The Effects of Prolonged Darkness Phase of Photoperiodic Cycle on Serum Levels of Creatine Kinase and Alkaline Phosphatase in Female Rats

Manafi M*, Ahmadi R, and Molaee S

***Abstract*—**There is association between changes in photoperiodic cycle and serum level of certain enzymes. This study aims to determine the effects of prolonged darkness phase on serum levels of creatine kinase and alkaline phosphatase in female rats. In the present experimental study, female Wistar rats were randomly divided into control animals and rats experiencing prolonged darkness phase. Control animals were under normal photoperiodic cycle (12h light/ 12 h darkness), however, in the group experiencing prolonged darkness phase the animals were under 7h light and 17h darkness. After 7 weeks, blood samples were collected and serum alkaline phosphatase and creatine kinase levels were measured using spectrophotometry. The data were compared statistically between groups using ANOVA. The serum creatine kinase and alkaline phosphatase levels significantly increased in animals experiencing prolonged darkness phase compared to control rats ($p<0.001$and $p<0.05$, respectively). Our findings indicated that prolonged darkness phase leads to increased serum levels of creatine kinase and alkaline phosphatase; according to which, can impose serious pathophysiological effects on internal organs including heart, brain, liver or muscles.

***Keywords*—**Creatine kinase, Alkaline phosphatase, Prolonged Darkness Phase, Rat.

I. Introduction

STUDIES show that photoperiodic changes could disturb the function of some organs [1,2]. It has negative effect on some systems like endocrine and immunity system [3]. Hormonal changes are the most typical effect of the prolonged darkness phase [4]. Studies indicate that photoperiodic changes have effect on secretion of a lot of hormones [5].

Creatine kinase is a dimer enzyme composed of 2 polypeptide chains. It's subunits are M (muscle) and B (brain), combination of them make 3 isoenzymes. The molecular weight of creatine kinase is 82 daltons [6].

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Creatine kinase is an enzyme that acts in consumption and saving energy specially in muscles [7]. Several factors like muscular activity, damage to the heart muscle or skeletal muscle, pulmonary diseases, chronic renal failure and chronic liver failure causes elevation of serum creatine kinase [8]-[10]. Alkaline phosphatase is a dimer enzyme in cell membrane with high molecular weight [11],[12]. It has liver and renal isophorms [13]. Studies show that this enzyme is increased in bone, liver and renal disorders [14].

II. Material And Methods

A. Animals

Female Adult Wistar rats weighting 200±30g were purchased and raised in our colony from an original stock of Pasteur institute (Tehran, Iran).The temperature was at 23±2 ^{0}C and animals had 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 animals and rats experiencing prolonged darkness phase. Control animals (n=7) were under normal photoperiodic cycle (12h light/ 12 h darkness/day), however, in the group experiencing prolonged darkness phase the animals (n=7) were under 7h light and 17h darkness/ day. After 7 weeks, blood samples were collected using cardiac puncture method and following serum collection, the levels of CK and ALP were measured using spectrophotometry 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 ± 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

Figure I shows the serum levels of creatine kinase and figure II shows the serum levels of alkaline phosphatase in female control and under prolonged darkness phase rats.

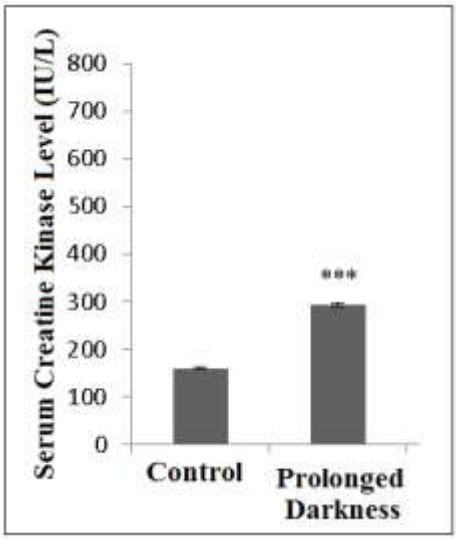

Fig. 1 Serum levels of creatine kinase in control rats and rats exposed to prolonged darkness phase. *** indicates significance difference at P<0.001.

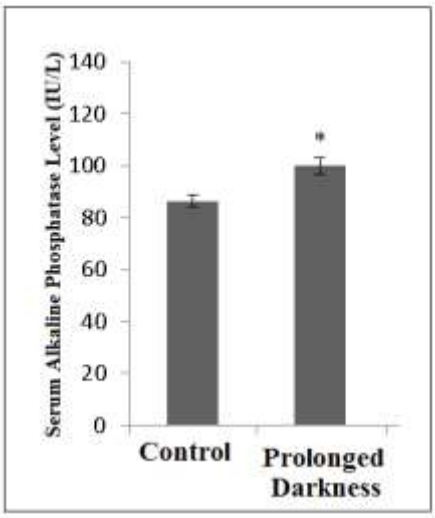

Fig. 2 Serum levels of alkaline phosphatase in control rats and rats exposed to prolonged darkness phase. * indicates significance difference at P<0.05.

The results indicated that the serum creatine kinase and alkaline phosphatase levels significantly increased in animals experiencing prolonged darkness phase compared to control rats ($p<0.001$and $p<0.05$, respectively).

IV. Discussion

Our findings clearly indicated that prolonged darkness phase in photoperiodic cycle results in increased serum levels of creatine kinase and alkaline phosphatase in female rats.

Previous studies also show that prolonged darkness phase can change the serum level of some hormones in body [15]. For example several studies show that prolonged darkness phase in photoperiodic cycle causes decreased serum level of T3 [16],[17], whereas there is a study indicating that prolonged darkness phase has no effect on secretion of T3 hormone [18].

The probable mechanism of action could be referred to the effect of prolonged darkness phase on systems such as immunity and gonadal system [19]-[21], which in turn, may result in increased serum level of creatine kinase and alkaline phosphatase. On the other hand experiences show that prolonged darkness phase causes increased melatonin secretion and pineal activity [16] and this may also lead to increased serum level of creatine kinase and alkaline phosphatase.

V. Conclusion

Our findings indicated that prolonged darkness phase leads to increased serum levels of creatine kinase and alkaline phosphatase; according to which, can impose serious pathophysiological effects on internal organs including heart, brain, liver or muscles.

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