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# Histomorphometric Study of Hepatocytes of Mice after Usimg Heroin

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**Abstract:** This study was aimed to determine the histomorphometric properties of hepatocytes mice (Balb/c) after using heroin. The factors studied include the size of hepatocytes, nucleus diameter of hepatocytes, size of binuclear hepatocytes and nucleus diameter of binuclear hepatocytes of controls and treated experimental animals. A total of 30 male mice were divided into three control and two treatment groups. At first, different experimental groups were addicted to heroin with the dosage of 50 mg $kg^{-1}$ IP for 3 days, twice daily and then were divided into two groups. One of them received heroin with a dose of 5 mg $kg^{-1}$ IP and the other 5 mg $mL^{-1}$ IP, twice daily for a period of 40 days. The results showed that the heroin could exert a significant effect on increasing the size of hepatocytes, size of binuclear hepatocytes, nucleus diameter of binuclear hepatocytes and nucleus diameter of hepatocytes in experimental groups.

**Key words:** Addicted, liver, diameter, nucleus

## INTRODUCTION

Heroin is one of the opioids that its effects on the various body organs (Fazelipour *et al*., 2006) such as liver, due to its detoxification function, has been studied by Pereska *et al*. (2007). The various researches have shown that liver hepatocytes show necrotic changes due to the effects of alcohol and the other toxins (Vento and Cainelli, 2002), also necrosis and death of the hepatocytes have been seen in addicts (Atici *et al*., 2005). Furthermore, change of liver cells, bile duct damage and then irregular regeneration of hepatocytes has been reported in the patients infected by hepatitis (Moriyama *et al*., 2000). Although hepatocytes derive from diploid hepatoblast progenitors, they often become binuclear and/or polyploid, only a minority remaining diploid in adult rat liver (Nadal and Zajdela, 1966). The amount of binuclear hepatocytes has been increased due to some drugs (Kostka *et al*., 1999). The increase in size of hepatocytes has been measured morphometrically. The chronic ethanol consumption results in hepatocellular hypertrophy and a significant increase in size of the hepatocytes. Furthermore, damage from toxic substances may cause swelling of hepatocytes, irregular clumped cytoplasm and large clear spaces. These are criteria of toxic liver (Robbins *et al*., 2004).

According to the changes of the liver hepatocytes due to using various poisons, it was decided to study the process of changes of liver hepatocytes (histomorphometrically) after using heroin.

## MATERIALS AND METHODS

A total number of 30 male adult mice (Balb/c) with average weight of 25-30 g were obtained from experimental animal center of Razi institute (Located in Hesarak/Karaj) and kept in special metal cages in animals laboratory house of Medical Faculty, Islamic Azad University in 2006. They were fed with prepared food (plate) and allowed access to clean tap water. The mice were divided in to 5 equal groups; 3 control (Intact, Sham I and Sham II) and 2 experimental groups (1, 2).

**Intact (n = 6):** Did not receive any drug.

**Sham I (n = 6):** Received normal saline by intra-peritoneal injection, 3 times daily for 3 days with the following pattern: In the 1st day with the dosage of 150 μL, the 2nd day with 20% increase in dosage than the first day and the 3rd day with 20% increase in dosage than the 2nd day. The last dose was then injected twice daily for 40 days period.

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**Sham II (n = 6):** Received normal saline and lemon juice by intra-peritoneal injection with the following pattern: In the first 3 days they were injected 3 times daily; with the dosage of 150 μL normal saline plus 0.4 μL lemon juice in the first day; the 2nd day with 20% increase in dosage than the first day and the 3rd day with 20% increase in dosage than the 2nd day. The last dose was then injected twice daily for 40 days period.

In order to addict the experimental groups, the homogenous solution was made.

For 1 mg heroin in 1 mL normal saline, 2.6 μL lemon juices were used and then they were mixed with ultrasonic mixer system.

At first, different experimental groups were addicted to heroin with the dosage of 50 mg $kg^{-1}$ IP for 3 days, twice daily (Homayoun *et al*., 2002) and then were divided into two groups.

**Experimental I (n = 6):** Heroin (5 mg $kg^{-1}$) was added to the solution of normal saline and lemon juice. The method of injection in this group was as the same as sham II group and continued to 40 days.

**Experimental II (n = 6):** Heroin (5 mg $mL^{-1}$) was added to the solution of normal saline and lemon juice. The method of injection in this group was as the same as sham II group and continued to 40 days.

After passing the mentioned period, livers were surgically removed from mice under anesthesia. Portions of liver were taken from the right lobe, washed with normal saline and fixed in 10% formalin. After embedding, 5 micron sections were prepared and stained with H and E (hematoxylin and eosin) in order to study histomorphometric changes of the hepatocytes under a light microscope. For this purpose, from the prepared sections of right lobe of the liver of each mouse, five sections which were equal in each group were randomly selected. Then, from each section, two visual field were photographed in 40 high power fields by stereomicroscope and the pictures were studied with computerized Microsoft system (Magens *et al*., 2005).

For histomorphometric studies of the hepatocytes in all groups, the size of hepatocytes, nucleus diameter of hepatocytes, size of binuclear hepatocytes and nucleus diameter of binuclear hepatocytes of controls and treated experimental animals were estimated. Furthermore the cytoplasms of hepatocytes were investigated.

## RESULTS AND DISCUSSION

The statistical result from the histomorphometric study of the kinds of hepatocytes in the different groups revealed that there was no significant difference in the size of hepatocyte and binuclear hepatocyte among control groups, (Fig. 1-3) but there was significant difference in control groups in comparison with experimental groups 1, 2. (Fig. 4, 5) (One-way ANOVA; $p<0.05$, Table 1). Furthermore no significant difference was seen in the nucleus diameter of binuclear hepatocytes among control groups, but there was significant difference in control groups in comparison with experimental groups 1, 2 (One-way ANOVA; $p<0.05$, Table 1). At last, there was no significant difference in the nucleus diameter of hepatocytes among control groups, but there was significant difference in control groups in comparison with experimental groups 1, 2 (One-way ANOVA; $p<0.05$, Table 1). Study of the process of histomorphometric changes of the hepatocytes was another purpose of this study.

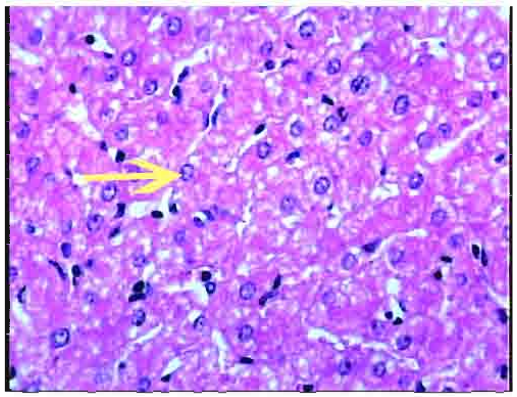

Fig. 1: A transverse section through the hepatocytes liver of mice H and E stain. The section shows hepatocytes nucleus (arrow) in intact group. Magnification x400

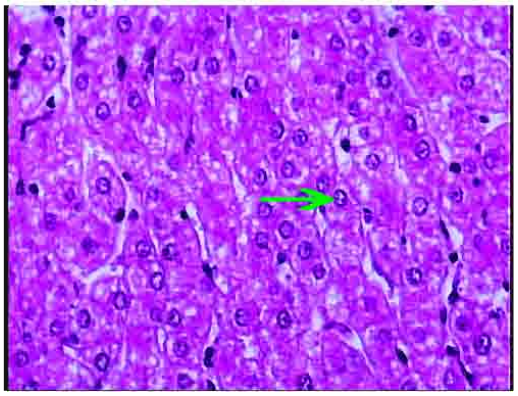

Fig. 2: A transverse section through the hepatocytes liver of mice H and E stain. The section shows hepatocytes nucleus (arrow) in sham I group. Magnification x400

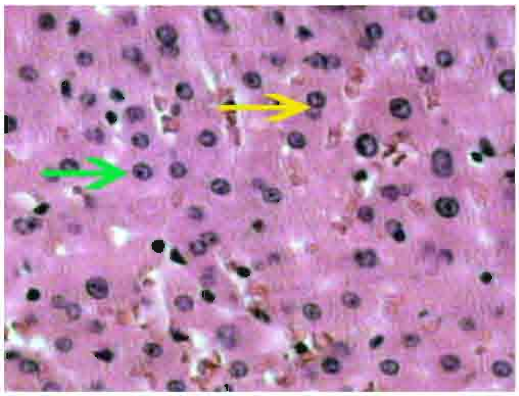

Fig. 3: A transverse section through the hepatocytes liver of mice H and E stain. The section shows hepatocytes nucleus (lower arrow) and binucleus hepatocytes (upper arrow) in sham II group. Magnification x400

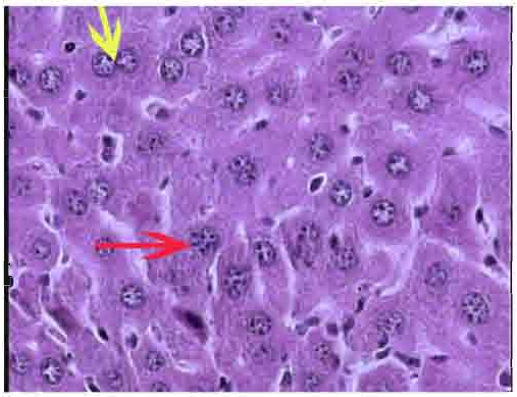

Fig. 4: A transverse section through the hepatocytes liver of mice H and E stain. Big nucleus hepatocytes (lower arrow) and big binucleus hepatocytes (upper arrow) in experimental 1 group. Magnification x400

The size of hepatocytes in experimental groups has been increased 2-3.5 times more than control groups. The size of binuclear hepatocytes in experimental groups has been increased 2-3 times more than control groups. The nucleus diameter of binuclear hepatocytes in experimental groups has been increased 1.8-2.5 times more than control groups. The nucleus diameter of in experimental groups has been increased 2-3.2 times more than control groups (Table 2).

Vacuolization of the cytoplasm of hepatocytes and their uniformal distribution to 92% in experimental group 2 and to 25% in experimental group I in comparison to the control groups were another result of this study (Fig. 6).

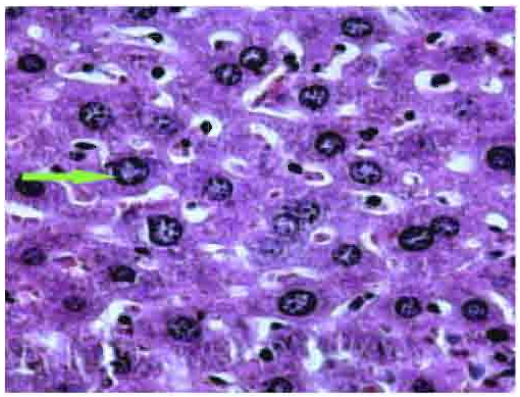

Fig. 5: A transverse section through the hepatocytes liver of mice H and E stain. Big nucleus hepatocytes (arrow) in experimental 2 group. Magnification x400

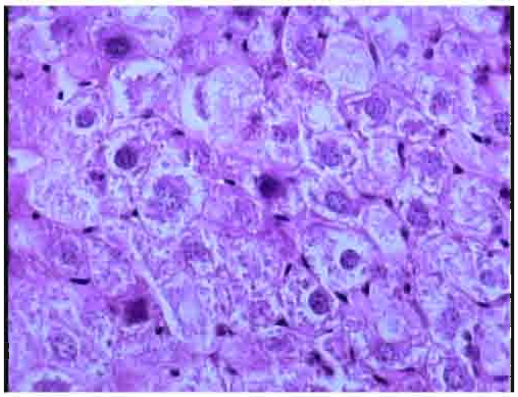

Fig. 6: A transverse section through the hepatocytes liver of mice H and E stain. Vacuolization of the cytoplasm of hepatocytes and their uniformly distribution in experimental groups. Magnification x400

The results from the study of the effects of heroin on the histomorphometric structure of mice liver (Balb/c) showed that the size of hepatocytes in experimental groups is significantly increased than the control groups. These results are as the same as the effects of alcohol on hepatocytes and their hypertrophy as reported by the other researchers (Vento and Cainelli, 2002). Big size and eu-cromatin nucleus of the hepatocytes were another result from this study which may be the result of hyperactivity of the hepatocytes due to necrosis and damage. With regard to this subject, the other scientists have reported that morphine can make such changes in hepatocytes (Bogomolova, 2003). In addition to the changes of the other parts of the body, pancreatic cells sclerosis has been reported in histomorphometric studies

Table 1: Effect of heroin on the size of hepatocytes, binuclear hepatocytes and nucleus diameter of hepatocytes, nucleus diameter of binuclear hepatocytes in intact

| | Groups | | | | |
|---|---|---|---|---|---|
| Data (μm) | Intact | Sham I | Sham II | Experimental 1 | Experimental 2 |
| The size of hepatocytes | 137.36±17.79a | 137.46±13.46a | 104.93±17.06a | 357.48±65.36b | 320.13±7.58b |
| The size of binuclear hepatocytes | 137.00±18.66a | 132.66±26.46A | 111.65± 61.70a | 284.26±162.33b | 327.43±94.24b |
| The nucleus diameter of binuclear hepatocytes | 0.66±1.47a | 3.76±1.45A | 3.35±1.80a | 6.80±3.48b | 8.20±1.24b |
| The nucleus diameter of hepatocytes | 3.70±1.02a | 4.86±0.60A | 4.66±0.65a | 11.87±1.23b | 10.79±1.72a |

Sham I and sham II (did not receive any heroin) and experimental I and experimental II (received heroin 5 mg $kg^{-1}$, 5 mg $mL^{-1}$, i.p.) in mice. (Mean±SE, n = 6, unequal letter(s) in each data indicate significant difference at the level of $p<0.05$)

Table 2: Effect of heroin on ratio the size of hepatocytes, binuclear hepatocytes and nucleus diameter of hepatocytes

| | Groups | | | | | |
|---|---|---|---|---|---|---|
| | Experiment 1 | | | Experiment 2 | | |
| Parameters | Intact | Sham I | Sham II | Intact | Sham I | Sham II |
| Ratio the size of hepatocytes | 2.60 | 2.60 | 3.40 | 2.32 | 2.33 | 3.05 |
| Ratio the size of binuclear hepatocytes | 2.07 | 2.14 | 2.54 | 2.39. | 2.46 | 2.93 |
| Ratio the nucleus diameter of binuclear hepatocytes (E) | 2.06 | 1.80 | 2.02 | 2.48 | 2.18 | 2.44 |
| Ratio the nucleus diameter of hepatocytes | 3.20 | 2.44 | 2.54 | 2.91 | 2.22 | 2.31 |

Nucleus diameter of binuclear hepatocytes in experimental I and experimental II (received heroin 5 mg $kg^{-1}$ 5 mg $mL^{-1}$, i.p.) to intact, sham I and sham II (did not receive any heroin) in mice

due to alcohol and in opium addicts (Bogomolova, 2003). increasing their nucleus diameter were also observed in present study. This shows that some of the drugs can induce such changes in hepatocytes (Kostka *et al*., 1999). Also, vacuolization of cytoplasm of the hepatocytes and their umformal distribution in experimental groups in comparison to control groups may be due to toxic effect of heroin on liver tissue. With regard to this subject, the other researchers have reported vacuolization of hepatocytes. It is the clue of degeneration in the fetus which its liver has received toxins (Saleki *et al*., 2007). The results of this study showed that heroin may make some changes such as increasing in the size of hepatocytes, the nucleus of liver cells and vacuolization of their cytoplasm, which can be one of the reasons of degeneration of hepatocytes due to toxic substances. So, it is suggested that for follow-up of the patients with liver problems, heroin may be considered as an active parameter in changing the hepatocytes.

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