Effect of Hypoxia on the Hepatic Tissue of Rat: Histological and Histochemical Studies

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Abstract: Hypoxia has been shown to have a role in the pathogenesis of several forms of liver disease. The current study aimed to investigate the histopathological alterations in the hepatic tissue of rats subjected to hypobaric hypoxia. Animals were divided into 4 groups. The first group represented intact controls. Rats of the second, third and forth groups were exposed to hypobaric chamber simulating 5000 m high altitude. Animals of the second and third group were sacrificed by decapitation after 2 and 4 weeks respectively. The fourth group was kept for recovery after 4 weeks exposure to hypobaric atmosphere. Results showed that hypoxia induced a degeneration of hepatic cells with vacuolation of cytoplasm associated with congestion. Recovery group for 2 weeks showed gradual regaining of normal hepatic pattern. Histochemical investigations showed that, hypoxia was able to decrease the hepatic tissue reaction of carbohydrate and succinyl dehydrogenase. These tissue injuries were improved after recovery. Collectively, the hepatic tissue of rats exhibited a severe damage during exposure to hypoxia and further studies are required to understand the mechanism of hypoxia.
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1. Introduction

Hypoxia, is a pathological condition in which the body as a whole or a region of the body is deprived of adequate oxygen supply. More than 140 million people worldwide live at altitudes above 3000m today (Pasha and Newman 2010). Furthermore, every year several hundreds of thousands of people from lowland areas move to higher altitudes for work and travel (Liao et al., 2010). But high altitude hypoxia is a challenge for people residing in or visiting high altitudes. Among those physiological effects of hypoxia is the liver injury (Cannito et al., 2013).

The liver is the largest metabolic organ in the body (Munding and Tannapfel, 2011). It performs a number of important and complex biological functions that are essential for survival. It also plays important roles in metabolism of carbohydrates, proteins, lipids, drugs, as well as in bile formation and secretion (Schugar et al., 2013). Hypoxic liver injury (HLI) is known as hypoxic hepatitis, is due to inadequate oxygen uptake by the centrilobular hepatocytes resulting in necrosis (Melgar-Lesmes et al., 2011).

Hypoxic liver injury has been described in patients who are markedly hypoxemic due to chronic respiratory failure or sleep apnea syndrome (Mathurin et al., 1995; Henrion et al., 1999). In the congested liver, hypoxic injury is precipitated by an acute event, whereas in patients with chronic respiratory failure, HLI is associated with progressive hypoxemia without an acute process (Henrion et al., 1999).

The current study aimed to investigate the histopathological alterations in the hepatic tissue of rats subjected to **hypobaric** hypoxia.

2. Material and Methods

2.1. Animals

Forty adult male albino rats weighing 150-170 g were used in this study. Animals were kept in wire bottomed cages in a room under standard condition of illumination with a 12-hours light-dark cycle at 25±1°C. They were provided with water and balanced diet *ad libitum*. The experiments were approved by the state authorities and followed Egyptian rules on animal protection.

2.2. Experimental design

Animals were divided into 4 groups. The first group represented intact controls. Rats of the second, third and forth groups were exposed to hypobaric chamber simulating 5000 m high altitude. Animals of the second and third group were sacrificed by decapitation after 2 and 4 weeks respectively. The fourth group was kept for recovery after 4 weeks exposure to hypobaric atmosphere.

2.3. Histology and histochemistry

Small pieces of the liver were quickly removed, then fixed in Carnoy's fixative fluid. Following fixation, specimens were dehydrated, embedded, and then sectioned to five microns thickness. For histological examinations, sections were stained with Ehrlich haematoxylin and eosin (Drury and

Wallington, 1980). Histological damages were scored as follows: 0: abscent; +: mild; ++; moderate; and +++: severe.

Masson's trichrome was used for demonstration of connective tissue. Sections were stained with periodic acid-Schiff's method to demonstrate total carbohydrates (Hotchkiss, 1948).

Freshly frozen specimens were cut in a cryostat operating at -20 °C. Sections of the liver were stained for succinate dehydrogenase (SDH), which is a commonly used marker enzyme for oxidative capacity (Nystrom, 1968). Many slides have been carefully examined for each animal (each group contained 6 animals and for each animal at least 3 slides from different areas of the organ were examined.

3. Results

The hepatic tissue in control group showed normal liver architecture (Fig. 1A) and normal distribution of the connective tissue (Fig. 2A). Exposed group for 2 weeks hypoxia showed degeneration of hepatic cells with vacuolation of cytoplasm associated with congestion. There is periatrial and portal monocytic infiltration (Fig. 1B) and collagenous fibres degeneration (Fig. 2B). Progressive degenerative changes in a form of cytoplasmic vacuolation, loss of architecture and monocytic infiltration (Fig. 1C) were detected after 4 weeks exposure to hypoxia. Collagenous fibres progressively arranged within the portal area (Fig. 2C). Recovery group for 2 weeks showed gradual regaining of normal hepatic pattern, hyaline degeneration, pachy necrosis (Fig. 1D) and regaining of normal collagenous fibres distribution (Fig. 2D). All the histopathological alterations are considered in the histological score (Table 1).

The histochemical study revealed that the hepatic tissue of the control animals showed normal PAS positive material (Fig. 3A) and normal tissue reaction with succinyl dehydrogenase (Fig. 4A). Exposed group for 2 weeks hypoxia showed slight decrease of PAS positive material (Fig. 3B) and slight decrease in tissue reaction with succinyl dehydrogenase (Fig. 4B). After 4 weeks exposure to hypoxia, there was a progressive decrease of PAS positive material (Fig. 3C) and marked decrease in tissue reaction by succinyl dehydrogenase (Fig.4C). Recovery group for 2 weeks showed more or less regaining of PAS positive material (Fig. 3D) and gradual regaining of tissue reaction with succinyl dehydrogenase (Fig. 4D).

4. Discussions

It is known to all that oxygen is essential for cell living, and hypoxia will lead to cell dysfunction, or even death. The liver is a highly aerobic organ whose metabolism and viability depend on the availability of oxygen. Oxygen consumption of the liver is 100 to 150 μmol O_2 per hour per gram of wet weight (Lemasters 2001). The hepatic artery and the portal vein together deliver blood to the liver. These vessels furnish about 25% and 75% of blood flow, respectively, although flow rates vary physiologically, particularly in response to digestive activity.

Hypoxic liver injury may occur with obstruction of either the portal vein or the hepatic artery, depending on several factors: blood flow in the other vessel, collateral vessel formation, and the ability of the liver to increase oxygen extraction to compensate for the decrease in perfusion. Ischemia followed by reperfusion of tissue results in oxygen-dependent generation of superoxide free radicals and other mediators that induce a neutrophilic response and tissue damage (Elias- Miró et al., 2013.

Cytoplasmic vacuolation is mainly a consequence of considerable disturbance in lipid inclusions and fat metabolism occurring during pathological changes (Ebaid et al., 2007). Also, vacuolar degeneration has been regarded by Durham et al. (1990) to be an alteration produced to collect the injurious substances in the cells. In this study, the vacuolation of the cytoplasm of the liver cells appeared at first in the hepatocytes of the peripheral zone of the hepatic lobules, extending gradually toward the center. This may be due to the direction of the lobular blood supply.

Results also showed a remarkable cellular infiltration in the hepatic tissue. This is due to the abundance of leucocytes, in general, and lymphocytes, in particular, are a prominentresponse of body tissues facing any injurious impacts.

Regarding the histochemical changes observed in this study under hypoxia, results clearly indicated reduction in the polysaccharides and succinyl dehydrogenase in the hepatic tissue. These changes were consistent with those induced histopathologically in the hypoxia subjected group. The decrease in carbohydrate content was attributed by some investigators to be due to increased stress on organs, leading to high energy consumption which allowed an equalized pressure to be exerted upon them (Gracey et al., 2011). Moreover, Pickett et al. (1979) support our results with the reduction in succinyl dehydrogenase in the hepatic tissue.

Collectively, the hepatic tissue of rats exhibited a severe damage during exposure to hypoxia and further studies are required to understand the mechanism of hypoxia.

Table 1. Histopathological changes in hepatic tissue of mice subjected to hypobaric hypoxia.
0 : Abscent, + : Mild, ++ : Moderate and +++ : Severe

Group	Microscopic Observation					
	Haemorrhage	Disorganized Sinusoids	Lymphocytic Infiltration		Kuppfer cell hyperplasia	Hepatocytic vacuolation
			Central	Intrlobular		
Group 1	0	0	0	0	0	0
Group 2	++	++	+++	+++	++	+
Group 3	++	++	++	+++	+++	+
Group 4	+	+	+	+	+	+++

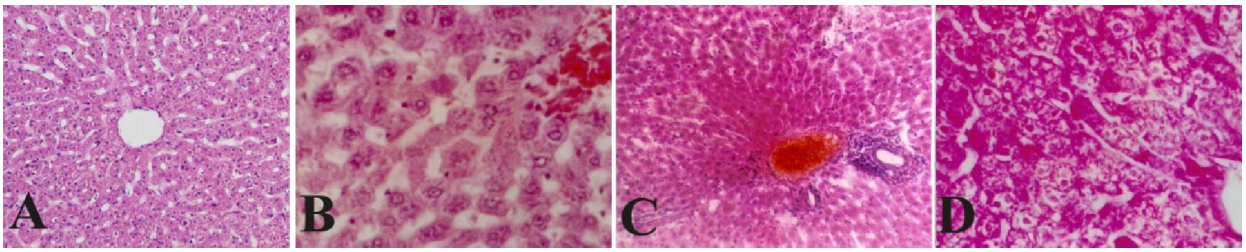

Figure 1. Hypoxia induced alterations in hepatic tissue archeticture. (A), Control liver section with normal structure. (B, C), Liver section of hypoxia subjected groups for 2 and 4 weeks, respectively. Sections appeared with severe damage. (D), Sections from recovery group for 2 weeks with partial improvement of the hepatic architecture. Sections stained with hematoxylin and eosin. Magnifications, X 250.

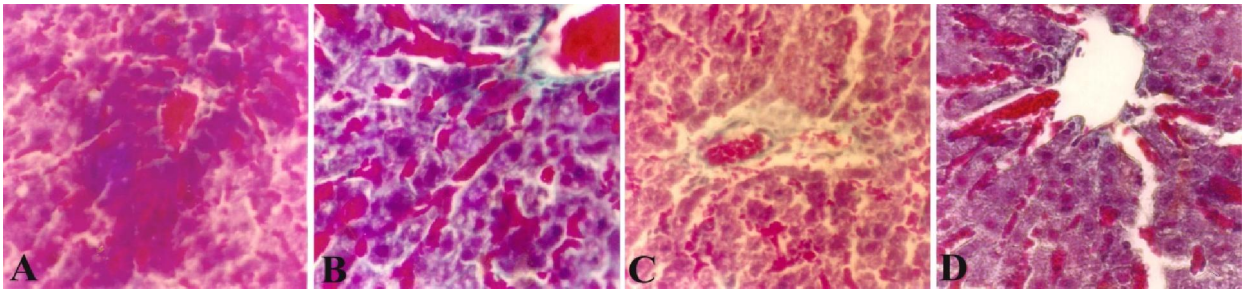

Figure 2. Connective tissue elements in liver sections. (A), Control liver section with normal distribution of connective tissue. (B, C), Liver section of hypoxia subjected groups for 2 and 4 weeks, respectively. Sections appeared with degeneration of collagenous fibres. (D), Sections from recovery group for 2 weeks with normal distribution of collagenous fibres. Sections stained with Masson Trichrom stain. Magnifications, X 250.

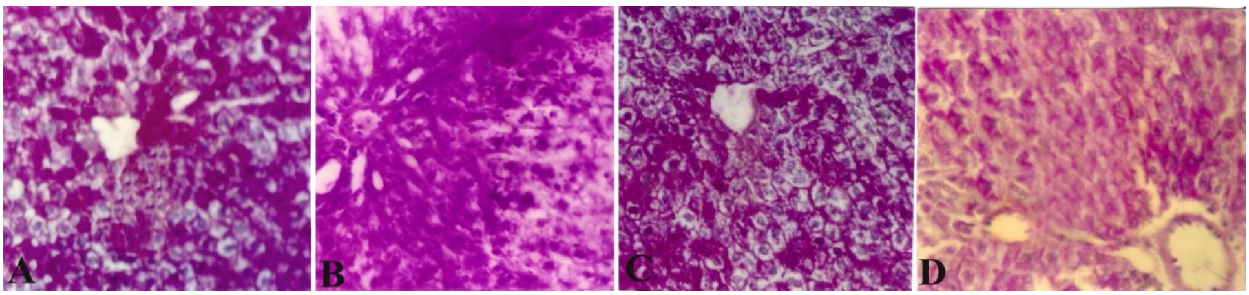

Figure 3. Total carbohydrates in liver sections. (A), Control liver section with normal distribution of carbohydrates. (B), Liver section of hypoxia subjected groups for 2 weeks. Sections appeared with slight decrease of PAS positive materials. (C), Liver section of hypoxia subjected groups for 4 weeks. Sections appeared with moderate decrease of PAS positive materials. (D), Sections from recovery group for 2 weeks with normal PAS positive materials. Sections stained with Periodic acid Schiff's method. Magnifications, X 250.

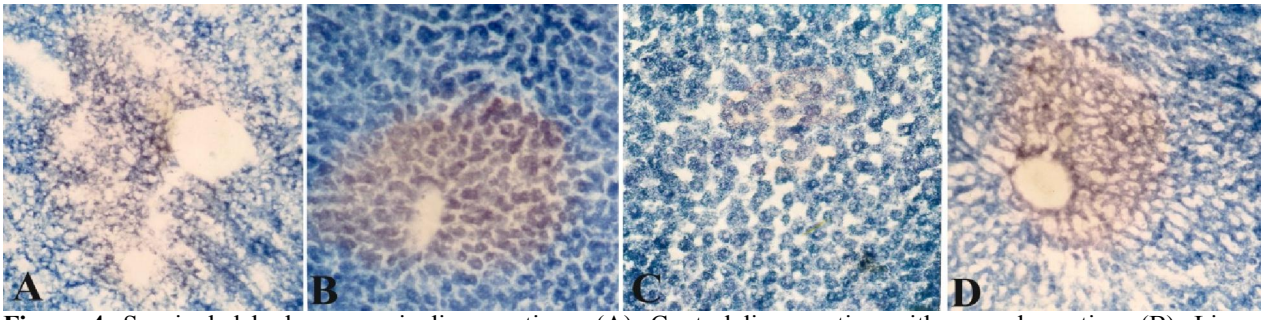

Figure 4. Succinyl dehydrogenase in liver sections. (A), Control liver section with normal reaction. (B), Liver section of hypoxia subjected groups for 2 weeks. Sections appeared with slight decrease in tissue reaction. (C), Liver section of hypoxia subjected groups for 4 weeks. Sections appeared with marked decrease in tissue reaction. (D), Sections from recovery group for 2 weeks with more or less normal tissue reaction. Magnifications, X 250.

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