Vol. 3, No. 1: 30-35 (2015).
ISSN: 2313-3937

WORLD JOURNAL OF EXPERIMENTAL BIOSCIENCES

Research article

Histological study in liver of albino mice post exposing to shisha smoke

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ABSTRACT

The effect of shisha smoking on liver parenchyma tissues were evaluated in (120) male adult mice. The animals were divided into four groups according to kind of exposed smoke (cigarette, shisha and mixture of cigarette and shisha). The mice exposed to fresh air considered as control group. A special inhalation chamber designed locally was used to expose the animals to different kinds of smokes. Exposure to cigarette smoke was done for 5 min/day, while exposure to shisha smoke was done for 15 min /day for 4, 8, 12 weeks. The tissues of control and exposed groups were processed for histological study. The results showed different histological changes such as hepatocytes degeneration, congestion, sinusoid dilation and infiltration of inflammatory cells in liver parenchyma tissues. These changes observed clearly when the period of exposure increased especially in groups exposed to shisha smoke and shisha plus cigarette smoke for 12 weeks. The results showed that shisha smoke causes mild damage in liver parenchyma tissue and this damage increased concomitantly with the increase of duration of exposure.

Keywords: Histological effects, Liver, Mice, Shisha smoke.

Citation: Al-Easawi NAF, Al-Azzawi MNA. (2015) Histological study in liver of albino mice post exposing to shisha smoke. *World J Exp Biosci* **3:** 30-35.

Received March 28, 2015; Accepted April 18, 2015; Published April 27, 2015.

INTRODUCTION

Shisha (water-pipe) smoking is method of tobacco consumption in many parts of the world. It has been claimed that more than 100 million people worldwide smoke shisha daily [1]. Recently, shisha cafes and water pipe tobacco smoking have been growing in popularity in the Iraq. Shisha smoking has become more accepted and widely used among young smokers, especially in university and high school students. This increasing of shisha use due to the misconception that shisha smoking is less harmful and less addictive than cigarettes [2-4]. Beside the hazardous effects on respiratory tract; smoking causes a variety of adverse effects on organs that have no direct contact with the smoke itself such as liver. Liver is an important organ

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that has many tasks. The liver is responsible for processing drugs, alcohol and other toxins to remove them from the body.

Liver as body's natural filter has responsibility to prevent harmful toxins from entering the bloodstream. In addition, it helps the body metabolize sugars and carbohydrates, and regulates the flow of bile. The substance aids in the digestion process. Humans cannot survive without a liver and when the organ is agitated, the body's immune system weakens and becomes more susceptible to disease and infection. Several study focused on the role of immune system and immune cells in pathogenicity of liver [5-7]. Heavy smoking yields toxins, which induce inflammation and increase the severity of hepatic lesions (fibrosis and activity scores). Tobacco smoking increases the risk of developing hepatocellular carcinoma (HCC) among chronic liver disease (CLD) patients [8].

Long-term exposure to tobacco smoke causes permanent inflammation. Nicotine, a major constituent of tobacco smoke, is mainly metabolized in the liver, and induces lesions characterized by focal or confluent necrosis, and varying degrees of fibrosis [9].

Watanabe et al. [10] observed that the antioxidant activity is reduced during exposure to cigarette smoke, facility-ating more hepatotoxic damage to the organ. The enzymes activities were reduced in animals exposed to smoke from cigarette; this shows that the degree of oxidative stress resulting from the other components of cigarette smoke, especially nicotine was affects on health more than that caused exclusively by carbon monoxide, which is also a constituent of cigarette smoke. The changes that occurred in the liver enzymes and anti-oxidants corroborated the histological observations of the liver that showed various degrees of effects on hepatocytes and canalicular network. This concept was evaluated clearly in current study.

MATERIAL AND METHOD

The experiments carried out on 120 healthy mature male albino mice (Mus musculus). The study was conducted following approval from the animal ethics committee of department of biology, University of Baghdad, Baghdad, Iraq. The age of mice was ranged from 7 to 8 weeks and weighing 21 to 29 grams. Mice were divided randomly into 12 groups; each group includes 10 animals for the exposed and control groups. According to inhalation exposure periods 12 groups of animals were divided into three groups each of them has four subgroups **table 1.**

Local made glass chamber of 80Lt. capacity was used for mice inhalation. The chamber designed according to the WHO specifications [11, 12]. Animal groups were exposed to shisha smoke and cigarette smoke according to the following criteria. The Subgroup (S) and subgroup (C+S) that belongs to group (1) were exposed to shisha smoke for 15 min/day. Exposure was done by burning 10 gm of Moassel using ordinary charcoal put in shisha head. Electronic pumps was switched on, the smoke generated transported through the parts of shisha and fed the inhalation chamber. The one puff of smoke is almost equal to 10 L in each operation cycle. After the end of exposed each of subgroups (S) and (C+S) were removed from inhalation exposure chamber and replaced with subgroup(C) in order to started to exposed to cigarette smoke .

Table 1. Groups of animals exposed to fresh air (F), Shisha smoke (S) and cigarette smoke (C).

Group No.	Exposure period in weeks	Number of animals in each subgroups according to the type of exposure			
		Control (F)	Shisha smoke (S)	Cigarette smoke(C)	Shisha and cigarette smoke(S+C)
1	4	10	10	10	10
2	8	10	10	10	10
3	12	10	10	10	10

The Cigarette smoking experiment was performed, seven days a week. The Subgroup (C) and subgroup (C+S) that belongs to group (1) were exposed to the smoke of 5 cigarettes for 5-7 min for each once [13]. The main and side stream smoke was generated by burning cigarettes in a smoking box. The cigarettes were lightened and left to glow then the produced smoke was drawn into the chamber as puff where the mice placed inside. While the subgroup (F) control group were exposed to fresh air only.

The groups 2 and 3 followed the same inhalation exposure procedures of group 1, but for 8 and 12 weeks, respectively. The operation in each smoking cycle lasts for 50 seconds and represents three steps:

1. Shisha or cigarette smoke is drawn through the inhalation chamber continuously for 10 sec.
2. The vacuum pump was turned off for 30 seconds, and mice allowed breathing shisha or cigarette smoke.
3. The vacuum pump will be turned on for the last 10 sec and smoke was exhausted to outside the inhalation chamber.

Animals were scarified after the end of exposure period by cervical dislocation. Liver was removed aseptically. It was washed well with normal saline (0.9%Nacl) to remove blood and kept immediately in 10 % formalin for study of the histological changes.

The tissues of control and exposed groups (test groups) were processed for histological sectioning according to the method that described previously [14]. The sections

of liver were examined using a light microscope. Two slides were chosen and one section from each slide was examined. The histopathological changes in liver were photographed by digital camera (Canon DSLR).

RESULTS AND DISCUSSION

Fig. 1 shows the normal structure of liver, which contains several hepatic lobules. The center of lobule represents the central vein, each lobule separated from other by thin connective tissue (septa), which contains portal area. The portal area includes the hepatic artery, portal vain and bile duct. The hepatic lobule contains hepatic cells (hepatocytes) which arranged as cords radically surrounds the central vein and these cords separated from each other by vascular canal (sinusoids). The sinusoids house an important part of livers that represents the defense system composed of fixed macrophage (kupffer cells).

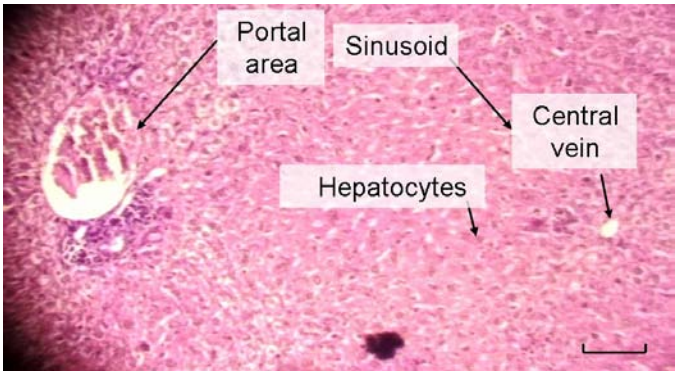

Fig. 1 cross section in liver showing normal structure in control mice exposed to normal air (bar, 150 µm).

Fig 2 represents the hepatic tissue changes in mice exposed to cigarette smoke for 4 weeks that showing normal hepatocytes with congestion in central vein. **Fig. 3** shows normal arrangement of hepatocytes with slight dilation of sinusoids in mice exposed to shisha smoke.

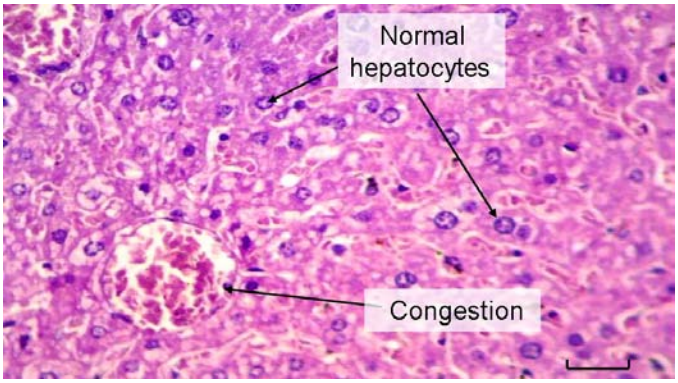

Fig. 2 Cross section in liver showing normal hepatocytes with congestion in central vein in mice exposed to cigarette smoke for 4 weeks (bar, 75 µm).

Fig. 4 shows focal degenerative and necrosis in hepatocytes with inflammatory cells infiltration near central congested vein in hepatic tissue from mice exposed to cigarette plus shish smoke.

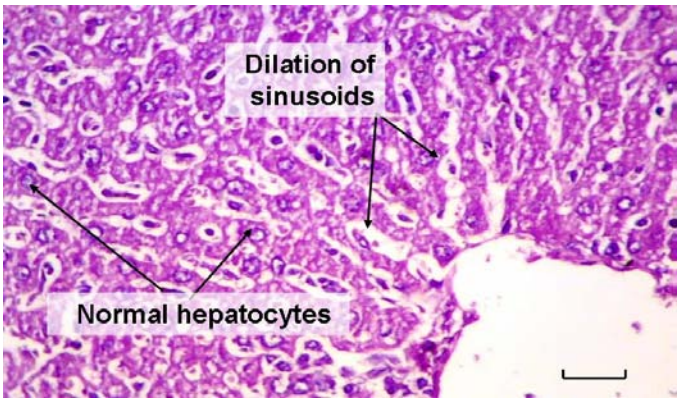

Fig. 3 Cross section in liver of mice exposed to shisha smoke for 4 weeks. Normal hepatocytes with dilation in sinusoids were seen (bar, 100 µm).

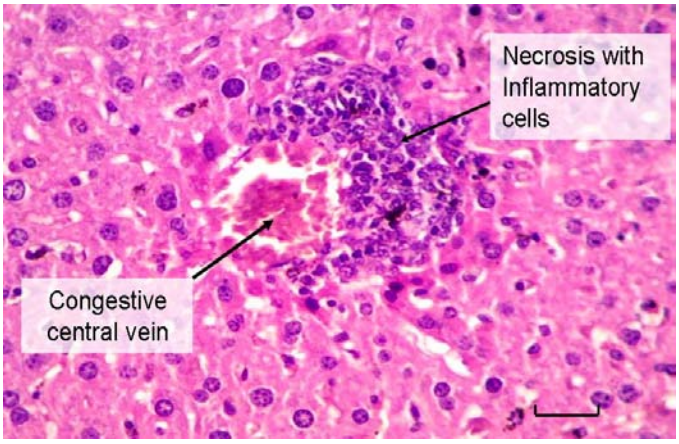

Fig. 4 Cross section in liver post exposing to cigarette plus shisha smoke for 4 weeks. Necrosis, inflammatory cells infiltration and central vein congestion were observed (bar, 65 µm)

The increasing in the duration of exposing to different smokes for 8 weeks resulted different changes. The hepatic histological changes were observed in all exposed groups. **Fig 5** shows the hepatic tissue obtained from mice exposed to cigarette smoke, mild degenerative changes of hepatocytes especially near the portal area with infiltration of inflammatory cells were seen. **Fig 6** showed the histological changes in mice liver post exposing to shisha smoke, the mild degenerative changes of hepatocytes appears in this group as compared with previous one with dilation of central congested vein. **Fig 7** shows also mild degenerative and necrosis of hepatocytes cells with widening of sinusoids and increase of kupffer cells. These changes appear in hepatic tissue section obtained from mice exposed to cigarette plus shisha smoke.

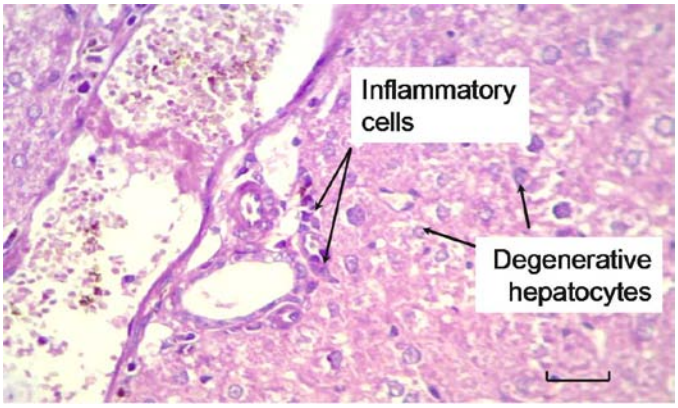

Fig. 5 Cross section in liver post exposing to cigarette smoke for 8 weeks. Inflammatory cells near portal area and mild degenerative hepatocytes were observed (bar, 100 µm).

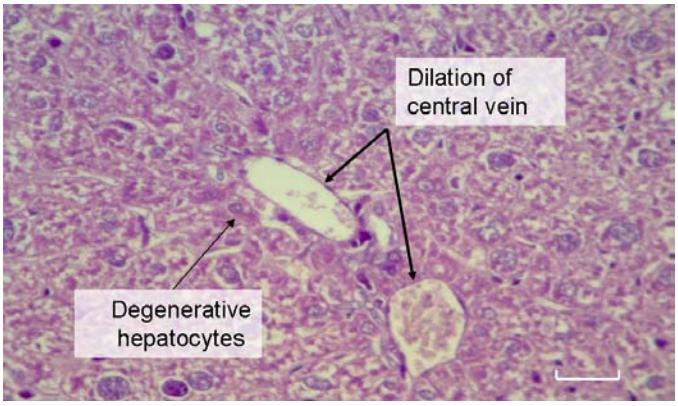

Fig. 6 Cross section in liver post exposing to shisha smoke for 8 weeks. Degenerative hepatocytes with dilation of central vein were seen (bar, 100 µm).

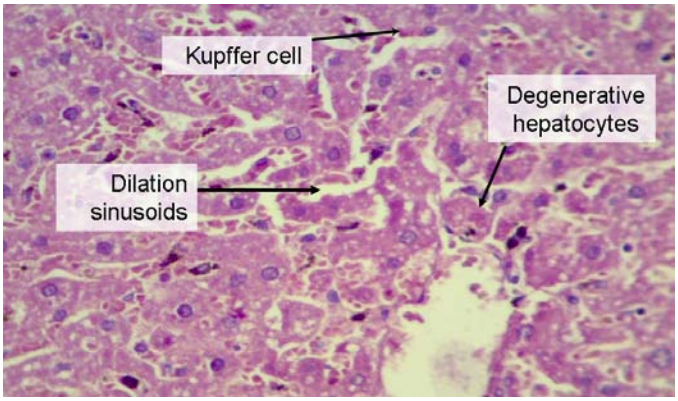

Fig. 7 Cross section in liver showing mild degenerative, necrosis hepatocytes with dilation of sinusoids in mice exposed to cigarette plus shisha smoke for 8 weeks.

The exposing animal to different types of smoke for 12 weeks produced more hepatic tissue changes. In section of liver tissue, which obtained from exposed to cigarette smoke, mild infiltration of inflammatory cells especially near portal area with congestion in sinusoids were seen (**Fig 8**). **Fig 9** showing the hepatic tissue changing post exposing to shisha smoke, focal degenerative and necrosis in hepatocytes cells with inflammatory cells infiltration especially near portal area were seen. **Fig 10** represents hepatic tissue obtained from mice exposed to cigarette plus shisha smoke, dispersed isolated hepatocytes apoptosis with widening of sinusoids with congestion especially around portal area were observed.

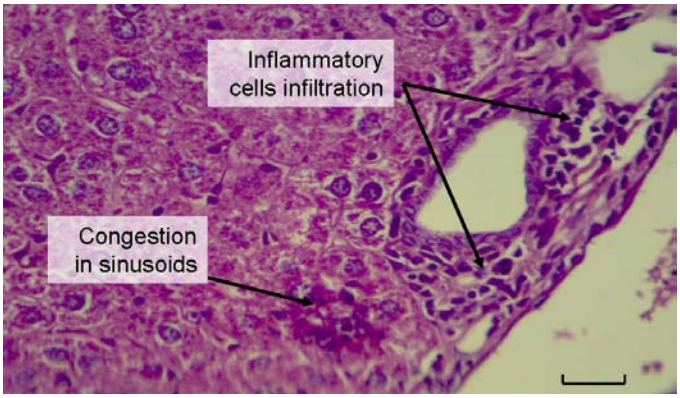

Fig. 8 Cross section in liver post exposing to cigarette smoke for 12 weeks. Inflammatory cells with sinusoids congestion were observed (bar, 85 µm).

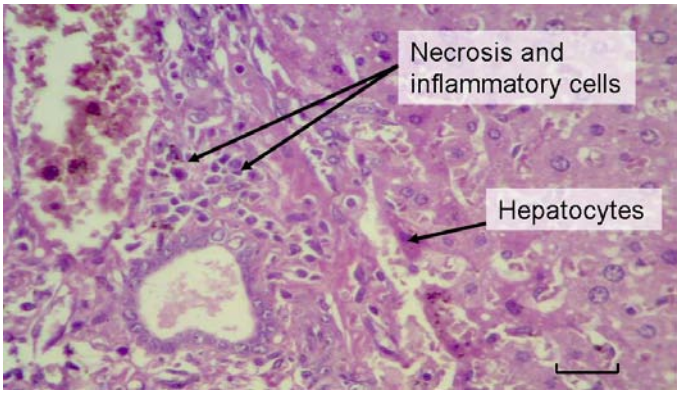

Fig. 9 Cross section in liver after exposing to shisha smoke for 12 weeks. Area of necrosis and inflammatory cells around portal area were observed (bar, 100 µm).

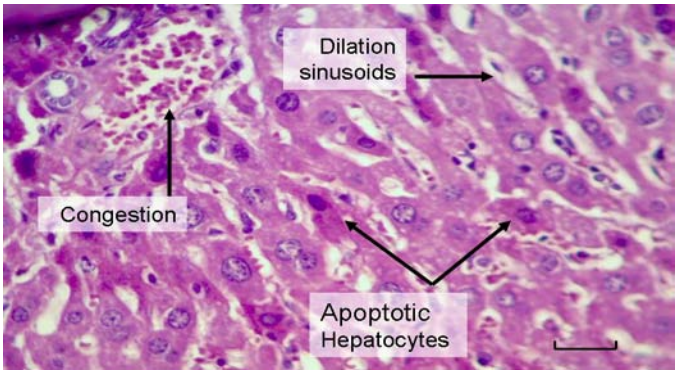

Fig. 10 Cross section in liver post exposing to cigarette plus shiahs smokes for 12 weeks. The hepatocytes apoptosis, dilation in sinusoids and congestion around portal area were seen (bar, 75 µm).

Previous study reported that there are two toxic effects of tobacco smoking on the liver either direct or indirect [15]. Direct toxic effect of smoking belongs to chemical substances with cytotoxic potentials. These chemicals created by smoking induce oxidative stress associated with lipid peroxidation this leads to activation of satellite cells and development of fibrosis. In addition, smoking increases the production of pro-inflammatory cytokines and causes liver cell injury. While, the Indirect toxic effects reveal that heavy smoking is associated with increased carboxy haemoglobin and decreased oxygen carrying capacity of red blood cells (RBCs) leading to tissue hypoxia. Hypoxia stimulates erythrop-oietin production which induces hyperplasia of the bone marrow. The latter contributes to the development of secondary polycythemia and in turn to increase red cell mass and turnover. This increases catabolic iron derived from both senescent red blood cells and iron derived from increased destruction of red cells associated with polycythemia. Furthermore, erythr-opoietin stimulates absorption of iron from the intestine. Both excess catabolic iron and increased iron absorption ultimately lead to its accumulation in macrophages and subsequently in hepatocytes over time, promoting oxidative stress of hepatocytes.

In current study, the histological examination of liver tissue showed that no changes took place in hepatocytes for exposure period of 4 weeks and for all groups. By increasing the exposure period from 8 to 12 weeks, damages in hepatocytes appeared in all animal groups, which were exposed to shisha and cigarette smoke.

Blood congestions appeared in the groups that exposed to smokes for 12 weeks. Infiltration of inflammatory cells was observed in most groups. The infiltration of inflame-matory cells was seen clearly in the group of animals that exposed to shisha and cigarette smoke for 12 weeks. Other damages in liver tissue such as dilation and congestion of sinusoids, dilation of central vein, and the appearance of kupffer cells slightly in the different test groups.

The results agree with previous, they showed that male rats were exposed to smoke from a completely burnt 0.74 g leaf of Tobacco nicotiana for 5 minutes three times daily. The duration of investigation was 5 days. Sections of the liver obtained from the treatment group include disruption of parenchyma tissue with degenerative of the hepatocytes. The hepatocytes are the main functional cells of the liver. It is known that the hepatocytes play a vital role in the proper functioning of the liver. A compromise in the integrity of the hepato-cytes could lead to improper functioning of the liver.

The results of current study agreed with study of Omotoso et al. [9] found that the degeneration of the liver parenchyma and disruption of the canalicular network after exposing the animals exposed with cigarette smoke, the hepatocytes nuclei were reduced in their size, with more vacuolar spaces in the cytoplasm and areas of necrosis, as well as neutrophilic infiltrates. Dhouib et al., [16] gave evidence of hepatic damage caused by chronic nicotine exposure and showed that long-term nicotine treatment changed the histological parameters in liver tissue such as loss of trabecular arrangement, congestion of centrilobular veins and sinusoids, and moderate infiltration of lymphocytes. These histological changes may be results from promoted oxidative stress and increased lipid peroxidation.

Shisha smoke containing high levels of nicotine, tar, respirable suspended particulates (RSP) and heavy metals [17]. Omotoso et al., [9] referred that nicotine could probably be responsible for the enhancement of lipid peroxidation, which is a part of the mechanism responsible for the tissue damage. Dhouib et al. [16] found that, nicotine-treated rats exhibited significantly increases haematocrit and hemoglobin and these hematological changes were supported by histopath-ological results which revealed congested blood vessels. Jankeer and El-Nouri [18] found that lead is one of the common toxic metals in tobacco smoke and it is absorbed from gastrointestinal tract, bounds to erythrocytes and widely distributed initially to soft tissues such as liver, kidney and brain and caused the histological effects in the liver such as necrosis of the parenchyma of hepatic lobule and a loss of normal architecture of the hepatocytes. This observation agreed with current study and hepatocytes necrosis that seen in histological sections due to accumulation of heavy metals such as lead. The current study concludes that inhalation of shisha smoke not different from cigarette smoke and causes mild histological changes in liver but these changes increased by increasing the exposure periods.

Conflict of interest

The authors declare that they have no conflict of interests.

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