

# The Effects of Inactivity with Smoking: High Risk for Heart or Liver Dysfunction in Females

Foroutanjazi M\*, Naghavi E, Molaei S, and Ahmadi R

**Abstract**—Various studies show that sedentary life style is associated with heart or liver failure. In this study we evaluated the effects of waterpipe smoking and restraining stress on serum creatine kinase (CK) and alkaline phosphatase (ALP) levels in female rats. Female Wistar rats were divided into control, restrained, exposed to waterpipe smoke and “restrained + exposed to waterpipe smoke” groups of 6 rats in each group. After 7 weeks, blood samples were obtained using cardiac puncture technique. Serum creatine kinase and alkaline phosphatase levels were measured using radioimmunoassay method. The data were compared statistically between groups (ANOVA). The results indicated that serum creatine kinase and alkaline phosphatase levels significantly increased in all experimental groups compared with control ( $P < 0.05$ ). Serum creatine kinase and alkaline phosphatase levels were significantly higher in “restrained + exposed to waterpipe smoke” than restrained animals or rats exposed to waterpipe smoke ( $P < 0.05$ ). Our findings clearly indicate that sedentary life when is accompanied with smoking has greater negative impact at least on heart or liver function in females appearing as elevated serum creatine kinase and alkaline phosphatase levels.

**Keywords**— Restraining, Smoke, CK, ALP, Rat.

## I. INTRODUCTION

WHO considered smoking especially waterpipe or tobacco smoking as a global threat(1,2). Studies indicate that there is an association between smoking and respiratory tract, lung, stomach, liver, kidneys and urinary tract malignancies or Myeloid leukemia(3). On the other hand, Creatine 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(4). 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(5). studies indicate that smoking would predispose to heart disease(6). Researchers have found significant

association between smoking and alterations in heart and brain biochemical enzyme activity(7). Alkaline phosphatase (ALP) is a membrane hydrolase enzyme which transfers phosphate group from many types of molecules, such as nucleotides, proteins and alkaloids(8). 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(9). Many studies suggest that there is an association between smoking and liver disease(10). Although it is supposed that Smoking can affect the body biochemical enzyme levels, especially in liver tissue(11), some of the results showed no significant changes in liver enzyme levels, especially ALP activity(12). 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. In this regards, considering more sensitivity of female rats to darkness stress(13) and due to the lack of motion in modern lifestyle, our study aims to examine the multiplicity or comparative effects of waterpipe smoking, immobilization and darkness stress on serum CK and ALP activities in female rats. The results of this study have an important role in measures of health, prevention of tobacco use and selection a more appropriate lifestyle through the new findings added to basic knowledge of health and disease.

## II. MATERIAL AND METHODS

### A. Animals

Female Adult Wistar rats weighting  $200 \pm 30$ g were purchased and raised in our colony from an original stock of Pasteur institute (Tehran, Iran). The temperature was at  $23 \pm 2$  °C and animals kept under a schedule of 12h light:12h darkness (light on at: 08: 00 a.m.) with free access to water and standard laboratory chow Food was withheld for 12-14h before death.

### B. Protocol of Study

In this laboratory experimental study, female Wistar rats were randomly divided to control, restrained, exposed to waterpipe smoke and “restrained + exposed to waterpipe smoke” groups of 6 rats in each group.

According to previous studies (14), for restraining of animals the female rats were restrained chronically (twice a day for 2h with 2h resting time interval) for a period of 7 weeks. A standard restrained was used to perform restraining procedure. For exposing to waterpipe smoke, according to previous

Foroutan Maryam (\*corresponding author) is with Department of Medical Genetics, Faculty of Medical Sciences, Zanjan University, Zanjan, Iran. (e-mail: m.foroutanjazi@yahoo.com).

Elnaz Naghavi (MSc) is with the Department of Physiology, Faculty of Basic Sciences, Islamic Azad University, Hamedan Branch, Hamedan, Iran. (e-mail: E.naghavi@yahoo.com).

Sedigheh Molaei (MSc student) is with Department of Biotechnology, Iranian Blood Transfusion Organization, Tehran, Iran. (e-mail: molaei.sedigheh@gmail.com).

Rahim Ahmadi (PhD) is with the Department of Physiology, Faculty of Basic Sciences, Islamic Azad University, Hamedan Branch, Hamedan, Iran. (e-mail: Drrahahmadi@yahoo.com).

experiences (15), a smoking chamber was designed to expose animals to tobacco smoke. Initially, the rats were 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 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. After 7 weeks, blood samples were collected and following serum collection, the levels of CK and ALP were measured using radioimmunoassay method. 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 (16).

### C. Statistical Analysis

All values are presented as mean  $\pm$  S.E.M. Statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS 19. Significance was measured using Fisher's least significant for the exact P values and significant differences are noted in the results. Differences with  $P < 0.05$  were considered significant

## III. RESULTS

Table I shows serum levels of CK and ALP in female rats.

TABLE I:  
SERUM LEVELS OF CK AND ALP IN CONTROL, RESTRAINED,  
EXPOSED TO WATERPIPE SMOKE AND "RESTRAINED + EXPOSED  
TO WATERPIPE SMOKE" GROUPS.

Animals	CK U/l $\pm$ SD	P	ALP U/l $\pm$ SD	P
Control	160.00 $\pm$ 2.16	-	86.45 $\pm$ 2.08	-
Exposed to waterpipe smoke	526.42 $\pm$ 4.51	<0.05	112.00 $\pm$ 2.16	<0.05
Restrained	379.40 $\pm$ 4.03	<0.05	102.00 $\pm$ 2.17	<0.05
Restrained + Exposed to waterpipe smoke	853.20 $\pm$ 2.18	<0.05	127.46 $\pm$ 8.42	<0.05

The data are indicated as mean  $\pm$  SEM. P values are expressed in comparison with control group.

The results indicated that serum creatin kinase and alkaline phosphatase levels significantly increased in all experimental groups compared with control ( $P < 0.05$ ). Serum creatin kinase and alkaline phosphatase levels were significantly higher in "restrained + exposed to waterpipe smoke" than restrained animals or rats exposed to waterpipe smoke ( $P < 0.05$ ).

## IV. DISCUSSION

Our findings clearly indicated that waterpipe smoking, restraining or darkness stress increases serum CK and ALP activity in female rats. However, waterpipe smoking was found to have the most powerful impact on these factors. As there are significant difference 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 CK activity. Indeed, this factor may alter in different life styles or stress situations. According to our study, serum ALP activity was found less affected by the type- mild stress, i.e., darkness or restraining have shown semi-equal effect on ALP activity- or multiplicity of stress- under darkness immobilized waterpipe smoking rats have ALP activity similar to immobilized waterpipe smoking or under darkness stress waterpipe smoking animals- compared to CK activity, although it has shown enhanced activity levels in all experimental groups compared to control same as CK activities. In this regard, there are reports suggesting a significant decrease in liver weight or damage and liver enzyme alteration in mice exposed to cigarette smoke(17). Moreover, smoking is a leading cause of heart disease and cardiac enzyme changes(18). In contrast, some research have shown that smoking has no effect on some liver enzyme activities especially ALP(19). Also, there are studies reporting even better recovery of smokers with acute myocardial infarction and lower mortality rate among them compared to non-smokers(20). On the other hand, consistent with our findings, there are studies which suggest enhanced plasma enzymes such as creatine kinase, lactate dehydrogenase and glutamic-pyruvic transaminase activity in rats following cold immobilization stress(21), immersion restraint stress or restraint and isolation stress. These findings support the concept that light- or dark-rearing environment alters chemical enzyme activity(22).

## V. CONCLUSION

Our findings clearly indicate that sedentary life when is accompanied with smoking has greater negative impact – at least- on heart or liver function in females appearing as elevated serum creatin kinase and alkaline phosphatase levels.

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**Maryam Foroutanjazi**. Msc in Medical Genetics. Zanjan University Medical Sciences. Bsc in Biology. Tehran university. Skill: Cell culture, RT-PCR, RealTime PCR, ELZA, Animal Model.