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The Influence of Fear on the Liver of Male Rat: Histopathological and Physiological Study

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ABSTRACT

The present study aimed to analyse the impact of fear on the structure and function of liver in Wistar albino rats. Fifteen adult male Wistar albino rats were divided into one control and two experimental groups, with 5 rats in each group. The animals of the control group were sacrificed by knife directly while the animals of the other two groups were exposed to extreme fear from killing for 60 and 120 seconds before being sacrificed. Blood samples were collected for liver enzyme determination and samples of liver were removed and prepared for histological study. The results of this study showed significant histopathological changes in the liver of rat exposed to fear. These changes included necrosis and vacuolization in hepatic cells, dilation of the central vein and hepatic sinusoids, congestion of blood vessels, proliferation of bile duct, mitotic division in hepatocytes and an increase in Kupffer cells. Moreover, the present study showed that the fear induced slight elevation of liver enzymes AST, ALT and ALP. These results demonstrated that exposure to extreme fear, such as killing, could induce harmful alterations in the liver, and these alterations are time-dependent.

INTRODUCTION

Fear and stress occur within the body as a result of exposure to extreme fear or social phobia, such as killing, pushing, horror films and slaughter (Heimberg *et al*., 1990; Hope *et al*., 1995; Mattick and Clarke, 1998). Results of previous studies proved that extreme fear could induce many physiological dysfunctions in different organs in both humans and animals. Many authors (Palomba *et al*., 2000; Castaned and Segerstrom, 2003; Krolak-Salmon *et al*., 2004; Viswanathan and Dhabhar, 2005; Boatman and Kim, 2006; Romero and Butler, 2007; Clem and Huganir, 2010; Järlestedt *et al*. 2011) suggested that the effects of fear can be attributed to the brain defensive system, which releases different hormones directly into the blood stream. These hormones rapidly prepare the body for action in emergency situations. The adrenaline released from the adrenal gland when danger threatens or in an emergency is carried by the blood to all parts of the body, and takes a second or two before its effects are felt. The hormone boosts the supply of oxygen and glucose to the brain and muscles, while suppressing other non-emergency bodily processes, in particular digestion. Adrenaline increases the heart rate and stroke volume, dilates the pupils, and constricts arterioles in the skin and gastrointestinal tract, while dilating arterioles in skeletal muscles.

It elevates the blood sugar level by increasing the catabolism of glycogen to glucose in the liver, and, at the same time, begins the breakdown of lipids in fat cells, the arousal of gas exchange efficiency in the lungs and the induction of piloerection (Romero and Butler, 2007). Like some other stress hormones, epinephrine has a suppressive effect on the immune system (Viswanathan and Dhabhar, 2005).

In addition, farmers found that beef produced by cattle which suffered from acute stress, such as injury, surgery, or hardware disease, had a much lower quality compared to beef from normal cattle (King *et al*., 2006)**.** In agreement with this finding, recent studies showed that the pre-slaughter environment negatively affected the welfare status and decreased the meat quality (Neđeljko *et al*., 2011; Sabuncuoglu *et al*., 2012).

To our knowledge, no studies have been carried out on the effect of fear on the structure and function of liver. Therefore, the current study aims to investigate the hazardous effect of extreme fear on the liver of male Wistar albino rats.

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MATERIAL AND METHODS

Experimental animals

Fifteen adult male Wistar albino rats (300 - 350g) were used in the current study.They were obtained from King Fahd Center for Research ,King Abdulaziz University , KSA.The animals were housed in a standard animal facility under a controlled temperature (23 ± 2˚C), 50 ± 10% humidity and a photoperiod of 12 h light and 12 h dark, with free access to standard diet pellets and water. They were acclimatized to the laboratory conditions for one week before the beginning of the experiment.

Experimental Design

The animals were divided randomly into three groups, with 5 rats in each group. In the first group (group A), animals were served as control but in the second and third groups (groups B and C) the animals were exposed to extreme fear from killing for 60 and 120 seconds, respectively.

At the end of the exposure time, both control and experimental rats were sacrificed by slaughtering. Peripheral blood samples were collected from the neck blood vessels. Serum was separated out by centrifugation at 3000 xg for 10 min. Serum samples were used to determine liver enzymes ALT, AST and ALP according to the methods described by Steven (1996) and Juliet & John (1996) respectively. On the other hand samples of the liver of each rat was promptly removed and prepared for histopathological study. The livers were cut into small pieces to allow good fixation in 10% neutral formalin. Dehydration, clearing and paraffin embedding were followed according to Bancroft and Gamble (2002). Sections (5µm thick) were stained with haematoxylin and eosin and then examined by light microscope.

Statistical Analysis

The data (expressed as mean ± SE) were analyzed by oneway ANOVA and LSD post hoc test using SPSS software. Valuesof $p < 0.05$ were considered to be statistically significant.

RESULTS

Histopathological results:

Examination of liver sections from control rats showed the normal histological structure of hepatic lobules, hepatic cells with well-preserved cytoplasm, prominent nucleus, central veins and compact arrangement of hepatocytes (Figs.1-A and 1-B). Liver sections from rats exposed to fear for 60 seconds showed different histopathological alterations represented by dilated blood sinusoids, congested central veins with damaged endothelial lining cells, hepatic cells with degenerated nuclei, pycknotic nuclei, proliferation Kupffer cells and loss of cell boundaries (Fig.2). Cytoplasmic vacuolization, fatty degeneration and diffuse necrosis of hepatocytes, cloudy swelling of hepatocytes, pycnotic nuclei, dilated and damged blood sinusoids, desruction of endothelial lining of central vein and lacking of cell boundaries were also noticed (Figs. 3 and 4). Moreover, bile duct proliferation , Kupffer cell hyperplasia, binucleated hepatocytes and mitotic cell divisions were recorded (Fig. 5). Similar alterations were noticed in rats exposed to fear for 120 seconds , but they were more sever and pronounced (Figs. 6 , 7 , 8 and 9).

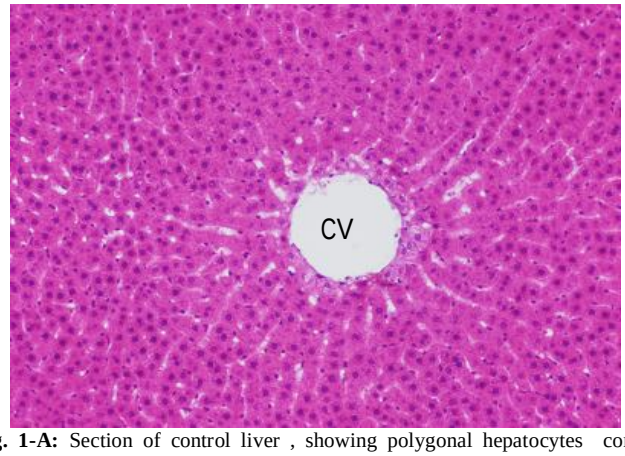

Fig. 1-A: Section of control liver , showing polygonal hepatocytes cords, blood sinusoids and central vein (CV) (X100).

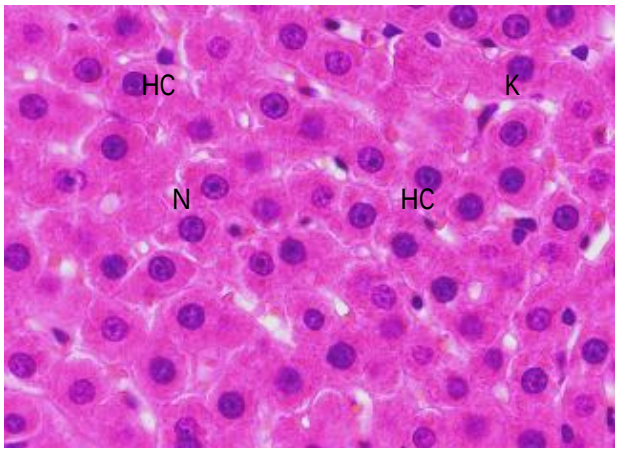

Fig. 1B: Higher magnification of the previous section, showing polygonal hepatocytes (HC) with central nuclei (N) and nucleoli, Kupffer cells (K), and blood sinusoids. (X400).

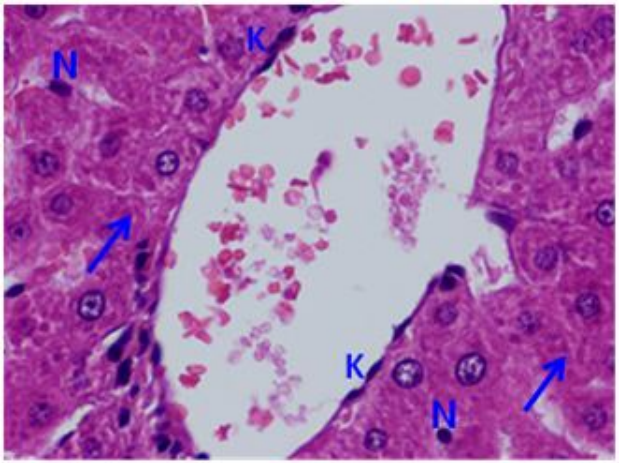

Fig. 2: Section of liver of rats exposed to fear for 60 seconds showing Branched, dilated and congested central vein, cells with degenerated nuclei, pycknotic nuclei (N), loss of cell boundries (arrows) and proliferation of kupffer cells(K) . (X400).

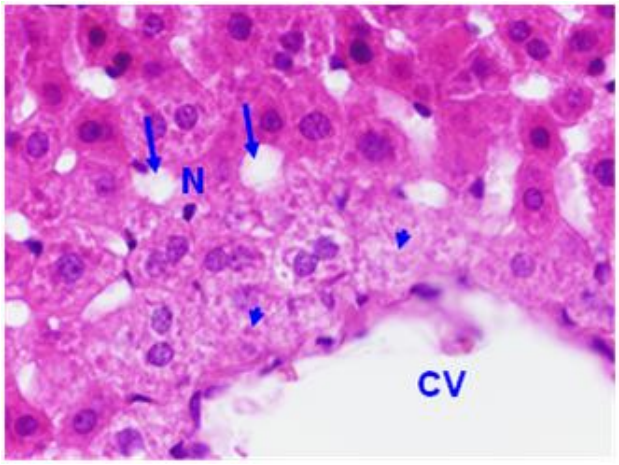

Fig.3: Section of liver in a rats exposed to fear for 60 seconds showing Fatty degeneration of some hepatocytes (arrows), cloudy swellingof hepatocytes (arrow heads), loss of cell boundaries, pycnotic nuclei (N) ,desruction of endothelial lining of central vein (CV). (X400).

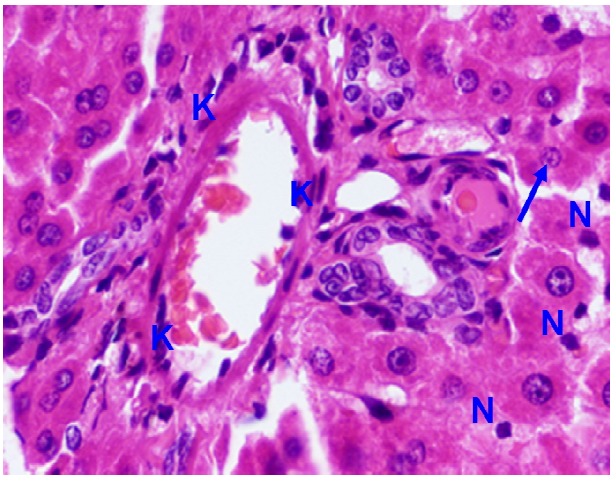

Fig.5: Section of liver of rats exposed to fear for 60 seconds showing destruction of internal lining of blood vessel, proliferation of bile ductule and kupffer cells (K), mitotic cell divisions (arrows) and pycknotic nuclei (N). (X400).

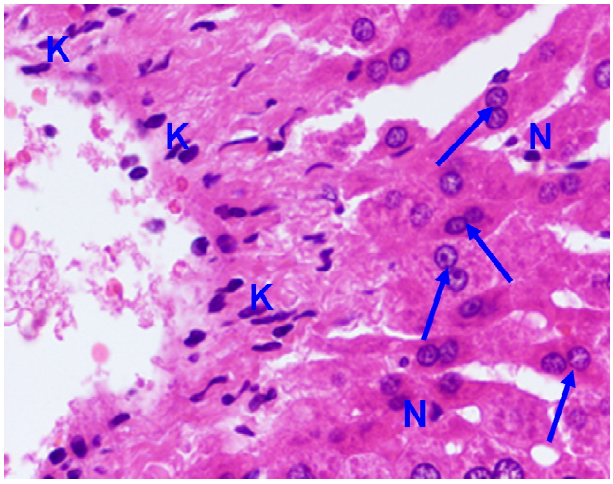

Fig. 7: Section of liver in a rats exposed to fear for 120 seconds showing, dilation of blood sinusoids (BS), pycknotic nuclei (N), proliferation of kupffer cells (K) and binucleated hepatocytes (arrows). (X400).

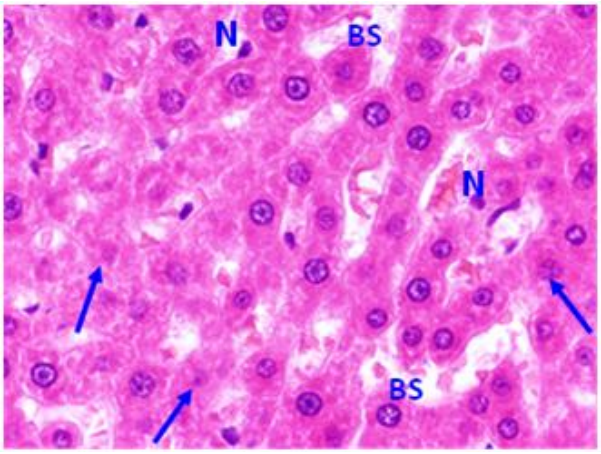

Fig. 4: Section of liver in a rats exposed to fear for 60 seconds showing, hepatocytes with degenerated nuclei (arrows), completely degenerated cells with pycknotic nuclei(N), dilated and damged blood sinusoids (BS). (X400).

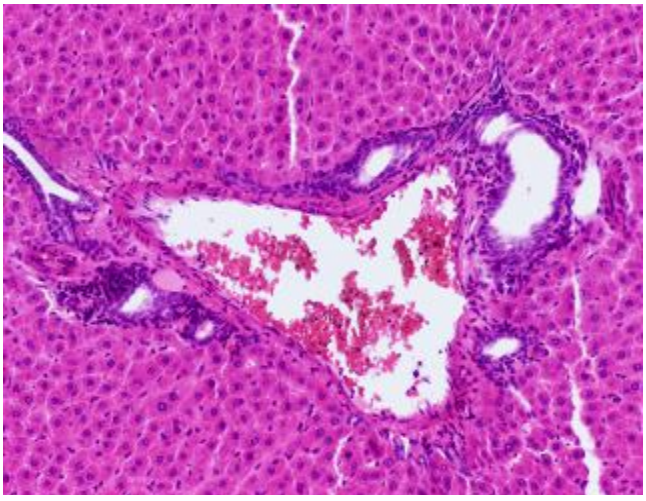

Fig. 6: Section of liver in a rats exposed to fear for 120 seconds showing Dilation and congestion of portal vein, proliferation of bile dicutule, hyperplasia of kupffer cells. (X100).

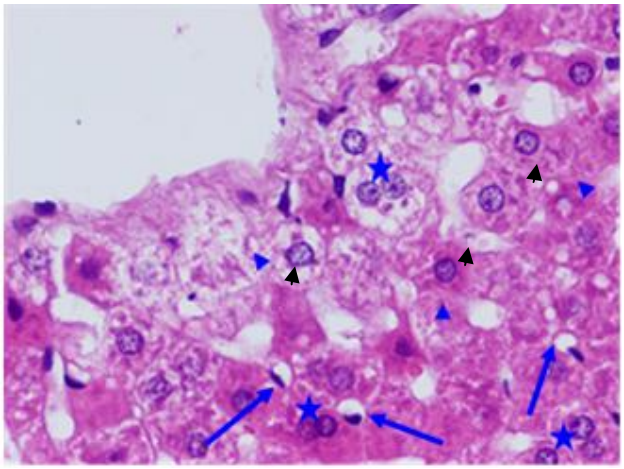

Fig. 8: Section of liver in a rats exposed to fear for 120 seconds showing, Fatty degeneration of hepatocytes, cells with degenerated nuclei, ballooning degeneration of some hepatic cells(arrows), loss of cell boundaries (arrow heads) and increase of binucleated cells (stars)(X400).

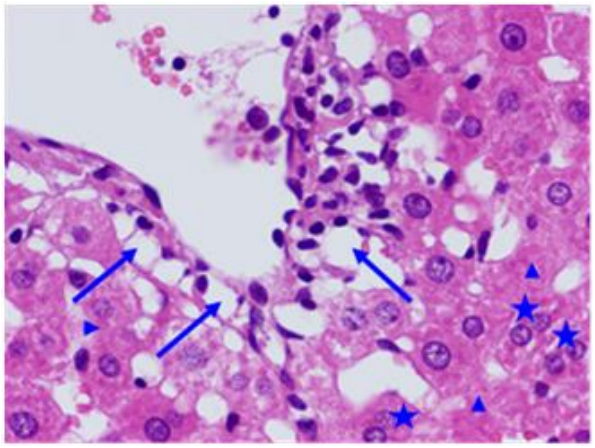

Fig. 9: Section of liver in a rats exposed to fear for 120 seconds showing, vacuolar degeneration, Fatty degeneration of hepatocytes (arrows), karyolysis and loss of cell boundries (arrow heads) binucleated cells (stars) (X400).

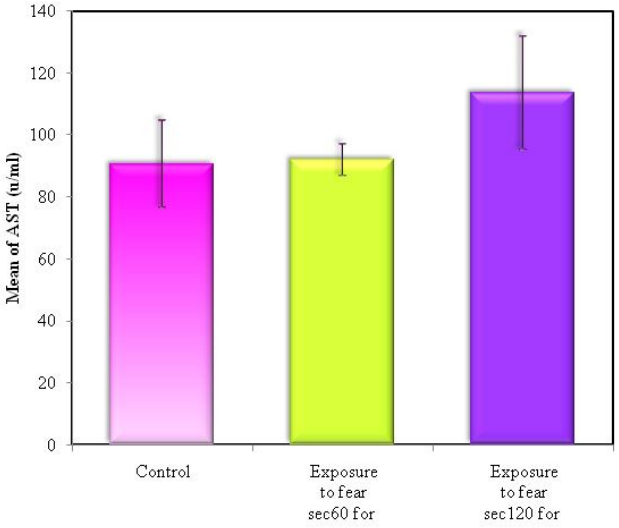

Fig. 11: Changes in the level of AST in rats exposed to fear.

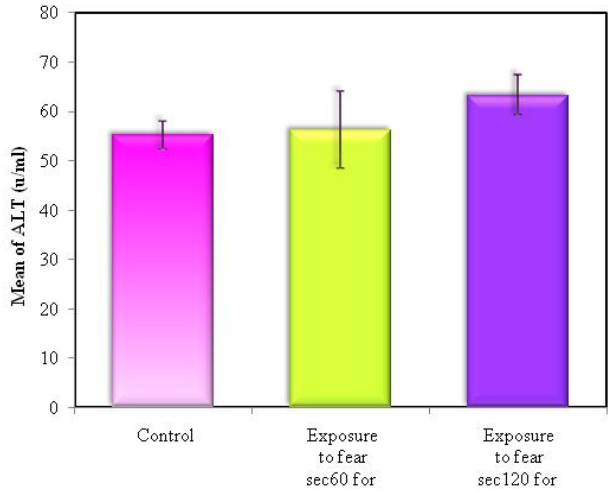

Fig. 10: Changes in the level of ALT in rats exposed to fear.

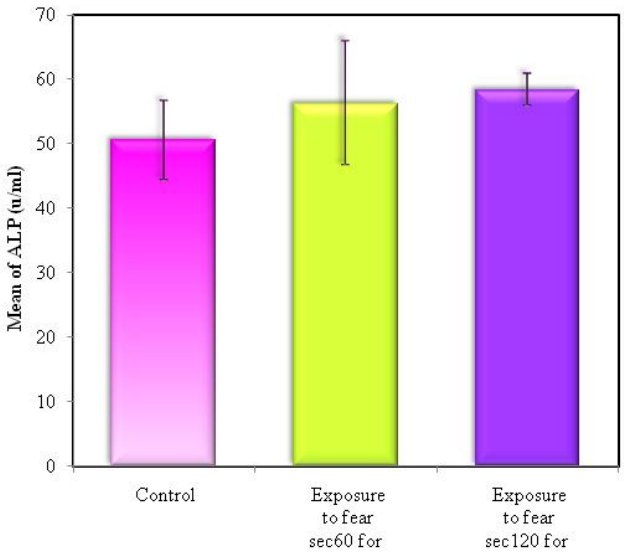

Fig. 12: Changes in the level of ALP in rats exposed to fear.

Physiological results

As shown in Table (1) and Figs (10-12) the plasma liver enzymes, ALT, AST and ALP in rats exposed to fear for 60 & 120 seconds were slightly elevated as compared to those in control group.

Table. 1: Effect of fear on the level of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in Wistar rats.

	Control	Exposure to fear for 60 sec	Exposure to fear for 120 sec
ALT (U/L)	$55.20^{a} \pm 2.78$	$56.20^{a} \pm 7.78$	$63.20^{a} \pm 3.97$
AST (U/L)	$90.40^{a} \pm 13.87$	$91.80^{a} \pm 5.07$	$113.40^{a} \pm 18.20$
ALP (U/L)	$50.50^{a} \pm 6.11$	$56.20^{a} \pm 9.55$	$58.35^{a} \pm 2.43$

Data represented as mean ±S.E. (n=5)
Similar superscripts within each raw indicate statistical non- significant differences between groups at 0.05

DISCUSSION

In the current study, exposure of rats to fear induced many histopathological alterations in the liver, including expansion in the central vein with damaged in the endothelial cells, congestion of blood vessels, necrosis and vacuolization of hepatocytes, fatty degeneration, and proliferation of kupffer cells and bile duct. Early reports demonstrated that, in the part of the brain that controls the liver, stress was found to impair blood flow and may lead to trigger liver damage (Hirose *et al*, 1961). Moreover, biochemical and electron microscopy studies showed that glucagon or adrenaline administration are capable of enhancing autophagic activity in the hepatocytes , degradation of glycogen inside autophagic vacuoles and degeneration of cell organellae (Rosa, 1971 , Kotoulas *et al*., 2004; Kondomerkos *et al*.,2005). Matot *et al*.(2008) mentioned that, liver injury, as assessed by liver enzyme levels, liver histology, and apoptosis, was attenuated to a greater extent with adrenaline resuscitation. Also, Montfort *et al*. (2008), demonstrated that epinephrine pre-exposure enhances liver damage (necrosis) caused by LPS with some necro-inflammatory foci and enhanced neutrophil infiltration. Recent studies were carried out on both human and animals and showed a link between stress (mainly of a psychosocial nature) and the evolution of three important liver-

related pathological entities: viral hepatitis, cirrhosis and hepatocellular carcinoma (Vere *et al*., 2009). On the other hand, Oben *et al*. (2003) suggested that sympathetic tone may modulate many aspects of liver disease. In the current study, the insignificant elevation of liver enzymes was recorded. It could be due to that, the time of exposure to fear is not enough to release these enzymes from the damaged hepatocytes to the circulation.

In conclusion, the present results suggest that exposure to fear has serious detrimental impacts on liver.

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