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Original article

Effect of circadian on the activities of ion transport ATPases and histological structure of kidneys in mice

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A B S T R A C T

The impacts of unnatural every day cycles (circadian) for 60 days on the histological structure of kidneys and ATPase activities in MF1 mice were studied. The exposure times were 16 h dark, 16 h light, 24 h dark, and 24 h light, and control exposure times were 12 h dark followed by 12 h light. Our results showed an increase in the total ATPase activity of mice in all groups. Additionally, the activity of the enzyme Na$^+$/K$^+$-ATPase was increased after 24 h darkness, 24 h light, and 16 h light exposures compared to control. The enzyme Mg^{+2}-ATPase activities of the groups were higher when exposed to 16 h light, 24 h light, 24 h darkness and 16 h darkness. The activities of total ATPase, Na$^+$/K$^+$-ATPase and Mg^{+2}-ATPase in kidneys were increased in all groups after 24 h light, 24 h darkness, 16 h darkness and 16 h light exposures. Interestingly, the activity of V-type ATPase was reduced after 16 h darkness, 24 h darkness and 16 h light. Taking everything into account, changes in the day by day cycle prompt neurotic changes, enzymatic and histological changes in the kidneys of mice. More studies should be directed to explore the impacts of light and darkness that can prompt these progressions.

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1. Introduction

Circadian rhythms (Latin, circa: "approximate"; dies: "day") refer to physiological processes that occur with a repeating period of approximately 24 h and ensure that internal physiology is synchronized with the external environment (Ferrell and Chiang, 2015). Rhythmicity is a feature of living matter that is generally present in uni- and multicellular life forms (Kondo et al., 1994; Vitaterna et al., 1994; Johnston and Pollock, 2018) as an adjustment to cyclic changes in ecological factors, e.g., light, temperature, and nourishment accessibility (Edmunds, 1988; Bünning, 1993). In mammals, light is the major consideration in the synchronization between numerous body capacities and the environment. At the cellular level, rhythmic changes in activity following a circadian schedule have been described for several enzymes, i.e., glycogen-synthase (Uchiyama, 1990; Harley and Rusak, 1993), glucose-6-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase (Peleg et al., 1990), and cardiac Ca-ATPase (Chen et al., 1993), renal Na$^+$/K$^+$-ATPase (Segura et al., 2004; Reyes et al., 2009; Damulewicz et al., 2013), V-ATPase (Bebas et al., 2002). In the animal kingdom, one of the most important mechanisms for active cellular Na$^+$ extrusion occurs in exchange for extracellular K$^+$ in a function catalyzed by the Na$^+$/K$^+$-ATPase. This process regulates the cellular Na$^+$/K$^+$ concentrations and hence their gradients across the plasma membrane, which are required for vital functions such as membrane co-transport, cell volume regulation and membrane excitability (Katz, 1982; Jørgensen, 1986; Doucet, 1988). Changes in Na$^+$/K$^+$-ATPase activity may regulate these cell functions with essential physiological implications. In this respect, renal discharge of water and electrolytes demonstrated a marked circadian rhythm, which was independent of the time of daily nourishment ingestion (Rabinowitz et al., 1986). Since the renal Na$^+$/K$^+$-ATPase activity certainly plays a vital role in the renal discharge of electrolytes, it is of physiological and biochemical importance to investigate whether the Na$^+$/K$^+$-ATPase activity of kidney cell membranes shows a circadian rhythm. Therefore, we designed the current study to assess the impacts of the unnatural day by day cycles (circadian) on the histological structure of kidneys and ATPase activities in MF1 mice for 60 days.

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2. Materials and Methods

2.1. Animals

Male and female Swiss mice (*Musmusculus*), 6–8 weeks old and weighing approximately 32 ± 2 g, were provided by the Animal House of the King Fahad medical research center, King Abdul Aziz University, Jeddah, Saudi Arabia. The animals were kept in polypropylene cages in an experimental room under a controlled state with temperature at 25 ± 2 °C and humidity at $50 \pm 5\%$. They had free access to regular laboratory diet chow and tap water. The Institutional Animal Ethics Committee at the Biology Department of Faculty of Science, King Abdul Aziz University has approved the study. The animals were kept for 21 days before the beginning the experiments. The MF1 mice were divided into 5 groups, each consisting of 10 animals (N = 10) including 5 males and 5 females. The males and females were separated from each other. The light exposure was done by a fluorescent light environment while the darkness exposure was setup in a completely dark room. The animals were divided into groups according to the exposure time (h) as follows:

Group (1) (control) exposed to 12 h fluorescent light, followed by 12 h dark.

Group (2) exposed to continuous fluorescent light for 24 h.

Group (3) exposed to continuous dark for 24 h.

Group (4) exposed to 16 h light and 8 h dark.

Group (5) exposed to 16 h dark and 8 h light.

2.2. Animal weight

All groups of animals were weighed before and after the exposure to light and darkness according to Casiraghi et al. (2016).

2.3. Histological preparation of kidney tissues

At the end of the experiments, the experimental animals were divided into two groups. The 1st group was the source of kidney tissue. The other group was the source for the extraction of Na^+/K^+-ATPase and V-type-ATPase. The kidneys were isolated from the animals after dissection and prepared for histological examination. The prepared tissues were stained by hematoxylin and eosin stains, and one drop of Canada balsam dissolved in xylene was added to the slide and covered by a cover-slip while avoiding air bubbles. At this point, the slides were ready for further investigation as described by Römisch-Margl et al. (2012).

2.4. Preparation of different cell fractions

Animals were anesthetized with diethylether and immediately decapitated, and their kidney were quickly dissected out and placed in 20 ml of an ice-cold homogenization medium consisting of 250 mM Sucrose, 25 mM Imidazole and 1 mM EDTA, pH = 7.2. Homogenization was carried out in a glass homogenizer with a Teflon pestle (clearance 0.1–0.15 mm) with 20 passes of the plunger at 100 rev/min. The homogenization tube was surrounded by ice throughout this procedure.

The subsequent homogenate was centrifuged to isolate the distinctive membrane components using a method previously described by Rodriguez and Edelman (1979) and Kaniike et al. (1980) and modified by Fogg et al. (1991). First, the homogenate was centrifuged at 600 g for 10 min at 0 °C. The pellet from this spin was discarded, and the supernatant (S1) was retained and centrifuged at 15,000 g for 20 min at 0 °C. The resulting mitochondrial-rich pellet (P2) was stored on ice, and the associated supernatant (S2) was re-centrifuged at 135,000 g to yield a pellet

(P3) which was then resuspended by homogenization in 10 ml of homogenization medium containing 10 mM $MgCl_2$. This was then incubated on ice for 20 min prior to centrifugation at 10,000 g for 15 min to yield pellet P4, which was the "Na^+/K^+-ATPase-rich" basolateral membrane fraction. The resulting supernatant (S4) was then centrifuged at 55,000 g for 30 min. The pellet (P5) resulting from this spin represented the "V-type-ATPase-rich" apical membrane fraction (Fogg et al., 1991, AL-Fifi et al., 1998). The last pellet was resuspended by homogenization in a known volume of deionized water at 4 °C.

2.5. Assay of ATPase activities

Each incubation medium, consisting of 100 µl of an appropriate ionic medium (see below) and 50 µl of membrane preparation, was thermo-equilibrated for 15 min at 35 °C in a water bath prior to starting the reaction by the addition of 50 µl of 12 mM ATP (Tris salt). Incubations were done for 30 min at 35 °C, and reactions were stopped by adding 400 µl of a 1:1 mixture of 1% Lubrol:1% ammonium molybdate in 0.9 M sulfuric acid (Atkinson et al., 1973). The tubes were then kept at room temperature for 10 min to allow the yellow color, which was proportional to the amount of inorganic phosphate released, to develop. Absorbance was measured at 390 nm. Enzyme activity was measured by determining the amount of inorganic phosphate released.

In determining different ATPase activities, a variety of different ionic media having the following ionic compositions were used (final concentrations):

2.5.1. Na^+/K^+-ATPase activity

(1) 4 mM $MgCl_2$.
(2) 4 mM $MgCl_2$, 120 mM NaCl and 25 mM KCl.
(3) 4 mM $MgCl_2$, 120 mM NaCl, 25 mM KCl plus 1 m Mouabain.

Each medium contained 50 mM imidazole/HCl and 1 mM EDTA (pH 7.2). Na^+/K^+-ATPase activity was determined as the difference in the amount of inorganic phosphate liberated in the presence of ionicmedium 2 and ionic medium 3.

2.5.2. V-type Atpase activity

This assay was performed as anazide- and orthovanadate-insensitive ATPase activity as described by Schweikl et al. (1989) in 1 mM $MgCl_2$, 20 mM KCl, 0.5 mM NaN_3, 0.1 mM Na_3VO_4, 0.1 mM EGTA and 0.3 mg BSA ml^{-1} in 50 mM Tris–MOPS buffer (pH 7.5).

Appropriate controls were performed to determine the extent of non-enzymatic hydrolysis of ATP.

All ATPase activities are expressed in µmoles Pi liberated mg protein^{-1} min^{-1}.

2.6. Determination of protein content

Protein determinations were carried out using the Bradford, (1976) with Coomassie Brilliant Blue and using bovine serum albumin fraction V as the standard.

2.7. Statistical analysis

Data are expressed as mean ± SEM. The data collected were compared by paired Student's t-test. A value of $P < 0.05$ was considered statistically significant.

3. Results

The current study was conducted to examine the effect of different durations of light and darkness (60 days) on the tissues and dynamic transport catalysts of mouse kidneys. Our study demonstrated that there was a reasonable change in behavioral conduct. At the beginning of the experiment, an increase in motor activity was observed in all groups, especially the 24 h light group and 16 h light group. Moreover, a strong effect continued in both groups compared to the control until later stages of the experiment. In other groups that started with natural activity, they tended to become isolated, calm, and lacked movement. An increase in the amount of urine with unpleasant smell was noted, especially in the 24 h light group and 24 h dark group.

3.1. The impact of the duration of light and darkness on MF1 mice weight

Male and female mice were weighed in each group separately, including the control group, before the beginning of the experiment and then again after 60 days of exposure to light and darkness. (Figs. 1, 2) show the average weights and standard error for each male and female group. In (Figs. 1 and 2), there are clear differences in the weights of mice before and after the experiment, and the weight of males and females has increased in all groups, including the control group. The weight in males increased from 6.8 g to 9.4 g, while the weight in females increased from 3.9 g to 6.8 g. It was noted from (Fig. 1) that the average weight of males in the control group before the experiment was 33.84 g and increased after the experiment to 41.67 g, which is a significant difference ($P < 0.05$) of 7.83 g. In addition, male weight levels increased in other groups, especially in the 24 h dark group and
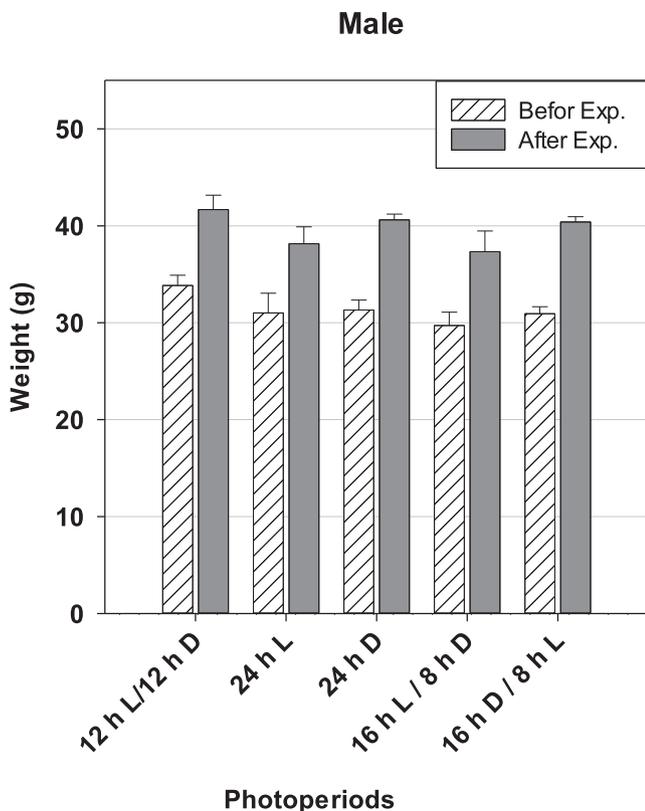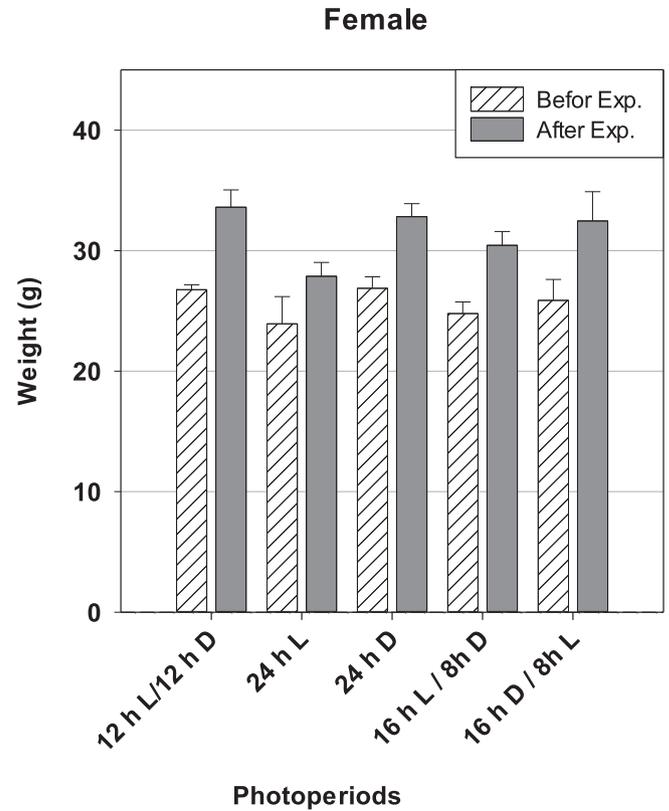

Female

Fig. 2. Effect of different photoperiod exposures on the weight of female MFI mice. (Each point represents mean ± SEM, n = 5, $P < 0.05$).

16 h dark group. The weight increases in those groups were 9.4 g and 9.3 g, respectively, compared to the weight before the experiment. The weight increases in the 24 h light group and 16 h light group were 7.1 g and 7.6 g, respectively. As shown in (Fig. 2), the weight of female mice in the control group increased from 26.77 g before the experiment to 33.6 g after. The difference in weight is approximately 6.84 g, which is significant ($P < 0.005$). The increases in female weights in all groups, especially in the 16 h dark group, were found to be statistically significant ($P < 0.05$). In male mice, there were significant differences in weight before and after the experiment ($P < 0.05$). When comparing the weights after experiment between the control group and other groups, the current study found significant differences in male weight in the 24 h light group ($P < 0.05$) and 16 h light group ($P < 0.05$). The differences in females were significant ($P < 0.05$ and $P < 0.05$, respectively) compared to the control group. No significant difference was found in the other groups.

3.2. Effect of difference in light and darkness duration on ATPase activity

3.2.1. Na^+/K^+-ATPase

The activity of Na^+/K^+-ATPase in the control group was 41.33 ± 1.09 µmoles (Pi) lib. mg Protein^{-1} min^{-1}, a percentage of 100%. The activities of enzyme in the 16 h D group and 16 hL group increased to 69.3 ± 0.62 µmoles (Pi) lib. mg Protein^{-1} min^{-1} and 53.76 ± 0.43 µmoles (Pi) lib.mg Protein^{-1} min^{-1}, a percentage of 167.8% and 130.1%, respectively, compared to the control group as shown in (Fig. 3). It was also observed that the activity of enzymes was highly increased in the 24 h L group and 24 h D group compared to the control group at 100.19 ± 0.58 µmoles (Pi) lib. mg Protein^{-1} min^{-1} and 99.61 ± 0.45 µmoles (Pi) lib. mg Protein^{-1} min^{-1}, a percentage of 242.4% and 241%, respectively. Our results

Male

Fig. 1. Effect of different photoperiod exposures on the weight of male MFI mice. (Each point represents mean ± SEM, n = 5, $P < 0.05$).
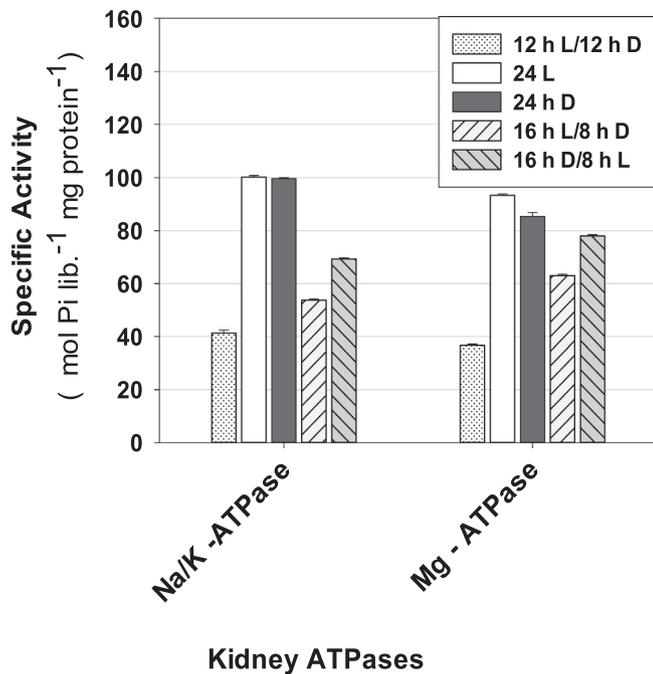
Fig. 3. Effect of photoperiod changes on Na^+/K^+ and Mg^{2+} ATPase activity in the kidney. Enzyme activity is expressed as μmoles Pi liberated mg $protein^{-1}$ min^{-1}. (Each point represents mean ± SEM, n = 3, P < 0.05).

demonstrated a statistically significant difference (P < 0.001) in the Na^+/K^+-ATPase activity in all groups comparing to the control group.

3.2.2. Mg^{+2}-ATPase

The activity of Mg^{+2}-ATPase in the control group was 36.76 μmoles Pi lib. mg $Protein^{-1}$ min^{-1}, a percentage of 100%. Our results also showed a highly statistically significant difference (P < 0.05) in the activity of enzyme in groups exposed to 24 h L and 24 h D compared to the control group. The activity of enzyme in the 24 h L group and 24 h D group greatly increased to 93.29 ± 0.49 μmoles (Pi) lib. mg $Protein^{-1}$ min^{-1} and 85.47 ± 1.37 μmoles (Pi) lib. mg $Protein^{-1}$ min^{-1}, a percentage of 253.8% and 232.5%, respectively (Fig. 3). It was observed that the activity of Mg^{+2}-ATPase in the 16 h L group and 16 h D group increased to 63.06 ± 0.41 μmoles (Pi) lib. mg $Protein^{-1}$ min^{-1} and 78.03 ± 0.28 μmoles (Pi) lib. mg $Protein^{-1}$ min^{-1}, a percentage of 171.6% and 212.3%, respectively. This increase was found to be statistically significant (P < 0.05) compared to the control group.

3.2.3. V-type-ATPase

The specific activity of V-type-ATPase isolated from the kidney of the control group reached 21.34 ± 0.24 μmoles (Pi) lib. mg $Protein^{-1}$ min^{-1}. When comparing the activity of V-type-ATPase in the control group with other groups that were exposed to different periods of light and dark, it was observed that the activity of V-type-ATPase greatly increased in the 24 h light to 24.13 ± 0.13 μmoles (Pi) lib. mg $Protein^{-1}$ min^{-1}, a percentage of 112.8%, compared to the control group. The activity of the enzyme decreases in the 16 h L group, 24 h D group and 16 h D group to 17.29 ± 0.16 μmoles (Pi) lib. mg $Protein^{-1}$ min^{-1}, 13.51 ± 0.16 μmoles (Pi) lib. mg $Protein^{-1}$ min^{-1} and 18.47 μmoles (Pi) lib. mg $Protein^{-1}$ min^{-1}, a percentage of 86.3%, 80.8% and 63.2%, respectively, compared to the control group. The difference in the activity of V-type-ATPase was significant (P < 0.05) in all groups compared to the control group (Fig. 4).
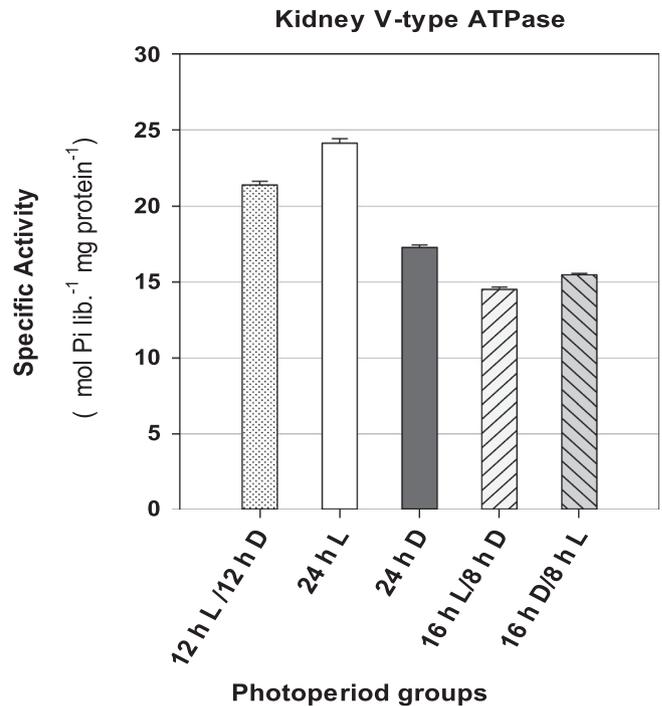

Fig. 4. Effect of photoperiod changes on V-type ATPase activity in the kidney. Enzyme activity is expressed as μmoles Pi liberated mg $protein^{-1}$ min^{-1}. (Each point represents mean ± SEM, n = 3, P < 0.05).

3.3. Histological investigation of the impact of light period on mouse kidney tissue

The light period changes led to evident histological changes. The progressions differ in the groups dependent on the duration of the light period to which they were exposed.

The investigations of histological sections in the kidney of MF1 mice exposed to various times of light demonstrated histological changes in the cells of the renal tubule and in Bowman's capsule, particularly in glomeruli.

Fig. 5 shows a kidney section in the control group. It shows a normal renal cell. The figure shows the general shape of Bowman's
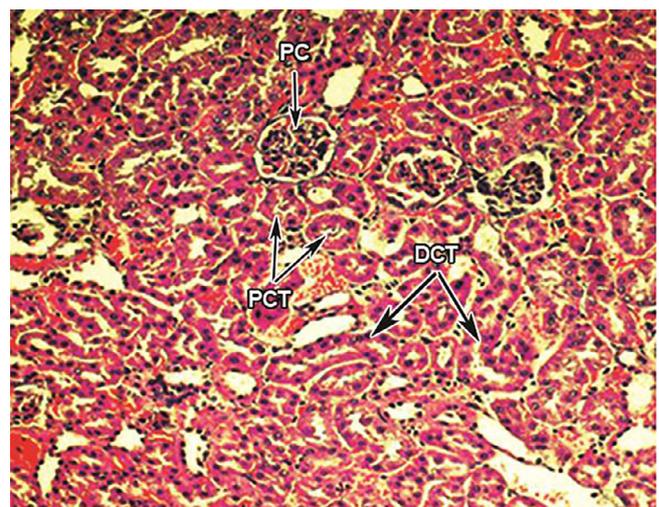

Fig. 5. T.S in the male kidney of the control mice (12 h light/12 h dark) showing normal structure of Bowman's capsule (BC), proximal convoluted tubule (PCT) and distal convoluted tubule (DCT).

capsule, the proximal convoluted tubule (PCT) and distal convoluted tubule (DCT). As shown in (Fig. 5) the kidney of the control group contains Bowman's capsule with a normally formed glomerulus surrounded by Bowman's capsule spacing (CS).

(Fig. 6) shows a section of mouse kidney in the 24 h D group. It shows a loss of normal renal cell shape, increase in the number of inflammatory renal cells generated fiber, small cell size and the beginning of nuclei decay due to the degradation of the contents of the cytoplasm in the renal tubes. Nevertheless, an increase in septicemia in the renal tubes was noted, resulting in loss of normal shape and increased inflammatory cell density in the 24 h D group.

While, (Fig. 7) shows part of the kidney section of the 24 h L group. Destruction of the glomerulus contents was observed causing the appearance of vacuolations free of cytoplasmic and nuclear contents. In addition, the destruction of the layer surrounding the glomerulus was noted, and this caused the mixing of the glomerulus contents with the tubule cells and a reduction in the nucleus and appearance of tissue rupture.
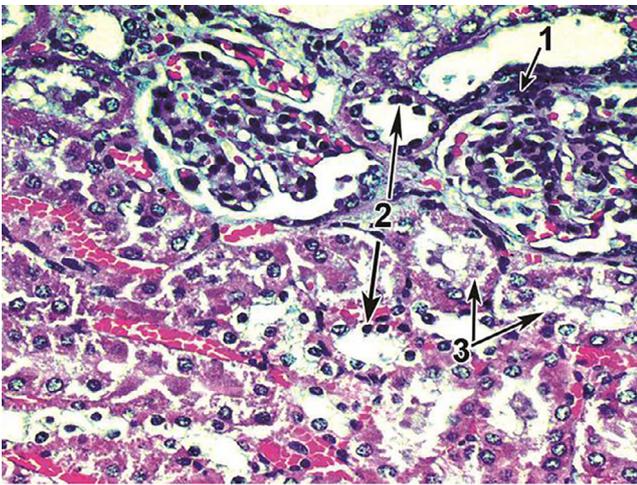

Fig. 6. T.S in the male kidney of the 24 h dark group mice showing the loss of normal kidney shape, accumulation of renal inflammatory cells (1), decay of nuclei (2) and lysis of renal tubules (3).
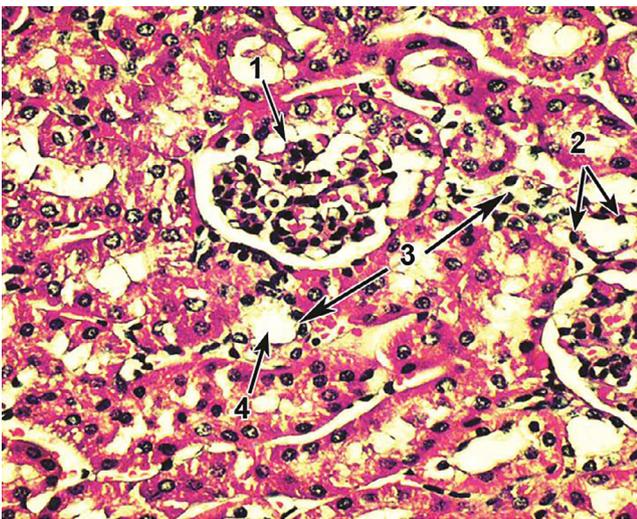

Fig. 7. T.S in the male kidney of the 24 h light group mice showing the disruption and disintegration of glomeruli (1), smaller size of renal nuclei (2), histopathological decomposition of renal tubules (3) and formation of cytoplasmic cavities (4).

4. Discussion

The shift of day and night is considered a standout among the most critical factors affecting biological activities. The change in photoperiod has coordinated impacts on physiology, organs, temperature, vital activities, blood pressure, heart beat and dynamic transportation in heart muscles (Li et al., 1999; Johnston and Pollock, 2018). Our study demonstrated that the change in photoperiod influences some physiological and psychological activities in mice exposed to different periods of light and darkness compared to control mice. The groups of mice exposed to continuous light showed aggressive behavior at the beginning of experiments. This behavior may be due to the new conditions in which they live. At late stages of our experiments, most groups of mice tend to have low motility and are isolated. These observations agree with other studies that showed that the biological clock was influenced by differences in photoperiods (darkness and light), which in turn affected the psychological and physiological activities of the animal (Young and Kay, 2001; Alves-Simoes et al., 2016). Likewise, changes in the light and dark periods significantly affect the behavior and sexual activity of seasonal animals, particularly Siberian hamsters (Prendergast et al., 2013). Gonadal steroid hormones, particularly testosterone, were reduced in the short light day, and this prompts aggressive behavior (Jasnow et al., 2000). Moreover, the amount of urine with offensive odor was increased in mice exposed to continuous darkness and night. This may be due to the disintegration and disruption of the proximal and distal renal tubules which causes a large amount of protein to be excreted in urine and in turn causes renal failure. There were significant differences in the weight of mice that were exposed to different photoperiods of light and darkness. Since there was increase in male and female weight after exposure to 16 h and continuous darkness, we assumed that the increase in weight was due to the continuous feeding and low activities of the animals which lead to low energy consumption and the increase of absorbed food that was stored as fat in the liver. In addition, defects in metabolism, such as defective fat oxidation (peroxidation), cause the accumulation of massive amounts of fats (Storch et al., 2002). Belanger et al. 1991) concluded that changes in the day cycle alter peroxidation and liver glutathione concentration. Interestingly, activity of kidney enzymes of the mice was affected by the change in the dark and light period. The total ATPase (Mg^{+2}-ATPase, Na^+/K^+-ATPase) activity was increased in all animals and was higher in the two groups of animals exposed to continuous darkness and light. The specific activity of the same kidney enzymes was 2-fold higher than the control. We assume that such changes in enzyme specific activities are due to alterations in the number of enzyme molecules or alterations in the activity rate of the enzymes as an adaptation to the new living conditions that experimental animals were exposed to. These results agree with similar studies that reported the effect of salinity on Mg^{+2}-ATPase and Na^+/K^+-ATPase activities in the gills of *Poecilia vivipara*. It was concluded that the active transport enzymes adapted to the new conditions by greatly increasing their activity with the salinity differences (Amaral et al., 2001). They concluded that these enzymes have complementary functions with the plasma membrane. Chose et al. (2004) reported that Na^+/K^+-ATPase, V-type-ATPase and carbonic anhydrase in cell membranes are able to adapt between fresh and salt waters in adult lampreys, especially in *Anadromous lamprey* during their migration to lay eggs. The increase of membranous vesicles in the cells due to the change of dark and light increases the number of enzyme molecules. This agrees with a study conducted to estimate the activity of Na^+/K^+-ATPase extracted from the cortex of rat kidneys through 24 h only (Segura et al., 2004). They concluded that the fluctuation of the enzyme activity was due to several

factors, such as the effect of the daily changes that may affect the enzyme molecules. Moreover, the change in the weight of the spleen and testes affect the activity of Mg^{+2}-ATPase and Na^+/K^+-ATPase. This result agrees with a study by Bertoni et al. (1992) that found that the enzyme activity increases with an increase in testes weight.

Kidneys are considered important organs for absorption and excretion. Our results showed pathological and histological changes due to the change of photoperiod. The effect was in the renal tubules which had strong disintegration and erosion, especially in groups exposed to continuous dark or light. We assume that these changes are due to the change in photoperiod which affects the enzyme activities of the cells, and consequently the permeability of the renal tubule membranes increased. As a result, the renal tubule cells swell and burst, and the cell contents protrude to inside the tubule cavity. All these events lead to renal failure. On this regard, Segura et al. (2004) and Reyes et al. (2009) showed that the activity of the Na^+/K^+-ATPase from rat kidney cortex increased to two peak values during the day at 9 h and 21 h, and decreased to two valley values at 15 h and 1–3 h. Incubations of 9 h rat kidney cortex slice homogenates with blood plasma obtained at 15 h resulted in a strong inhibition of the Na^+/K^+-ATPase activity, which was not seen when the incubations were carried out with 9 h blood plasma. The dysfunction of the circadian clock or its misalignment with behavioral cycles has been implicated in pathogenesis of many diseases. For instance, long-term night work is associated with a significant increase in the risk of breast cancer (Pesch et al., 2010), metabolic syndrome (Loffing and Korbmacher, 2009) and ischemic heart disease (Hager et al., 2001). In the individuals with essential hypertension, abnormal rhythm of sodium re-absorption by the kidney has been associated with a blunted decrease in night-time blood pressure, a condition characterized by a significantly increased risk of end-organ damage (Solocinski and Gumz, 2015; Mistry et al., 2016). In hamsters, it has been shown that a point mutation in the circadian regulatory gene, casein kinase-1e, leads to a disorganization of the circadian clock accompanied by cardiomyopathy, extensive cardiac and renal fibrosis, and renal tubular dilation. Disturbance in renal rhythms may not only influence calciuria, phosphaturia, natriuria, urinary pH, and diuresis but also other risk factors for stone formation, including citrate and oxalate urinary excretion (Johnston and Pollock, 2018).

5. Conclusion

In this study, the effect of an abnormal daily cycle on the histological structures and enzymatic activities of ATPases in the mouse kidney were examined. Changes in the daily cycle lead to pathological, enzymatic and histological changes in the mouse kidney.

In all groups, the specific activity of total ATPase enzymes increased. It was even higher in 24 h dark and 24 h light groups. The specific activity of V-type-ATPase slightly decreased in all groups. The group exposed to continuous light had a slight increase in enzyme activity. The normal uniformity of the renal cells was lost. The exposure of mice to longer periods of dark or light caused degeneration of renal tissue, especially tubules, resulting in an increased amount of urine with foul odor. This degeneration may cause renal failure. There was an increase in the density of inflammatory cells around the renal pellet, particularly in the 24 h light group and 24 h dark group. Our results also showed the presence of vacuolations in renal tissues due to the degeneration of renal pellet and tubule tissues.

The current study recommends that more studies and research should be conducted to investigate the effects of light and dark using electron microscopy techniques and to study the effect of abnormal blood circulation on other physical organs.

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