Histopathological Changes in Adult Male Rat’s Liver Induced by Continuous Darkness

Samia A Eleiwe1 MBChB PhD, Basim Sh Ahmed2 MBChB FIBMS, Salman S Salman3 MBChB PhD

1,3 Dept. Anatomy, Histology & Embryology, 2 Dept. of Pathology & Forensic Medicine, College of Medicine, Al-Mustansyria University

Abstract

Background It is well known that liver is an endocrine as well as exocrine gland; it also synthesizes, accumulates, detoxifies and transports certain substances. Melatonin is the principle hormone of the pineal gland, which is mainly secreted at night and it is definitely documented to regulate the physiology of all tissues and cells keeping their normality.

Objective This work is designed to study the effect of continuous darkness on hepatic tissues.

Methods Adult Wister albino rats were kept in complete 24 hours darkness for successive 4 periods. Rats were divided into 16 groups. Group II, III, IV and V were left in continuous darkness for 2, 4, 6 and 8 weeks, respectively. Group I†, Group I††, Group I†††, and Group I†††† were the control groups for group II, III, IV and V correspondingly. After the last day of the dark period for each group, the animals were dissected under effect of anesthesia. The liver was weighed and right lobe of liver was processed for study its histopathology.

Results The results showed no important histopathological effect on short and medium periods, while on long periods; there was histopathological changes represented by clear lobulation and inflammatory cell infiltrations.

Conclusion Continuous darkness affects the hepatic tissues of rats depending on the length of exposure.

Key words Melatonin, Darkness, Liver injury.

Introduction Despite decades of investigations on the darkness hormone namely melatonin; researches are still going on, about this hormone, with great deal of interest (1,2). Melatonin is the principle hormone of the pineal gland and it is level is well known to undergo an undulating level through daylight and night darkness, and it hits the highest point level at night (3). It has receptors in all bodily tissues and cell, through which it thought to regulate their function (4).

It is well known that liver is an endocrine as well as exocrine gland; it also synthesizes, accumulates, detoxifies and transports certain substances (5) and its integrity is principally vital for whole body tissues and organs, so the effect of any environmental events on hepatic tissues could be critical for the entire body healthiness, such as alcohol or drugs administration (6).

Melatonin has hepatoprotective potential in various models of oxidative stress and reduces liver damage after sepsis, hemorrhagic shock, ischemia/perfusion and in various models of toxic liver injury. These effects of melatonin on liver are mediated by its influence on hepatic antioxidants enzymes, nitric oxide signaling, hepatic cytokine & heat shock protein expression (7).
The aim of the present study is to assess the "histopathological changes" in liver tissue, in response to gradually increasing periods of continuous darkness in adult male rats.

**Methods**

Forty healthy mature ten week old male Wister albino rats were kept individually in wire meshed stainless steel cages room temperature 22±2°C, fed controlled pellet diet and tap water was provided for drinking ad libitum. They were divided into 8 groups, each consisting of 5 rats. Group I†, Group I†† Group I††† and Group I†††† were the control of group II, III, IV, and V respectively. All of the 4 control groups were put on 12:12 light – dark cycle. Group II, III, IV, and V were put in continuous darkness for a period of 2, 4, 6 and 8 weeks respectively. All the 8 groups were kept in and were put individually in wire meshed. At the last day of 1st couple of weeks, rats of group II with its control group (Group I†), were sacrificed under diethyl ether anesthesia effect, the whole liver was weighed and the right lobe of liver was removed, fixed in Boun’s solution immediately and processed through for histopathological study by light microscopy, using serial paraffin sections of 5 µm thickness stained with haematoxyline and eosin. At the same manner the rats of group III was dissected at the end of the 4th week also with its control group (Group I††). The rats of group IV with its control group (Group I†††), were managed at same way at end of the 6th week, and rats belong to group V with its control group (Group I††††), were operated on at end of the 8th week. Histopathological as well as anatomical examinations were performed. Biostatistical analysis was done to evaluate the significance of results by analysis of variance, using student-t-test.

**Results**

Descriptive as well as morphologic studies were done, as follows:

**Body & liver weight**: There was no significant effect of continuous darkness on the over all body weight as shown in (Table 1).

<table>
<thead>
<tr>
<th>Time of keeping rats in continuous darkness</th>
<th>Body wt. of rats at 1st day of experiment</th>
<th>Body wt. of rats at last day of experiment</th>
<th>Difference in body wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group I†)</td>
<td>411.17±62.9</td>
<td>437.95±74.9</td>
<td>26.78±11.9</td>
</tr>
<tr>
<td>2 week continuous darkness</td>
<td>419.84±77.1</td>
<td>443.81±75.2</td>
<td>23.97±10.8</td>
</tr>
<tr>
<td>Control (Group I††)</td>
<td>410.97±65.1</td>
<td>454.42±51.1</td>
<td>43.45±17.1</td>
</tr>
<tr>
<td>4 week continuous darkness</td>
<td>422.03±82.9</td>
<td>461.11±80.2</td>
<td>39.08±15.5</td>
</tr>
<tr>
<td>Control (Group I†††)</td>
<td>423.23±44.0</td>
<td>491.96±79.0</td>
<td>68.73±23.2</td>
</tr>
<tr>
<td>6 week continuous darkness</td>
<td>416.00±72.8</td>
<td>489.09±90.1</td>
<td>73.09±25.2</td>
</tr>
<tr>
<td>Control (Group I††††)</td>
<td>418.02±74.6</td>
<td>511.19±75.2</td>
<td>93.17±32.6</td>
</tr>
<tr>
<td>8 week continuous darkness</td>
<td>405.25±64.1</td>
<td>503.25±87.7</td>
<td>98.00±34.1</td>
</tr>
</tbody>
</table>

Results were expressed in mean ± SD of 5 rats.
All differences were statistically not significant (P>0.05) when compared with its control.

Liver weight was not affected in group II, whereas it was significantly affected in group III, IV, and V (Table 2). Liver weight to body weight ratio was also estimated (Table 3). Liver weight was increased in group III and still more increased in group IV, and the outer surface of the organ was clearly irregular. Then after, at group V the liver shrank significantly and the outer surface of the organ was markedly nodular.
Table 2. Effect of continuous darkness on liver weight of 10 week old male rats

<table>
<thead>
<tr>
<th>Time of keeping rats in continuous darkness</th>
<th>Liver weight at autopsy (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group I††††)</td>
<td>15080±121.3</td>
</tr>
<tr>
<td>2 week continuous darkness Control (Group I††)</td>
<td>15119±169.3</td>
</tr>
<tr>
<td>4 week continuous darkness Control (Group I†††)</td>
<td>15271±101.2</td>
</tr>
<tr>
<td>6 week continuous darkness Control (Group I††††)</td>
<td>15312±115.2*</td>
</tr>
<tr>
<td>8 week continuous darkness Control (Group I†††††)</td>
<td>15382±123.4</td>
</tr>
</tbody>
</table>

Results were expressed in mean ±SD of 5 rats.
* = P<0.001

Table 3. Effect of continuous darkness on liver weight to body weight ratio in 10 week old male rats

<table>
<thead>
<tr>
<th>Time of keeping rats in continuous darkness</th>
<th>Liver weight/100g BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group I††††)</td>
<td>3.443±0.18</td>
</tr>
<tr>
<td>2 week continuous darkness Control (Group I††)</td>
<td>3.406±0.20</td>
</tr>
<tr>
<td>4 week continuous darkness Control (Group I†††)</td>
<td>3.360±0.18</td>
</tr>
<tr>
<td>6 week continuous darkness Control (Group I††††)</td>
<td>3.320±0.19</td>
</tr>
<tr>
<td>8 week continuous darkness Control (Group I†††††)</td>
<td>3.126±0.17</td>
</tr>
</tbody>
</table>

Results were expressed in mean ± SD of 5 rats.
* = P<0.01, ** = P<0.001

Histological observations:
The liver of the control groups showed normal histological structure; consists of epithelial, liver cells (hepatocytes); arranged into interconnected plates forming hepatic cords separated by vascular sinusoids, the hepatocytes extend radially from the central vein toward the periphery forming the hepatic cords (Figure 1).

In group II: no any clear abnormalities were noticed in the liver architecture, apart from a little infiltration of mononuclear inflammatory cells seen on the portal tract. Nuclear vacuolation was noticed and some cytoplasmic fat vacuoles (Figure 2).

In group III: The liver tissue showed swelling of a few hepatocytes, blurring of the septal-parenchymal junction with heavy infiltration of connective tissues with lymphocytes, macrophages, granulocytes. Apoptotic
Eleiwe et al, Histopathological Changes in Adult Male Rat

hepatocytes noticed concomitantly with large vacuolated cells. Acidophil bodies also noticed (Figure 3).

**Figure 3.** Liver tissues in male rat exposed to 4 week continuous darkness. Necrosis was seen blurring the septal-parenchymal junction, and obvious swelling of adjacent liver cell cytoplasm (double head arrow). Severe infiltrated with different types of inflammatory cells was illustrated in the connective tissue (one head arrow), and unusual ductular proliferation is seen. (H&E×250)

In group IV: There was nodular formation; consisting of aggregated hepatocytes, and the nodules were surrounded by thick bands of fibrous connective tissue, infiltrated with large number of mononuclear cells and macrophages. There was a variation in the size of hepatocytes and their nuclei, with moderate amount of fatty vacuoles within those hepatocytes. A large number of apoptotic cells in parenchyma and mononuclear cells infiltration both in septal and parenchymal constituents (Figure 4).

**Figure 4.** Liver tissues in male rat exposed to 6 week continuous darkness. Bands of fibrous connective tissue surrounding a population of hepatocytes forming nodular compartments of liver tissues. Connective tissue infiltration with mononuclear cells (arrow). There was discrepancy in the size of hepatic cells and their nuclei. Fatty change was present. (H&E. ×250)

In group V: Abnormal liver architecture was clearly seen, with wide fibrous septal bands, radiating from the portal tract to surround the lobules. Those lobules consisted mainly from hepatocytes with a large number of mononuclear cells. Prominent fatty vacuolation, was seen within the hepatocytes. Apoptotic hepatocytes seemed to be much more abundant in this group (Figure 5).

**Figure 5.** Liver tissues in male rat exposed to 8 weeks continuous darkness. A more apparent nodular organization of the hepatic tissue was viewed. The lobules were surrounded by short connective tissue bands (one head arrow). A clear fatty change was seen (double head arrow). (H&E ×250)

**Discussion**

The continuous darkness was well documented to increase the endogenous melatonin, the principle pineal neuro-hormone \(^{1,4,11}\), and the
Melatonin is known to cause no effect on body weight in rats \(^{12,13}\); this might be because melatonin administration does not affect food intake in rats, as it was noticed in our work. The increase in liver weight induced by continuous darkness which was proportionate with length time exposure to darkness, might be explained by the fact that melatonin can reach all body tissues and cells, acting through specific receptors in all body tissues \(^{14,15}\). Once melatonin reaches any bodily tissue it exerts its action immediately, and melatonin has a dose-dependent physiologic action \(^{16,17}\). In the present study no significant hepatomegaly occurred, on those animals kept for 2 weeks of continuous darkness, however there was hepatomegaly accompanied with longer period of darkness. These results could be discussed by the fact that melatonin has damaging effect only when executed on high level \(^{15-17}\). The hepatomegaly is essentially the consequence of hepatocyte death which could be of 2 types; the ballooning degeneration leading to massive increase in cell size or, apoptosis; this is also leads to hepatomegaly since these apoptotic cells are entrapped within a large gathering of inflammatory mononuclear cells leading to increasing volume of cellular parenchyma \(^{6}\). The rise in built and thickening of connective tissue bands also could contribute to that hepatomegaly. This large increment in septal thickness is due to the increase in production of fibro-collagenous tissue whenever there are any injurious events to the liver \(^{6,18}\). Also the melatonin has specific effect on fibroblast cells which are the active collagen - secreting cells and the basic forming cells of the connective tissues \(^{6,19}\). The other provider to hepatomegaly could be the dilated blood vessels, because melatonin has a well-known vasodilator action \(^{20,21}\).

The shrink and regression in size of liver was so clear at toxic effect of long dark period, that might be discussed by the fact that liver gets regression and shrink after any toxic damaging effect leading to fibrosis and scaring preventing the regenerating hepatocytes from expanding the parenchymal mass \(^{6,18}\). The blurring of the septal-parenchymal junction might be caused by the beginning of increased in connective tissue bulk as discussed previously. The swelling of hepatocytes is always seen in any destructive effect that results from the buildup of fat and water as well as proteins which are normally is exported \(^{6}\).

The heavy infiltration of connective tissues and parenchyma, with lymphocytes, macrophages granulocytes and other mononuclear cells, is due to the inflammatory process within the liver tissues, since these are the principal cells in any inflammatory process \(^{6,18}\). Apoptotic cells are seen in any programmed cell death, to replace the damaged cells by new healthy ones \(^{6,21}\). The large vacuolated hepatocyte is the form of cell that represents the intermediate type of liver injure, their cytoplasm seen filled with large and small vacuoles of fat, predominantly triglycerides, what is called steatosis, accordingly, these hepatocytes are named steatotic hepatocytes \(^{6}\). Acidophil bodies noticed at this work in fact, were the dead hepatocytes demonstrating current liver damage \(^{6,18}\).

In the last group the hepatic lobules that entrapped within thick bands of fibrous connective tissues were formed by almost normal hepatocytes. The enlightenment for that could be the fact that a new generation of hepatocytes, is formed in an attempt of the liver to substitute the dead hepatocytes \(^{6,17,18}\). The present study about the histopathological effect of continuous darkness on the liver both macroscopic and microscopic reveals no injurious effect on short period of continuous darkness, whilst it induced ultimately destructive effect with long period of continuous darkness.

The severity of destructive and damaging effects seemed to be correlated with the length of continuous darkness, which could be due to graduated amount of melatonin secretion, because the effect of melatonin is proportional to its level \(^{12,23-25}\). The time-course (30 days), for rats, was regarded as a long period according to
Peltier et al. (25) We conclude that hepatic tissues are affected by periods of darkness depending on the length of exposure.

References

Correspondence to Dr. Basim Sh Ahmed
E-mail: drbasim71@yahoo.com
Received 31st Oct. 2011: Accepted 18th Apr. 2012