

# Effects of Waterpipe Smoking, Immobilization and Darkness Stress on Serum Creatine Kinase and Alkaline Phosphatase Levels in Female Rats

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## Abstract

**Background:** Various studies indicate that life style may influence serum enzyme levels.

**Objectives:** The aim of this study was to evaluate the effects of waterpipe smoking, immobilization and darkness stress on serum creatine kinase (CK) and alkaline phosphatase (ALP) levels in female rats.

**Materials and Methods:** Female Wistar rats were divided into control, waterpipe smoking, immobilized, under darkness stress, immobilized waterpipe smoking, under darkness stress waterpipe smoking, under darkness stress immobilized and under darkness stress immobilized waterpipe smoking of 7 each. Waterpipe smoking exposure was 10<sup>th</sup>/day for 10 minutes of each with 5 minutes resting time; chronically immobilization was applied 4 hours/day (twice a day for 2 hours with 2 hours resting time) and darkness stress for 6 hours/day in dark situation. Following 7 weeks experiment, serum enzyme levels were measured and compared statistically between groups using ANOVA.

**Results:** The results indicated enhanced serum CK and ALP levels in all groups compared with control ( $P < 0.05$ ). Also, there was a significant difference in serum CK levels between all the groups including different types or multiplicity of stress ( $P < 0.05$ ). Serum ALP levels were found less affected by these factors; where darkness or immobilization have shown semi-equal effect on these enzyme levels with no significant difference. Besides, under darkness immobilized waterpipe smoking, ALP levels have no significant difference compared to immobilized waterpipe smoking or under darkness stress waterpipe smoking animals.

**Conclusions:** Our findings clearly indicate a synergistic effect of waterpipe smoke, immobilization and darkness stress on serum CK levels, whereas serum ALP levels of experimental groups have shown no significant synergism for the mentioned factors.

**Keywords:** Smoking, Immobilization, Darkness, Creatine Kinase, Alkaline Phosphatase, Rats

## 1. Background

World health organization considered smoking, especially waterpipe or tobacco smoking as a global threat (1). Many studies have been conducted on smoking effects on people's health (2). Research has shown that tobacco smoke contains over 4800 different chemicals which 69 of them are carcinogenic and some contribute to tumor progression (3). Studies indicate that there is an association between smoking and respiratory tract, lung, stomach, liver, kidneys and urinary tract malignancies or myeloid leukemia (4).

On the other hand, creatin kinase (CK) is an enzyme which participates in energy use and storage cycle of tissue, especially in muscles and quickly provides the energy for endothermic processes (5). It is normal to have a low CK level in blood; however, its highest level has been found in skeletal muscle, brain and heart tissue (6). Several factors such as heavy exercise, muscle injury, chronic renal failure, lung disease and severe respiratory muscle activity may affect serum CK level (7). Many studies indicate that smoking would predispose to heart disease (8). Researchers have found a significant association between smoking and alterations in heart

and brain biochemical enzyme activity (9).

Alkaline phosphatase (ALP) is a membrane hydrolase enzyme which transfers phosphate group from many types of molecules, such as nucleotides, proteins and alkaloids (10). The main function of ALP is probably to facilitate exchange of metabolites through the cell membrane in transport and storage of fat and osteogenesis (11). Although this enzyme is active in most tissues, the highest enzyme activities have been found in intestines, liver, bone, spleen, placenta and kidney (12). Studies indicate that ALP activity which is one of the liver and bone disorder markers apparently alters in many diseases, including autoimmune diseases, some infectious diseases, liver, bone and biliary diseases, inflammation, anemia and malnutrition (13). Many studies suggest that there is an association between smoking and liver disease (14). Although it is supposed that smoking can affect the body biochemical enzyme levels, especially in liver tissue (15), some of the results showed no significant changes in liver enzyme levels (16), especially ALP activity (17). Also, some researches indicated that smoking can affect the morphology and anatomy of the liver and other organs (18).

Immobilization is one of the most common performed stresses on animals and among the various stress models, acute or chronic immobilization has been used extensively and accepted widely for studying the association between stress and physiological alterations (19); however, the exact pathophysiological mechanism is unknown (20). In this regard, some studies indicate that there is an association between stress and some serum enzyme activities (21).

Moreover, people are less exposed to light due to modern lifestyle and this phenomenon has effects on body physiology as a stress type; however, the exact mechanism is unknown (22). Research suggest that there is an association between darkness stress and excretory function of certain glands and organs (23). Darkness stress may induce depression of cell-mediated immunity in mice (24). At the molecular level, environmental lighting might regulate the expression of some enzyme genes (25, 26); indeed, it may regulate gene expression. Most of the researches on darkness stress have been conducted on long-term or acute darkness and there are few studies in the context of short-term or chronic darkness (27). Besides, studies indicate that female rats are more sensitive than males to the changes in photoperiod (28).

## 2. Objectives

Although there are various studies on the effects of smoking on enzyme levels, there is limited comparative research conducted on females, although, emerging epidemic of tobacco use among women is obvious (29). In this regards, considering more sensitivity of female rats to darkness stress (28) and due to the lack of motion in modern lifestyle, our study aimed to examine the multiplicity or comparative effects of waterpipe smoking, immobilization and darkness stress on serum CK and ALP levels in female rats. The results of this study have an important role in measures of health, prevention of tobacco use and choosing a more appropriate lifestyle through the new findings added to basic knowledge of health and disease.

## 3. Materials and Methods

### 3.1. Animals

Adult male Wistar rats, weighting  $190 \pm 10$  g, were purchased and raised in our colony from an original stock of Pasteur institute (Tehran, Iran). The temperature was at  $20^{\circ}\text{C} - 25^{\circ}\text{C}$  and animals kept under a schedule of 12 hours light:12 hours darkness (light on at: 8:00 AM) with free access to water and standard laboratory chow. Care was taken to examine the animals for general pathological symptoms. Food was withheld for 12 - 14 hours before operation or death. In all experiments, attention was paid to the regulation of local authorities for handling laboratory animals and the ethical guidelines for investigation of immobilization or darkness stress in rats (30).

### 3.2. Study Design

This work was conducted in laboratory complex of Islamic Azad University of Shahr Rey (Tehran, Iran). Ani-

mals were randomly divided into 8 groups, including control, waterpipe smoking, chronically immobilized, under darkness stress, immobilized waterpipe smoking, under darkness stress waterpipe smoking, under darkness stress immobilized and under darkness stress immobilized waterpipe smoking of 7 each.

In the present study, restrainer device was used for immobilizing. According to previous studies (31, 32), immobilization stress was performed as follows: animals were exposed to chronic immobilization stress for 4 hours/day (twice a day for 2 hours with 2 hours resting time among them) for a period of 7 weeks. To perform darkness stress, animals were in dark situation for 6 hours/day during a period of 7 weeks according to our previous experiment (33). A smoking chamber was designed to expose animals to tobacco smoke (33). This device was composed of the following parts, vacuum, smoke compressor cylinder in a glass box (aquarium) and an air conditioner on the top for ventilation. Initially, the rats entered the glass chamber, then tobacco was burned and suction was started simultaneously. When all the tobacco was burned, suction was automatically disconnected, beginning to transfer dense smoke into the glass chamber over the course of 15 minutes, including 10 minutes of smoking and exposure to smoke (indeed, condensation of smoke took a minute and animals were exposed to smoke for 9 minutes) and 5 minutes of resting time. This process was repeated 10 times per day which resulted in 90 minutes of tobacco smoke each day of 7 weeks experiment. Control animals were at the same position without any smoke.

### 3.3. Creatin Kinase and Alkaline Phosphatase Assay

Blood samples were collected using the cardiac puncture technique after anesthetizing animals by ether. Following serum collection, CK and ALP levels were determined using the spectrophotometry method.

### 3.4. Statistical Analysis

All results are presented as mean  $\pm$  SD. The significance of differences between groups was determined by analysis of variance (ANOVA). Games-Howell and Tukey tests were used for post-hoc comparisons. Differences were considered significant when  $P < 0.05$ .

## 4. Results

Table 1 represents serum CK and ALP levels, respectively in different groups of our study including, control, waterpipe smoking, chronic immobilized, under darkness stress, immobilized waterpipe smoking, under darkness stress waterpipe smoking, under darkness stress immobilized and under darkness stress immobilized waterpipe smoking female Wistar rats.

Our findings indicated that there was no significant difference in serum CK and ALP levels of control nonsmoking animals in the smoking device compared to normal

**Table 1.** Serum Creatin Kinase and Alkaline Phosphatase Levels in Control and Experimental Groups<sup>a</sup>

Animals	CK, U/L <sup>b</sup>	P Value <sup>c</sup>	ALP, U/L <sup>b</sup>	P Value <sup>c</sup>
Control	160.00 ± 2.16	-	81.45 ± 2.08	-
Waterpipe smoking	526.42 ± 4.51	< 0.05	112.00 ± 2.16	< 0.05
Chronically immobilized	379.40 ± 4.03	< 0.05	102.00 ± 2.17	< 0.05
Under darkness stress	292.30 ± 3.90	< 0.05	99.84 ± 3.20	< 0.05
Immobilized waterpipe smoking	853.20 ± 2.18	< 0.05	127.46 ± 8.42	< 0.05
Under darkness stress waterpipe smoking	710.35 ± 2.26	< 0.05	125.00 ± 6.55	< 0.05
Under darkness stress immobilized	610.04 ± 1.98	< 0.05	120.52 ± 3.42	< 0.05
Under darkness stress immobilized waterpipe smoking	932.04 ± 2.02	< 0.05	132.00 ± 2.64	< 0.05

<sup>a</sup>Abbreviations: CK, creatine kinase; ALP, alkaline phosphatase.

<sup>b</sup>Data represent the mean ± SD of 7 rats in each group.

<sup>c</sup>P values are versus control group.

animals. Therefore, smoking method used in the experience had no significant impact on the results of our study.

Serum CK and ALP levels were significantly increased in all groups compared to control. Likewise, there was a significant difference in serum CK levels between all the groups, including different types or multiplicity of stress ( $P < 0.05$ ).

Moreover, serum ALP level was increased in chronically immobilized or under darkness stress rats and showed a significant difference compared to other groups ( $P < 0.05$ ), although there was no significant difference between these two groups. Furthermore, there was no significant difference in the serum ALP level of under darkness stress immobilized rats compared to immobilized or under darkness stress waterpipe smoking animals; however, it was significant compared to other groups ( $P < 0.05$ ). Besides, in comparison with under darkness stress immobilized waterpipe smoking animals, there was a significant difference in ALP levels of under darkness stress immobilized or the other groups except immobilized or under darkness stress waterpipe smoking rats, which had no significant difference compared to the three diverse stress group.

## 5. Discussion

Our findings clearly indicate that waterpipe smoking, immobilization or darkness stress increases the serum CK and ALP level in female rats. However, waterpipe smoking was found to have the most powerful impact on these factors. As there are significant differences in serum CK levels between all the groups, including different types or multiplicity of stress, the type and frequency of the stress factors are very influential in the CK level. Indeed, this factor may alter in different life styles or stress situations. According to our study, the serum ALP level was found less affected by the type, mild stress compared to the CK level, although all experimental groups have shown enhanced ALP levels compared to control. Indeed, darkness or immobilization has shown semi-equal effect on ALP levels. Also, under darkness immobilized waterpipe smoking rats have the ALP level similar to immobilized waterpipe smoking or under darkness stress waterpipe smoking animals. In this

regard, there are reports suggesting a significant decrease in liver weight and liver enzyme alteration in mice exposed to cigarette smoke (34). Moreover, smoking is a leading cause of heart disease and cardiac enzyme changes (35). In contrast, some studies have shown that smoking has no effect on some liver enzyme levels (36). Also, there are studies reporting even better recovery of smokers with acute myocardial infarction and lower mortality rate among them compared to nonsmokers (37, 38). However, consistent with our findings, there are studies which suggest enhanced plasma enzymes such as CK, lactate dehydrogenase and glutamic-pyruvic transaminase activity in rats following cold immobilization stress (39), immersion restraint stress (40) or restraint and isolation stress (41). Our findings are consistent with studies indicating chemical enzyme activity alterations in light or dark rearing environment (42, 43). Also, some enzyme levels have reported to vary during dark or light period (44). However, there are very few and conflicting experiments on this issue. The main mode of mechanism of action, however, in one hand, can be thought to be on the cell damage and cytoplasmic enzymes leakage into the blood following increased cell membrane permeability. Indeed, hookah smoke and stress may cause free radical production, which can damage cell membrane through fatty acids peroxidation leading to enzyme release in plasma (45). Besides, it is well accepted that stress stimulates the activity of the autonomic nervous system (46) which probably participates in stress-induced increase in plasma enzyme activities.

In conclusion, the results indicate a synergistic effect of waterpipe smoke, immobilization and darkness stress on serum ALP and CK levels. Therefore, pathophysiological consequences of these factors are very important, particularly in heart and liver tissue.

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## Footnotes

**Authors' Contribution:**Rahim Ahmadi developed the original idea and the protocol, abstracted data and revised the manuscript. Sedigheh Molaei conducted the protocols, analyzed and abstracted data and wrote the manuscript.

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