

The Histological Examination of Male Albino Rats Liver Which was Exposed to Hunger Stress

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Abstract: Feed intake is the cornerstone of animal productivity. The consequences of inadequate intake include inhibited growth, delayed puberty, infertility, reduced milk production and lowered resistance to parasites and disease. The present study aimed to examine the effect of hunger on the histology of the liver of male albino rats. Pieces from liver were taken in the 1st, 2nd, 3rd, 4th and 5th days following last feeding. The liver of each rat was excised and processed for light microscopy and stained using the Hematoxylin and Eosin (H and E) stain. Liver congestion and abnormal central vein were recorded in the 1st day following last feeding. Steatosis, abnormal central vein and congestion were also seen. Sever congestion was noticed in the 2nd and 3rd day. Congestion of portal vein and abnormal vessels were also seen in the 4th day, while congestion of central vein was seen in the 5th day of hunger. Histological examination of the liver revealed points of focal necrosis among the experimental groups. The mild liver tissue damage was more evident among the 4th and 5th day of the last feeding.

Key words: Hepatotoxicity · Hematological indices · Histology · Liver · Hunger · Fasting

INTRODUCTION

The natural drive to eat is determined by complex interactions between biological mechanisms of appetite control and responses to challenges from the physical environment. Suppressed intake and performance associated with environmental, social and disease stress are well recognized [1-3]. When intake fails to meet the basic energy needs for maintenance and survival, potent adaptive mechanisms terminate nonessential functions such as growth, reproduction and lactation. Under optimum conditions, nutritional intake is adequate for basal metabolic needs, growth, development, reproduction and controlled deposition of energy stores (fat). Although animal managers strive to keep stress to a minimum and to provide adequate amounts of high quality feed and water, targeted improvements in appetite and intake would further enhance recovery from disease and stress and overcome satiety-related limitations of growth and reproduction at full genetic potential [4].

The liver is generally considered to be the primary organ responsible for maintaining cholesterol homeostasis by regulating plasma lipoprotein metabolism

and the lipid output in the bile [5]. In the liver, high FFA concentration contribute to resistance to the action of insulin by enhancing glucose output from liver [5]. The accumulation of TG in liver by high FAs also bring about non-alcoholic fatty liver disease (NAFLD). NAFLD does damage to liver, which is the main organ of glucose metabolism, such as steatosis, steatohepatitis and hepatocellular necrosis to fibrosis [6]. The balance between hepatic lipogenesis and lipolysis is important to improving insulin resistance and NAFLD [7]. In the pancreas, prolonged exposure to FFA might cause impairment of insulin release through the mechanism of lipotoxicity [7-10].

Several recent experiments have provided evidence that the ingestion of a distinctive food by rats can be a learnt instrumental act as well as an associatively conditioned reaction. Extra eating before the longer fast was interpreted as avoidance of hunger. This interpretation was based on the evidence showing that extra eating as a result of classical conditioning comes from pairing food stimuli with the presence of little or no hunger because of repletion with energy nutrients. The theory that the extra eating arose from a response-

depletion contingency was tested in the present experiment by training rats on only a long fast or only a short fast. Greater increase in intake was seen before the longer fast. The results also replicated previously seen cycles of increase, decrease and renewed increase in putative deficit-avoidant eating over about three trials, indicating that the extra eating reduces the response-reinforcing hunger and that the consequent part-extinction restores reinforcement. The shape of the learning curve was consistent with these cycles occurring from the start of training, further supporting the view that the increase in food intake before a long delay in refeeding is hunger-reinforced instrumental behavior [11].

This study was carried out to evaluate the effect of hunger stress on the histology of the liver of adult albino rats.

MATERIALS AND METHODS

15 Male albino rats, weighing $300 \text{ g} \pm 10$ were obtained and housed in the animal House of faculty of Medicine, University of Jordan in August 2011. All experiments in this study were accomplished according to the protocol recommended by Local Animal Care Ethical Committee. The rats were kept at room temperature ($23 \pm 2^\circ\text{C}$) in a controlled room with a 12-h light: 12-h dark cycle. The rats were sacrificed and tissue samples were collected for histological analysis. Following last feeding, small pieces were taken from liver tissues of the subjects after applying ether anesthesia at the end of the 24th h and 2nd, 3rd, 4th and 5th days. The liver tissues were fixed in 10% formalin solution and processed for histological studies using haematoxylin and eosin stains. The slides were then evaluated for pathological changes under light microscope. Photomicrographs were taken using Kodak digital 10.3 mega pixels camera.

RESULTS

The results obtained are shown in (Fig. 1-7). Light microscopic examination of the liver sections of the control group showed a normal histological appearance of liver with clearly outlined figs of anastomosing hepatocytes along with adjacent sinusoids radiating from the central veins towards the periphery of the liver lobules. Normal outline of the central vein can be clearly visualized as shown in (Fig. 1).

Fig. 2-7 show sections from liver rats which were taken in the 1st, 2nd, 3rd, 4th and 5th days following last feeding. Liver congestion and abnormal central vein were

seen in the 1st day following last feeding (Fig. 2), steatosis, abnormal central vein and congestion were also reported (Fig. 3). Sever congestion was noticed in the 2nd and 3rd day (Fig. 4 and 5), respectively. Congestion of portal vein and abnormal vessels were the most prominent records seen in the 4th day (Fig. 6), while congestion of central vein was noticed in the 5th day of hunger as shown in (Fig. 7).

Results from liver portal tracts did not illustrate any inflammatory infiltration such as neutrophils presence within surrounding scanty connective tissues, even after 5 days of fasting.

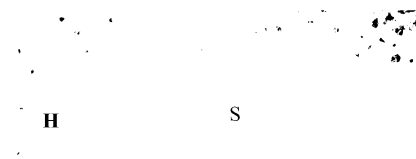


Fig. 1: Transverse section of male rats liver, showing normal histological structure of control liver. H: hepatocyte, S: sinusoid, (-): central vein. 400X, (H and E stain)



Fig. 2: 1st day, transverse section of male rats liver illustrating: Congestion (C) and abnormal central vein (-). 400X, (H and E stain)



Fig. 3: 1st day, transverse section of male rats liver illustrating: Steatosis (-), abnormal central vein (-) and congestion (C). 400X, (H and E stain)

DISCUSSION

Rats consume about 80% of their daily food intake at night [12, 13], with peaks around 8 p.m. and 6 a.m. [14]. A practical procedure to understand the etiology and pathogenesis of diseases is study on animal models. The benefits of using animal models in research works involved the ability to access its multifactorial genetics and complications. However, correlations between animal diseases and that in human kind are well established [15]. Besides, liver is considered one of the major organ involved in metabolism [16].

The liver is primarily composed of parenchymal cells or hepatocytes (80% by volume) and four types of nonparenchymal cells: endothelial, Kupffer, Ito and pit cells. Hepatic tissue is highly specialized and functions as a major effector organ, acting as principal center of nutrient metabolism, major component of the organism defensive response, control station of the endocrine system and blood reservoir [17]. The hepatic gland performs a strategic role in the digestive process by receiving the nutrients from the diet and orchestrating their transformation into useful biomolecules to be delivered to other organs and tissues. Hence, the liver is fundamental in the metabolism of carbohydrates, lipids and all other biomolecules. Hypothalamic and midbrain nuclei are connected via vagal and splanchnic nerves to the liver, allowing the hepatic organ to participate in the control of food intake by sensing and regulating the energy status of the body [18-19].

It has been reported that Non-genetic environmental factors including diet and food constituents are able to prevent or induce several diseases [20-24].

The histological observations on tissues of control and experimental rats in this study, displayed normal structure of hepatic trabeculae, normal sinusoids, normal hepatic portal area (portal vein, lymph & bile duct). Clear histopathological changes were noticed in liver of treated rats. Extremely congestion in sinusoids, portal vein and central vein were observed (Fig. 5, 6 and 7).

Diaz-Munoz *et al.* [19] demonstrated that the group of rats with 24-h of fasting showed no variation in the size of their liver cells compared to the ad-libitum fed counterpart. Food restriction also promoted obvious modifications in hepatocyte morphometry. The group showed a dramatic reduction (about 82%) in the glycogen content. In their study, Vendemiaie *et al.* [25] found no histological differences were observed between fed and 18-hour fasted rats.

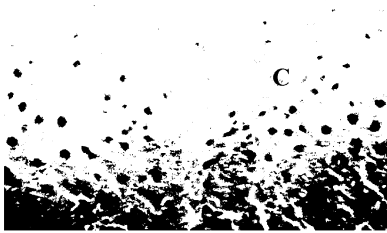


Fig. 4: 2nd day, transverse section of male rats liver illustrating: Sever congestion (C). 400X, (H and E stain)

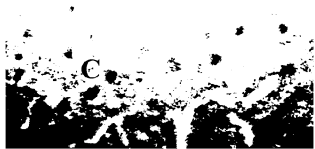


Fig. 5: 3rd day, transverse section of male rats liver illustrating: Sever congestion (C). 400X, (H and E stain)



Fig. 6: 4th day, transverse section of male rats liver illustrating: Congestion in portal vein (C) and abnormal vessels. 400X, (H and E stain)

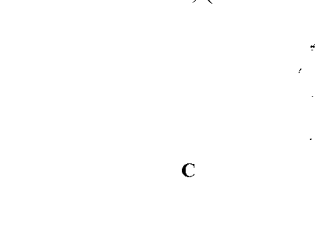


Fig. 7: 5th day, transverse section of male rats liver illustrating: Congestion of central vein (C). 400X, (H and E stain)

It has been shown that dietary state influences the hepatocyte dimensions. Uhal and Roehrig [26] reported that the dietary state influences the hepatocyte size and volume: 48 h of fasting resulted in a two-fold reduction in hepatocyte size and its protein content, whereas refeeding promoted a 70- 80%.

Vermeulen *et al.* [14] demonstrated that the stomach of male rats (200-250 g) is empty as soon as 6 hours after the initiation of fasting. There was no significant difference in intestinal emptying between animals fasted for 6 or 18 hours. Large changes in body weight have been reported following fasting. Vermeulen *et al.* [14] recorded a weight reduction of approx. 10% after 18 hours food deprivation. Others have reported weight loss up to 48g in male rats that weighed 264 g, which is over 18% of their body weight [27]. Claassen [28] cites studies where weight loss in rats varied between 3.3% and 18% after 24 hours of fasting.

The reduction in liver weight during a fast can be relatively larger than the reduction of body weight and the liver's content of free fatty acids increases markedly [13, 28].

Fasting can be expected to cause, among other things, stress, aggressive behavior, as well as reductions in body weight, body temperature and plasma glucose levels. The effect of food deprivation is greatest when it occurs in the dark phase, significant reductions in liver weight and glycogen content, as well as increases in levels of glycerol, free fatty acids and acetoacetate have been measured after 3 hours [29]. Claassen [28], cites a number of studies that show increases in plasma levels of glucose, urea, lactate and amino acids, a range of hormones including insulin, glucagon and corticosterone [13].

Vermeulen *et al.* [14], investigated activity patterns in rats during a period of fasting. Animals that were fasted for 18 hours showed increased locomotory behavior and an increase in the time spent grooming, which also resulted in the accumulation of significant amounts of hair in the stomach. Shorter periods of deprivation resulted in smaller changes in the animal's physiology and behavior.

Tooth and Gardiner [30], cite several publications that demonstrate that activation of the sympathetic nervous system increases in plasma glucocorticoids can be measured in rats fasted for 24 hours, but these changes are less than those observed when animals are stressed with other stimuli. These changes can therefore be interpreted as normal adaptive responses to reduced food supply. After 48 hours of food deprivation, glucocorticoid

levels increase dramatically. This suggests that periods of fasting that last longer than 24 hours are associated with increasing metabolic stress and perhaps worsening of psychological stress [30].

CONCLUSION

Although mild level of liver tissue damage was observed, congestion was the most prominent hisopathological change recorded in this study insuring that the liver is one of the few glands with a remarkable ability to regenerate.

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