significant differences with gonadectomized control group
Figure 4. Comparison of chronic and acute effects of morphine on serum urea in the presence and absence of carrageenan-induced inflammation in gonadectomized and normal male rats groups

***P < 0.001: Significant differences with intact recipient of acute morphine and carrageenan group; **P < 0.001: Significant differences with intact receiving acute morphine and carrageenan group; ***P < 0.001, ***P < 0.010, ***P < 0.050: Significant differences with intact receiving chronic morphine and carrageenan group; ^^P < 0.001, ^^P < 0.010, ^P < 0.050: Significant differences with intact group receiving carrageenan; #^P < 0.001, #^P < 0.010: Significant differences with intact control group; P < 0.050, †P < 0.010: Significant differences with gonadectomized recipient of chronic morphine and carrageenan group; ††P < 0.001, ††P < 0.010, ††P < 0.050: significant difference with gonadectomized receiving acute morphine and carrageenan group

Allan et al. demonstrated that treatment of mice with morphine in a 6-24 hours course increases glucose synthesis by stimulating the gluconeogenic enzymes by the liver and restraining pyruvate kinase. Based on these studies, we may come to this conclusion that morphine may directly affect liver glucose production. In addition, there is considerable evidence indicating that the effects of morphine and other opiates on the carbohydrates metabolism can be mediated by sensitive opiate receptors in the brain, activating the sympathetic nervous system, and releasing catecholamines. Furthermore, the exogen opiates may stimulate the release of vasopressin from the posterior pituitary, and function in the pancreas surface and cause an increase in insulin and glucagon secretion. Since stress and surgery change the blood glucose levels and so does the inflammation, the effect of opiates on blood sugar in the presence or absence of inflammation is very complicated and requires further investigation.

A study on the effect of opium on serum glucose, potassium and sodium in male and female rats, found that any increase in serum glucose by opium depends on time and sex. It has also been proven that analgesic effects of opium are sex-dependent; therefore, it can be concluded that other effects of morphine (as the main opium-alkaloid) are also sex-dependent and as the evidence has shown gonadal hormones affect opium response.

Our findings on the cholesterol levels have shown that in inflammation conditions, morphine increased the cholesterol level more than it was the case in normal and healthy animals. Moreover, in mice resisting the analgesic effects of morphine in the case of inflammation it could cause more increment of cholesterol. In other words, consumption of morphine in both acute and chronic conditions elevates blood cholesterol in inflammation status. However, studies on the effect of opiates on serum biochemical parameters have reported different results.
some researchers have shown an increase in cholesterol level in the mice treated with morphine. morphine can indirectly affect the homeostasis of cholesterol by reducing stomach-intestines movement, making a fatty liver and stimulating corticosterone secretion. they have also shown that morphine causes hypercholesterolemia in acute and chronic condition. according to a study, opiates have no effect on the blood lipid level.

another study indicated that morphine elevated low-density lipoprotein (ldl) and very ldll in mice with a normal diet. also, it has been shown that morphine increases some biochemical parameters related in cardiovascular disease. to our best knowledge, there is no research on the effects of carrageenan-induced inflammation on alteration of cholesterol level, but based on a study, there is a direct relationship between the cholesterol level and the performance of opiates in a way that the decreased cholesterol level results in defective signaling of opiates and hence reduction of their impact, while the elevated cholesterol level causes opposite effects. based on the results of the present study, in case of inflammation, opiates chronically enhanced the severity of the inflammation. this result is of importance in terms of strengthening the effects of the opiates in the inflamed tissue.

the results of this study showed a significant reduction in cholesterol in the gonadectomized male rats resistant to morphine carrageenan recipient compared with the male rats receiving chronic morphine. females generally have higher rates of cholesterol synthesis and catabolism in liver compared to males, and hence they show higher levels of serum cholesterol. gonadectomy causes a reduction in cholesterol biosynthesis in liver, while after reduction of hormone, catabolism increases in males and decreases in females. there are reports indicating that treating with estrogen results in the stimulation of cholesterol biosynthesis in male rats. based on a study, morphine affects triglyceride synthesis in the liver. also, another study showed no significant difference in the triglyceride level in those who were addicted to opium for more than 2 years.

comparing the gonadectomized group with the normal one, we found a significant reduction in the triglyceride level in the gonadectomized group that received chronic morphine and carrageenan more than the animals which received acute morphine and carrageenan. in a study of the influence of diet and the gonadal hormones on the lipid metabolism in rats, it was found that there was a clear relationship among diet, sexual hormones, and fat metabolism. however, there are many reports on sex-dependent differences in lipid metabolism in rats. studies indicated that estrogen decreases total plasma cholesterol level and increases or maintains triglyceride level. mami et al. reported that cholesterol, triglyceride, and ldll levels increased significantly in opium addicted group, while this finding was in contrast to the results gained about human, however, they are compatible to the results obtained by bryant et al. about rats. increase in the serum levels of these parameters could be due to the lipolytic effects of opiates. there is a direct effect of morphine on the separation of the epididymal fatty layers in the rats.

the results obtained from a study on urea level showed a significant increase in acute and chronic morphine recipient groups compared with the carrageenan recipient group. in general, the results showed that the carrageenan recipient groups had a lower level of urea; among these groups, the group that only received carrageenan exhibited low urea level in all groups. the present results indicated a significant reduction of urea level in the gonadectomized animals that received acute morphine compared with normal males that received acute morphine. interestingly, the gonadectomized group which received carrageenan had a higher level of urea in comparison with the group which received chronic morphine and acute-carrageenan morphine. while, this parameter had opposite results in the same group in the presence of sexual hormones before being gonadectomized. therefore, sexual hormones affect serum urea level. by and large, there are few reports on the effects of opiates on serum urea. based on a study on the effect of addiction on some serum parameters in rabbits, it was found that sex is not involved in the effect of opiates on the serum glucose, cholesterol, triglyceride, and urea. in addition, these researchers reported that serum urea was not affected by addiction to opium. however, sumathi and niranjali and divsalar et al. reported a significant reduction in urea in opioid addicts. this contradiction may be due to the different use of opium as it causes some
histopathological changes in kidney whose intensity is related to the changes in the biochemical alteration.\textsuperscript{10}

\textbf{Conclusion}

As a result, morphine alters the serum glucose, cholesterol, triglyceride, and urea levels differently in the normal and inflammatory conditions in the present or absent of gonadal hormones. This finding is of importance to be considered in the patients who use morphine as a painkiller especially in the inflammatory condition.

\textbf{Conflict of Interests}

The Authors have no conflict of interest.

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چکیده

مقدمه: اکوپوییده‌ها، شاخص‌های خونی و درد و التهاب را به صورت وابسته به جنس تحت تأثیر قرار می‌دهند. هدف پژوهش حاضر، بررسی تأثیرات مورفین بر گلوکز، کلسترول، تری‌گلیسرید و اوره سرم در شرایط التهابی و گناهکوده‌پوشی می‌باشد.

روش‌ها: بر اساس حیوانات به گروه‌های مختلف، دریافت کننده کاراپینان، دریافت کننده مورفین حاد، دریافت کننده مورفین مزمن، دریافت کننده مزمن و کاراپینان، دریافت کننده مورفین حاد و کاراپینان، گناهکوده‌پوشی، گناهکوده شب و دریافت کننده مورفین حاد، گناهکوده‌پوشی، گناهکوده شب و دریافت کننده مورفین مزمن، گناهکوده‌پوشی، گناهکوده شب و دریافت کننده کاراپینان و مورفین حاد، گناهکوده‌پوشی، گناهکوده شب و دریافت کننده کاراپینان می‌باشد.

یافته‌ها: دریافت حاد و مزمن مورفین، سطح گلوکز خون را در گروه‌های مورفین حاد و مزمن بالا برده. سطح کلسترول خون در گروه‌های دریافت کاراپینان و مورفین در مقایسه با گروه‌های کنترل بالاتر بود. تری‌گلیسرید و اوره سرم در گروه‌های مورفین حاد و مزمن بالا برده بود و در مقایسه با گروه‌های کنترل بالاتر بود. در نتیجه گیری، مورفین در شرایط التهابی و طبیعی می‌تواند سطح گلوکز، کلسترول، تری‌گلیسرید و اوره سرم خون را به طور متفاوتی تغییر دهد. بنابراین، نتایج حاصل شده در مورد بیمارانی که برای تسكین در آنها (به ویژه در شرایط التهابی) از مورفین استفاده می‌شود، با مورد توجه قرار گیرد.

واژگان کلیدی: التهاب، کاراپینان، مورفین، موس‌صرحای گناهکوده‌پوشی، شاخص‌های بیوشیمیایی

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Morphine Alters Biochemical Parameters in Inflammatory Condition Chahkandi et al.

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Abstract: Objective: To investigate the effects of morphine on biochemical parameters in inflammatory conditions. Methods: The study included rats divided into four groups: the control group received saline, the morphine group received morphine, and the celecoxib group received celecoxib. Results: The results showed that the levels of glucose, cholesterol, triglycerides, and uric acid were significantly higher in the morphine and celecoxib groups compared to the control group. Conclusions: Morphine increases the levels of glucose, cholesterol, triglycerides, and uric acid in the blood of inflammatory conditions, and this effect is more pronounced in the celecoxib group.

Keywords: Inflammation, Celecoxib, Morphine, Biochemical Parameters

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