ANSF APPLIED AND NATURAL SCIENCE FOUNDATION

Journal of Applied and Natural Science
14(3), 954 - 962 (2022)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Dysfunctions of liver and behavioural disorders of females rats suffering from malnutrition: Physiological and histological information as a model of animal anorexia nervosa disease

Halla Abdul-Hadi Chabuk
Department of Biology, College of Science, University of Babylon, Babil 51001, Iraq

E mail : sci.halah.a.hadi@uobabylon.edu.iq

Article Info
https://doi.org/10.31018/jans.v14i3.3721
Received: July 7, 2022
Revised: August 12, 2022
Accepted: August 19, 2022

How to Cite

Abdul-Hadi Chabuk, H. 2022). Dysfunctions of liver and behavioural disorders of females rats suffering from malnutrition: Physiological and histological information as a model of animal anorexia nervosa disease. *Journal of Applied and Natural Science*, 14(3), 954 - 962. https://doi.org/10.31018/jans.v14i3.3721

Abstract
Anorexia nervosa disease is an eating deficiency that occurs around 1 per 100 individuals. The present study was conducted to assess the effects of malnutrition (animal anorexia nervosa models) via removing certain elements from food on liver functions and behaviours of female rats. Eighteen Females of rats were divided into three groups: Control, in which food intake quantity was 25 g /day, moderately food-restricted Group, in which food intake quantity was 15 g /day, and severe food-restricted Group, in which food intake quantity was 5 g /day, for 90 days. Physiological parameters, liver histopathological analysis, and the behaviour measurement by multiple T maze tests were examined. All food-restricted groups observed a significant increase ($p<0.05$) in aminotransferase, Malondialdehyde and lactate dehydrogenase levels. Antioxidant activity, acid phosphatase, hepatic protein, glycogen and serotonin levels were significantly ($p<0.05$) decreased in all food-restricted groups. The hyperactivity behaviour appeared as a feature of restricted rats. Histopathological examinations indicated a marked alteration in the hepatocyte with features of autophagy in the restricted Group. Food-restriction (animal anorexia nervosa model) in rats caused disorders in hepatic aminotransferase and serotonin levels and antioxidant activity in addition to hyperactivity behaviours with changes in the liver histological structure in the restricted Group. The study focused on the effect deficiency in essential nutrients needed by the body may have side effects on liver functions and behavioural activity of the animals linked with food searching was measured by multiple T maze tests.

Keywords: Animal anorexia, Antioxidant, Behavioural test, Female rat, Liver enzymes, Malnutrition, Serotonin.

INTRODUCTION

Anorexia nervosa (AN) disease is an energy intake restriction that results from a severe fear of increasing body weight and ends in a status of very weight loss (Koga *et al*., 2019). Food restriction of the laboratory rats can be used as animal models of anorexia nervosa to mimic in human disorders. Anorexia is widespread among young and adolescent girls and has higher mortality rates than all chronic psychiatric diseases (Kim, 2012). Anorexia causes psychological changes, increases in physical activity (hyperactivity), and reduced in serotonin levels may be associated with hepatocyte damage leading to an increase in aminotransferases levels (Rosen *et al*., 2017 and Schalla & Stengel, 2019). Studies indicated that serotonin secretion from the platelet plays a role important in liver regeneration (Dhanda and Sandhir, 2015 and Zhang *et al*., 2020). The evidence suggests prolonged food restriction and severe weight loss are associated with liver disorder, brain atrophy, and cognitive deficits (Oudman *et al*., 2018).

There are several physical complications caused by malnutrition such as renal and hepatic failure caused by diet deficiency, hypokalemia, dehydration, and the use of diuretic or laxatives drugs leading to rising liver transaminase levels, especially alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the more frequently influenced levels from 2–4 times the normal, while sometimes they elevate to higher levels causing even sudden death due to cardiac failures such as hypotension and bradycardia (Koga *et al*., 2021). Malnutrition-induced hepatitis causes hormonal and biochemical changes in rats exposed to food restriction, (Rosen et al., 2017). The liver plays a major role in food restriction adaptation, it contains an enzyme system involved in gluconeogenesis, glycogenolysis, and ketogenesis causes the low density of liver glycogen and thus provides energy substrates to other tissues (Hanachi et al., 2013). Early malnutrition is featured by drooping in insulin secretion and increased glucagon secretions. In this condition, the body switches from carbohydrates to the usage of proteins and fats to output energy. So, glucose is replaced with ketone bodies and fatty acids as the major energy sources (Namazi *et al*., 2016).

The liver is also a main detoxification organ in the body and has a wide range of antioxidant defense systems necessary to control reactive oxygen species (ROS) metabolism. These systems include superoxide dismutase (SOD) catalase (CAT), and glutathione peroxidase (GSH), the activity of these enzymes is higher in the liver than in other tissues (Marczuk-Krynicka *et al*., 2003 and Mojahed *et al*., 2016), food restriction induces oxidative stress and leads to alteration in the antioxidant enzyme activity in the liver tissue (Siegfried *et al*., 2003., Celik *et al*., 2012 and Jáuregui-Lobera *et al*., 2013).

Lasègue acknowledged that food abstinence increased the patient’s aptitude for movement. These disorders are not limited to changes in the diet but also include severe somatic and psychosocial abnormal complications (Oliveras-López *et al*., 2015).

Malnutrition also causes liver fibrosis with chronic severe liver damage that occurs because of an inflammatory reaction. Liver histological analysis revealed necrosis features or apoptosis, hepatocyte autophagic death was the main cause of acute liver damage in rats exposed to severe food restriction (Rosen *et al*., 2017). Autophagy is a cellular process in which cytosolic organelles and proteins degenerate, because of the exacerbation of starvation and reduced body weight. Thus excessive autophagy activation leads to rising hepatocyte dysfunction and, liver injury (Harris *et al*., 2013). Several previous studies demonstrated the effect of starvation for different time periods on the rat's liver and behaviour, but the present study focuses on studying the impact of malnutrition or food-restricted (the animal anorexia disease model) on the biochemical parameters, histological of the liver in young females' rats and their behaviour, to mimic anorexia nervosa disease in humans. It was carried out using different quantities of food to reduce the nutrients the body needs during the day.

MATERIALS AND METHODS

Ethical approval

The study protocol was approved by the Department of Biology, College of Sciences, University of Babylon (Protocol No. 1223/10-6-2021), and the experiments were carried out following approved guidelines and in compliance with ethical standards according to the National Committee for Research Ethics in Science and Technology (NETNT).

Experimental animals

The study included 18 young female albino Wistar rats (8 to 9 weeks old, with a mean weight of 194 g). Rats were purchased from the animal's house in the College of Science / University of Thi Qar/ Iraq. Animals were housed individually in standard cages, under thermostatically controlled (24^0C ±1) with 12/12 hrs. light /dark cycle room and animals were adapted to laboratory conditions for two weeks prior to the experiment's start.

Experimental protocol (Animal anorexia nervosa model)

The experimental methods of food restriction in rats as anorexia nervosa models were described by Wojciak *et al.* (2014), with a quantitate modification in feed intake in this study. A food source used in this study was purchased from Al-Diwaniyah Modern Feed Factory. Animals were divided into three groups.

First Group (Control group): Six female rats with free access to food and water (quantity of food intake 100% about 25 g /day) during the 90 days. (The rats by standard feed were fed with fixed proportions consisting of 20% barley, 34% wheat, 25% corn, 10% milk powder, 10% animal protein, and 1% salt.

Second Group (Moderately feed restricted): Six female rats exposed to middle feed (quantity of food intake 60% of control about 15 g /day, (The diet intake consists of 12% barley, 20.4% wheat, 15% corn, 6% milk powder, 6% animal protein, and 0.6% salt) during the 90 days.

Third Group (Severely restricted): Six female rats exposed to severe feed restriction (quantity of food intake 20% of control about 5 g /day, (The diet intake consists of 4% barley, 6.8% wheat,5% corn, 2% milk powder, 2% animal protein, 0.2% salt) during the 90 days. All rats were weighed on the first day of the experiment (0 days), and after (30, 60, and 90 days).

After the end period of the experiment, animals were sacrificed by putting them in a closed container containing cotton with chloroform anaesthetic.

Food was provided to each rat in each Group. The control group was given about 8.3 g of food in each meal of each rat. while the rats in the second and third groups were given about 5, and 1.6 g respectively of food for each rat at each meal. The food was given to animals three times a day at 8 am, 1 pm and 7 pm.

Physiological parameters

Liver enzymes assays

After 90 days, liver tissue was collected from the animal

In a Potter homogenizer, this tissue was minced and homogenized (10% w/v) in ice-cold saline followed by 0.1M phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000×g for 20 min at 4 °C, and the resultant supernatants were used for different enzymes assays, like (alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and acid phosphatase (ACP) levels in the rat's liver was measured by using the Reflotron plus device manufactured by the German company Roche. This procedure was done according to the instruction manual of the Reflotron device (CHabuk *et al*.,2016). Serotonin was measured by using special kits of the Enzyme-Linked Immunosorbent Assay (ELISA) supplied by Elabscience-China company.

Determination activity of the liver antioxidant enzymes

For enzyme assays, the liver pieces were homogenized (10% w/v). The homogenates were centrifuged at 10,000 x g for 20 minutes, and the supernatants were used to measure both catalase (CAT) and glutathione peroxidase (GPX) activities were analyzed using an ELISA kit (Colorimetric method) from (Elabscience, China) according to the detection principle method of these kits. Superoxide dismutase (SOD) activity was analyzed using an ELISA kit (Colorimetric method (WST-1 Method)) from (Elabscience, China) a according to detection principle method of these kits. Malondialdehyde (MDA) level measured by Competitive-ELISA kit from (Elabscience, China).

Histopathological analysis

Liver tissue was collected from the animal and fixed in formalin (10%), paraffin- embedded, then section at 5 µm and stained with hematoxylin and eosin (H&E) for examination by light microscopic, according to Kumar *et. al.* (2010). Slides were examined by an expert pathologist. Histopathological grading of liver tissue lesions was assessed according to Zhang *et al*. (1996) using a score from 0-2 according to the severity grade (Grade 0 =none, Grade I=mild, Grade II=moderate).

Grading levels of liver tissue injuries

Grade 0 (none) no changes and normal liver structures.

Grade I (mild) showed slight hepatocyte swelling, congestion at portal area with focal infiltration of inflammatory cells, fibrosis around the portal area and sinusoidal spaces and hepatocyte with slight features of autophagic cell.

Grade II (moderate) showed increased hepatocyte swelling, congestion at portal area with infiltration of inflammatory cells, increased fibrosis around portal area and sinusoidal spaces, also observed mild microvesicular steatosis in the hepatocyte.

The protocol for examination of histopathological slides was as described by Gibson-Corley *et al*. (2013). A monitoring site for ten random fields in every slide was examined under the light of microscopy at 100x and 400 magnification power. The monitoring site was (a) the zone around the central vein and (b) the portal and periportal area. The number of cells that revealed each lesion was calculated. The mean of these lesions was recorded. The three grades with their own criteria determined the classification of each slide under each Grade.

Behavioural study

All behavioural experiments were conducted in a lower sound-room between 8:30–12:30 am. After the end experiment. Animals should be habituated to the room for one hour before starting the experiments for behaviour al study. Activity-based anorexia (ABA) is also known as food-restriction-induced activity anorexia or hyperactivity. This activity was estimated by using the multiple T maze test consisting of a box containing multiple arms of T shape made of wood; and these arms were fastened to a wooden base, and the box was covered with a clip. This test was used for spatial memory and took changing behaviours of rats in searching for food. These tasks depend on animals that have developed strategies to explore their environments and obtain food with minimum effort. Set up the multiple T maze test is shown in Fig. 1. A food reward was placed at the end of the maze and put the rat in

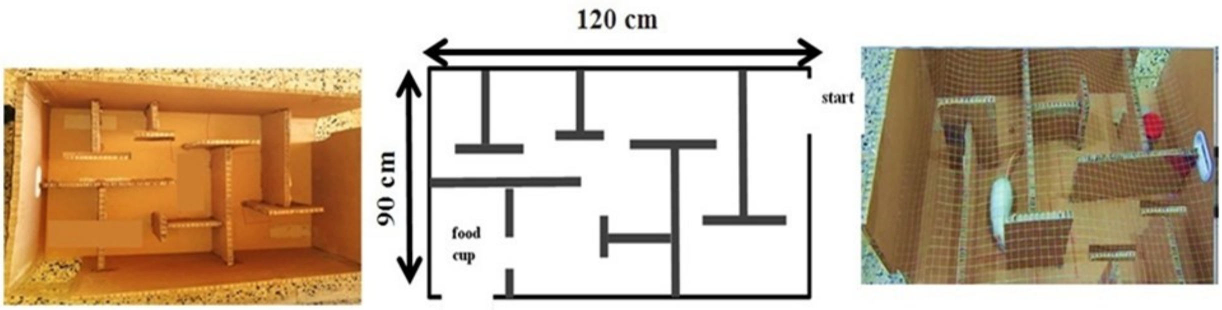

Fig. 1. *Multiple T maze test*

the starting position of the maze. Time (sec.) was recorded to measure the physical activity of rats to reach the food cups at the maze end for all the groups. The author designed the maze according to Dietze *et al*. (2016) with some modifications.

Statistical analysis

The data of these results were expressed as mean ± SE. Statistical analysis was performed by one-way ANOVA and Duncan post-hoc test, by using SPSS software, version 23. Under significant P<0.05.

RESULTS

Effect of food-restriction on body weight of female rats

Before food-restriction (0 day), the mean body weights of female rats in three groups (Group first to Group third) were 188.8± 12.6 g, 197.5± 6.26g and 193.5± 5.44g, respectively. After 30 days of food restriction, the mean body weight of rats in the second and third groups showed a significant (p<0.05) decrease to 177.4 g ± 8,4 and 164.48 g ± 7.1. respectively, compared to the control group (Group first) which showed an increase in body weight to 217.69 g ± 6.32. While the body weight of rats in the second and third groups during 60 days was markedly decreased to 162.5 g ± 9,32 and 152.81g ± 12.80. respectively, compared to the control group (256.03g ±7.68). Body weights of the rats in the second and third groups after 90 days of restriction were significantly (p<0.05) lower (157.65 g ± 12.0 and 114.69 g ± 9.5) respectively, compared to the control group (302.87g ± 10.91). The results showed no significant difference in body weights of rats in the second and third groups after 30 and 60 days of food restriction, but after 90 days of restriction showed a significantly (p<0.05) decreased in body weights between rats in the second and third groups . Fig.- 2

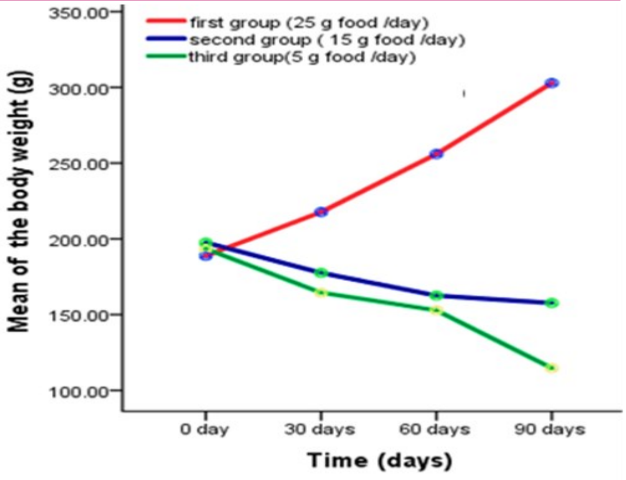

Fig. 2. *Bodyweight (g) of female rats exposed to food restriction for 90 days. Values are (Mean ± S.E) (n = 6)*

Effect of food-restriction on liver enzymes and serotonin

The study revealed the effect of food deprivation on liver enzyme levels as a significant increase (p<0.05) in transaminases AST, ALT and ALP levels in the rats' liver of the third Group compared to the rats in the first and second Groups. AST exhibited no difference significantly (p>0.05) between the second and control groups. While ALT and ALP levels in the tissue of food-restricted rats in the second Group exhibited a significant increase (p<0.05) compared to the first Group. The present data indicated a significant decrease (p<0.05) in the liver parameters levels (ACP, LDH, total protein,

Table 1. Assay of liver enzyme levels (AST, ALT, ALP, ACP, LDH and Serotonin), total protein and glycogen levels in liver tissues of female rats exposed to food restriction for 90 days as an animal anorexia model. (n = 6)

Parameters	First Group (25 g/day food)	Second Group (15 g/day food)	Third Group (5 g/day food)
AST (IU/mg tissue)	73.13±4.94	81.26±5.86	113.17±9.26 **
ALT (IU/mg tissue)	37.48±2.61	51.29±3.98 *	82.35±4.03 **
ALP (IU/mg tissue)	168.40±10.62	220.24±16.81 *	285.27±7.00 **
ACP (IU/mg tissue)	43.35±3.38	27.31±1.85 #	22.15±2.61 #
LDH (IU/mg tissue)	283.66±24.92	358.87±10.10 *	451.08±32.28 **
Total hepatic protein (mg/g tissue)	224.23± 11.64	186.87 ± 14.98 *	145.01 ± 9.02 **
Hepatic glycogen (mg/g tissue)	30.95 ± 2.59	18.03 ± 1.97 *	8.18 ± 0.66 **
Serotonin (ng/ mg tissue)	10.12 ± 1.44	6.54 ±0.47#	3.78 ± 0.75#

Values are (Mean ± S.E) (n = 6), *The mean is significantly different at the P ≤ 0.05 as compared to first and third groups.; ** The mean is significantly different at the P ≤ 0.05 as compared to first and second groups;# The mean is significantly different at the P ≤ 0.05 as compared to first Group.

and glycogen) between the food-restricted groups and the control group. The results showed a significantly decreased ($p<0.05$) in the level of serotonin liver of food-restricted rats in the second and third groups compared to the first Group (Table 1).

Effect of food-restriction on antioxidant enzymes activity in rat liver tissue

Food restriction caused significantly decreased ($p<0.05$) activity of principal antioxidant enzymes (CAT, GPX and SOD) in the liver tissue of food-restricted rats Groups compared with the first control Group, and MDA level showed significant ($p<0.05$) increase after food-restriction stress in food-restricted groups compared to the control group (Table 2).

Correlation between liver serotonin and liver enzymes level

The correlation between liver serotonin, and liver enzyme levels (AST, ALT and ALP) of rats that exposure to malnutrition stress for 90 days showed a significantly negative correlation r = - 0.579, - 0.690, - 0.623, respectively. While there was a significantly positive correlation r = 0.707 between the level of serotonin and ACP enzyme level (Table -3).

Effect of food restriction on behavioural rats

The multiple T maze test measures rats' activity, exploration and anxiety behaviour. Fig. 3 showed significant hyperactivity behaviours characteristic of rats in the third Group exposed to hunger compared to the first and second groups. While rats in the second Group showed a significantly decreased activity ($p<0.05$) compared to rats in the third Group.

Histopathological evaluation:

Rats in stressful food restricted groups (B and C) manifested changes in the color of liver (light colored) as compared to the control Group (A) (Fig. 4). The liver of rats in control group that gave 25 g/day food (no stressful food-restriction) showed normal histological structures that consisted of hepatocytes cords regularly arranged enclosing the sinusoidal networks and central vein (Fig. 5). Meanwhile the rats in Group two and three (after stressful food restriction) revealed changing in liver tissue involved hepatocytic swelling due to glycogen deficit with congestion at portal area and proliferation in bile epithelia, cellular infiltration, centrilobular fibrosis and atrophy of hepatocyte with features of autophagic cell death (Fig. 5,6).

Grading levels of the examined liver slides of the lesion criteria represented in Table -4 showing three grades from 1-30% represented the percentage of each tissue lesion in the ten random fields of liver tissue slides for three Groups.

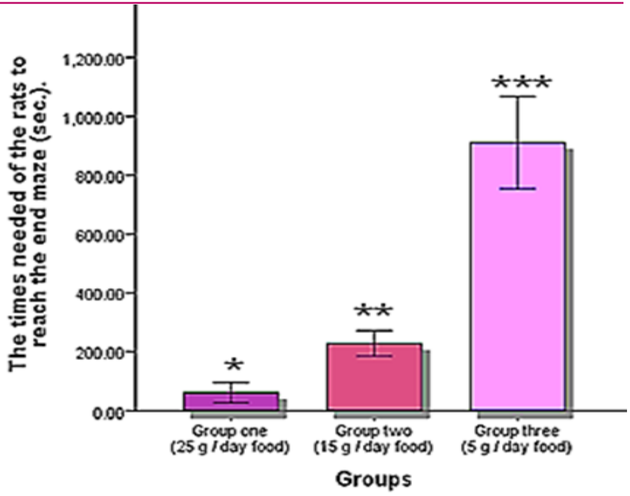

Fig. 3. *Effect of food-restriction stressful for 90 days on activity behavioural of rats measured by the multiple T maze test. The different signs indicated to different indicated statistical significance at p<0.05. (n = 6)*

Table 2. Antioxidant enzymes activity and MDA levels in female rat liver exposed to food-restriction for 90 days as animal anorexia model. (n = 6)

Parameters	First Group (25 g/day food)	Second Group (15 g/day food)	Third Group (5 g/day food)
CAT(IU/mg tissue)	34.63±3.59	25.55± 2.77 *	15.65 ± 1.51 **
GPX (IU/mg tissue)	102.61 ± 7.11	72.32 ± 4.46 *	45.06 ± 3.32 **
SOD (IU/mg tissue)	206.46 ± 10.19	120.93 ±8.60 *	82.89 ± 6.20 **
MDA (nmol /mg tissue)	40.26 ± 3.71	86.92 ± 6.37 #	94.84 ± 4.24 #

Values are (Mean ± S.E) (n = 6); *The mean is significantly different at the P ≤ 0.05 as compared to first and third groups; ** The mean is significantly different at the P ≤ 0.05 as compared to first and second groups; # The mean is significantly different at the P ≤ 0.05 as

Table 3. Correlation between serotonin and liver enzyme levels in liver tissue of rats that exposure to food-restriction for 90 days as animal anorexia model. (n = 6).

Correlations		AST	ALT	ALP	ACP
Serotonin	Pearson Correlation	- 0.579*	- 0.690**	- 0.623**	0.707**
	Sig. (2-tailed)	0.012	0.002	0,006	0.001

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

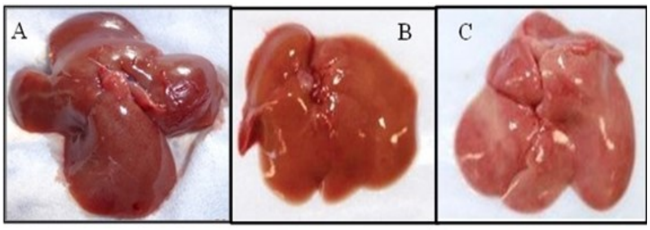

Fig. 4. *Changing in the morphology of livers organs of rats that exposed to food-restriction stress from (A) first Group (B) second Group (C) third Group*

DISCUSSION

The present results revealed a decrease in the body weight of the food-restricted groups as compared with the control group showing an elevation in the body weight at the end of the experiment (Fig. 2). The liver that accomplishes several of the metabolic activities in the body remains exposed to stress as the duration of malnutrition increases can lead to acute changes in the liver functions and markedly body weight loss as malnutrition progresses which may be due to the depletion in the glycogen and proteins concentration from their stores (liver and muscle) during food restriction (Mai-siyama *et al*. 2017 and van den Berg *et al*., 2019), since the study revealed a significant decrease in hepatic glycogen and protein.

This study in Table 1 showed a markedly significant ($p<0.05$) increase in LDH levels and liver aminotransferases (ALT, AST, and ALP) levels in food-restricted groups that play a major role in the biological processes. Consequently, these enzymes are an indicator of hepatocellular dysfunctions and damage due to the prolonged starvation state that causes the hepatocyte autophagy mechanisms (as shown in the liver slides of food-restricted groups of the study) or autophagy cell death considered a major cellular pathway for the degradation of proteins. The range of these hepatic enzymes elevation could also signal the illness severity in anorexia patients. Malnutrition may lead to changes in the plasma membrane by increasing the permeability of plasma membranes of hepatocytes which can explain this increase in female rats (Rautou *et al*., 2008 and Narayanan *et al.*, 2010). From the study results, the liver hyperfunctions in response to the activities of the liver may be due to starvation caused by glycogen breakdown to release glucose into the bloodstream. The malnutrition state is characterized by a decline in the secretion of insulin hormone and an increase in the secretion of glucagon in response to the decrease in blood glucose levels and releasing glycogen stores from the liver causes hepatic glycogen decrease after starvation and increased autophagic vacuoles in the liver tissue (Lennerz *et al*., 2010; Harris *et al*., 2013 and Mai-siyama *et al*., 2017).

LDH enzyme was significantly increased in both food-restricted groups (table-1), LDH is one of the tissue metabolic requirements and engages in energy production. LDH activity represents an indicator for cytotoxicity of toxic agents and cellular damage. Increasing the activities of LDH is fundamentally due to the leakage of this enzyme from the liver cytosol into the hepatic sinusoids indicates liver damage and disorder of normal liver function (Yousef *et al*., 2002). The study corresponded with previous other studies (Wojciak, 2014 and Carrera *et al.*, 2014) that mentioned the effect of malnutrition on abnormalities of liver parameters, increased physical activity, and loss of body weight that was most common in the female rats exposed to malnutrition. The recent results were similar to other studies (Narayanan *et al*., 2010 and Harris *et al*., 2013) that found that starvation resulted in elevated aminotransferase related to loss of body weight in anorexia patients. The rapid decline in AST/ALT levels occurred with refeeding and weight restoration in anorexic patients. This study showed that transaminase levels of the liver inversely correlate with body weight, suggesting an effect of the nutritional state on the liver tissue of these animals. In addition, some hepatocytes showed necrosis and autophagic cell death characteristics that are considered the major pathway of severe liver failure with acute malnutrition (Kheloufi *et al*., 2014).

The study also showed a decrease in the ACP levels in liver tissue which could be due to hepatocyte damage and bile ducts occlusion because of the proliferation of its cells and progressive liver necrosis due to starvation that causes metals deficiency which in turn has essen-

Table 4. The histopathological lesions grading in the three groups.

	Grading level		
Histopathological Lesion	**First Group (control)**	**Second Group**	**Third Group**
Hepatocyte autophagy	0*	11**	30***
Hepatocyte swelling	1*	10**	22***
Hepatic fibrosis	0*	5**	16***
Cellular infiltration	1*	6**	19***
Congestion at portal area	1*	5**	10**
Microvesicular steatosis	0*	3**	5**

* Grade 0: represented 0-1% rate of tissue lesions;** Grade I (mild): represented 3-11% of the hepatocyte field;*** Grade II ((moderate) : represented 12-30 % of the hepatocyte field

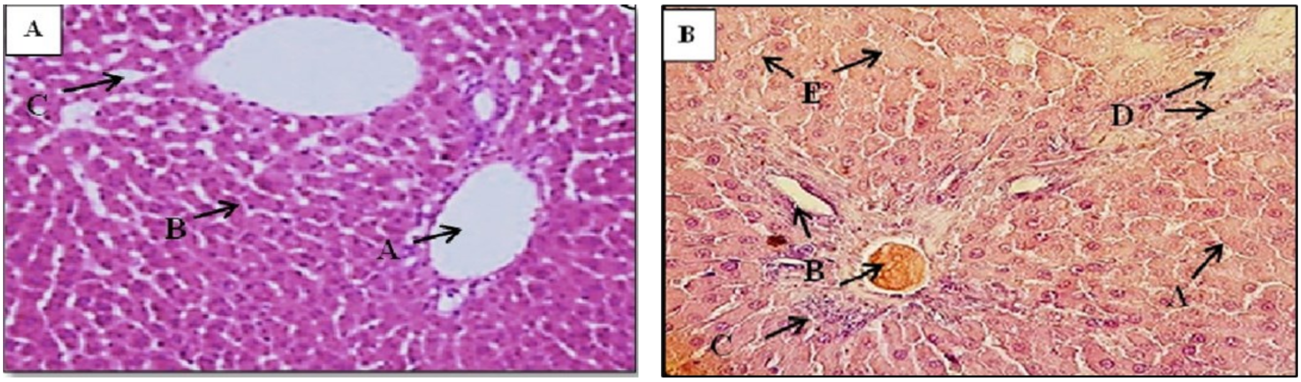

Fig. 5. ***Picture A-*** *Histological section of the female rats liver from the first Group (25 g/day food) showing normal liver structures. (A) central vein, (B) hepatocytes, (C) sinusoid;* ***Picture B-*** *Histological section of rats liver from the second Group (15 g/day food) showing (A)hepatocytic swelling, (B) congestion at portal area and bile duct hyperplasia, (C) cellular infiltration, (D)centrilobular fibrosis, (E)hepatocyte with features of autophagic cell. (H & E, 400X).*

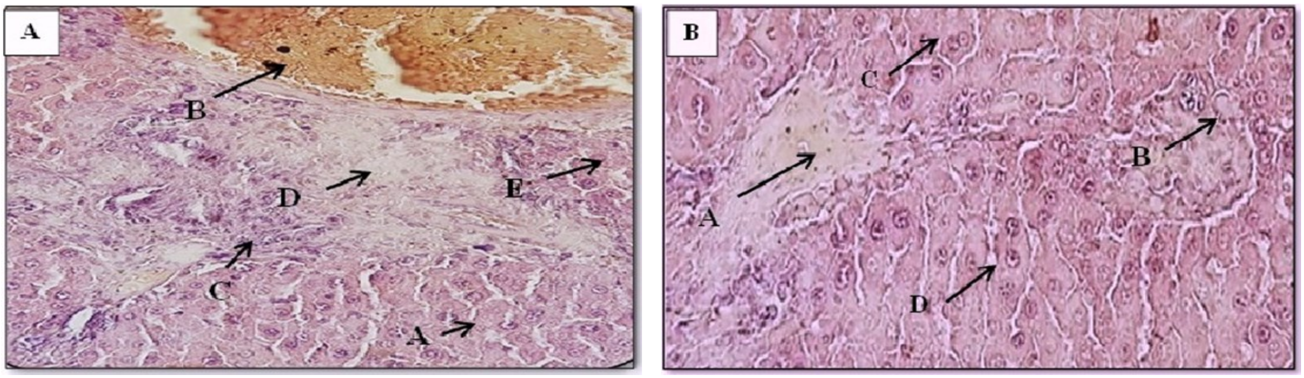

Fig. 6. ***(Image A and B****) histological section of rats liver from the third Group (5 g/day food)* ***(Image A)****showed (A) increase hepatocyte swelling, (B)congestion at central vein, (C)cellular infiltration, (D)hepatocyte with features of autophagic cell. (E)mild microvesicular steatosis in the hepatocyte (H & E, 400X). (****Image B****) showed (A) dilation area of centrilobular fibrosis. (B) hepatocyte with features of autophagic cell death. (c) observed mild microvesicular steatosis in the hepatocyte, (D) cellular infiltration (H & E, 400X)*

tial roles in the levels of these enzymes (Yousef *et al*., 2002).

The serotonin or 5-hydroxytryptamine (5-HT) is synthesized (about 90%) via enterochromaffin cells in the digestive system and it is exported to different sites around the body like the liver (Cornide-Petronio *et al*., 2020) .Serotonin considers neurotransmission within the central and autonomic nervous systems. Peripherally, 5-HT acts on vascular relaxation and contraction, cell proliferation, gastrointestinal motility, platelet aggregation and apoptosis (Dhanda and Sandhir, 2015 and Ko *et al*., 2021). Serotonin induces hepatocyte proliferation, So decreased levels of serotonin may lead to damage to hepatocytes due to food-eating deficiency, which leads to programmed death of some cells and necrosis of others. Since liver enzymes are concentrated in the cytoplasm of cells, the destruction of these cells leads to the infiltration of these enzymes into the blood and its high level (Cornide-Petronio *et al*., 2020).

Protein levels observed a remarkable decrease due to reduced amino acid availability necessary for protein synthesis and that could be due to increasing protein and lipid metabolism as a response to food deprivation and hypoglycemia. Thus, malnutrition results in reduced enzyme levels and protein production. In addition, rising in protein degeneration may affect the antioxidant enzymes (Marczuk-Krynicka *et al*., 2003 and De Caprio *et al*., 2006). Hepatic antioxidant enzyme activities (catalase, GPX and SOD) in table-2 showed significantly reduced in rats due to food deprivation that has a pro-oxidative impact as a result of increased free radical oxygen (ROS) generation, thus, it could decrease antioxidant enzyme activities. Starvation is known to increase the influx of fatty acids during the peroxisomal oxidation pathways, resulting in increased H_2O_2 generation. However, one study has reported starvation was not involved with increased oxidative stress in mallards (Sylvie *et al*., 2012).

The elevation of MDA in restricted groups of the study, indicates a rise in ROS production due to decreased GPx and SOD activities after food restriction stress, may play an important role in malnutrition syndromes (Marczuk-Krynicka *et al*., 2003). Previous studies showed that the antioxidant enzyme activity has a correlation with hypoinsulinemia in starved rats and diabetic rats, which suffered from antioxidant enzyme activity disorders, but treatment with refeeding and insulin injection in starved or diabetic rats may improve antioxidant enzyme disorders (Marczuk-Krynicka *et al*., 2003 ; Wasselin *et al*., 2014; Mojahed *et al*., 2016 and Sadowska *et al*., 2019). Exposure to the multiple T maze test arena showed an increase in locomotors activity in restricted rats (Fig.3). Feeding reduction initiates a cascade of metabolic and neurobiological events and has multiple influences on a difference in behavioural systems which include the hyperactivity that considers forms increased exploratory behaviour of rats (Dietze *et al*., 2016). The histological study of liver tissues of the control group displayed normal structure (Fig.5). The obvious histopathological variations are shown in the liver of food-restricted rats, hepatic glycogen reduced after food deprivation was associated with hypoglycemia and liver autophagic vacuoles increasing (Fig. 6 and Table 4). This study showed that hepatic steatosis is characterized by intracellular accumulation of lipids in cytoplasmic vacuoles representing stored lipid droplets by food restriction, steatosis was involved with lower antioxidant enzymes of hepatic cells. Previous studies have shown that glucose regulation during fasting can increase the hepatic contents of triacylglycerol and lead to eventually the accumulation of lipid in the liver parenchymas (Marks *et al*., 2015).

Conclusion

The present results concluded that food restriction stress (animal anorexia models) caused a significant increase in the levels of aminotransferases and MDA levels in liver tissue and a decrease in the activity of antioxidant enzymes. Total protein and glycogen levels were significantly ($p<0.05$) decreased in the liver tissues of the restricted females. The present results support the presumption that removing certain elements from food may reduce serotonin levels in liver regions which may impact liver enzyme function. Food restriction also caused hyperactivity behaviours in female rats. This study showed occur necrosis and apoptosis in the hepatocyte which is the major mechanism leading to autophagy during acute liver disorders.

ACKNOWLEDGEMENTS

I am pleased to thank the Head of the Department of biology /College of Science /Babylon University, for providing the necessary facilities and support during this study.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Carrera, O., Fraga, Á., Pellón, R. & Gutiérrez, E., (2014). Rodent model of activity□based anorexia. *Current Protocols in Neuroscience*, 67(1), 9–47. 10.1002/047 1142301.ns0947s67.
2. Celik, M., Sermatov, K., Abuhandan, M., Zeyrek, D., Kocyigit, A. & Iscan, A., (2012). Oxidative status and DNA damage in chidren with marasmic malnutrition. *Journal of Clinical Laboratory Analysis*, 26(3), 161–166. doi: 10.100 2/ jcla.21505
3. CHabuk, A-H. H., Al-Saadi, H. K. Z. & Al-Hamairy, A. K., (2016). Effect of the Experimental Infection withToxoplasma gondii on some Biochemical aspects and Histological Changes for the Liver and Spleen in Female Rats. *International Journal of PharmTech Research*, 5(11), 142-150.
4. Cornide-Petronio, M. E., Álvarez-Mercado, A. I., Jiménez-Castro, M. B. & Peralta, C. (2020). Current knowledge about the effect of nutritional status, supplemented nutrition diet, and gut microbiota on hepatic ischemia-reperfusion and regeneration in liver surgery. *Nutrients*, 12(2), 284. doi.org/10.3390/nu12020284.
5. De Caprio, C., Alfano, A., Senatore, I., Zarrella, L., Pasanisi, F. & Contaldo, F. (2006). Severe acute liver damage in anorexia nervosa: two case reports. *Nutrition*, 22(5), 572–575. doi.org/10.1016/j.nut.2006.01.003
6. Dietze, S., Lees, KR., Fink, H., Brosda, J. & Voigt J-P. (2016). Food deprivation, body weight loss and anxiety-related behaviour in rats. *Animals*, 6(4),1-14. doi:10.3390/ ani6010004.
7. Dhanda, S. & Sandhir, R. (2015). Role of dopaminergic and serotonergic neurotransmitters in behavioral alterations observed in rodent model of hepatic encephalopathy. *Behavioural brain research*, 286, 222-235. doi.org/10.1016/j.bbr.2015.01.042.
8. Gibson-Corley, K. N., Olivier, A. K. & Meyerholz, D. K. (2013). Principles for valid histopathologic scoring in research. *Veterinary Pathology*, 50(6), 1007–1015. 10.1177/0300985813485099.
9. Hanachi, M., Melchior, J. C. & Crenn, P. (2013). Hypertransaminasemia in severely malnourished adult anorexia nervosa patients: risk factors and evolution under enteral nutrition. *Clinical Nutrition*, 32(3), 391–395. doi.org/10.1016/ j.clnu.2012.08.020
10. Harris, R. H., Sasson, G. & Mehler, P. S. (2013). Elevation of liver function tests in severe anorexianervosa. *International Journal of Eating Disorders*, 46(4), 369–374. doi.org/10.1002/eat.22073.
11. Jáuregui-Lobera, I., Ezquerra-Cabrera, M., Carbonero-Carreño, R. & Ruiz-Prieto, I. (2013). Weight misperception, self-reported physical fitness, dieting and some psychological variables as risk factors for eating disorders. *Nutrients*, 5 (11), 4486–4502. doi.org/10.3390/nu5114486.
12. Kheloufi, M., Boulanger, C. M., Durand, F. & Rautou, P.-E. (2014). Liver autophagy in anorexia nervosa and acute liver injury. *BioMed Research International*, 2014,1-12. doi.org/10.1155/2014/701064.
13. Kim, S. F. (2012). Animal models of eating disorders. *Neuroscience*, 1(211), 2–12. doi: 10.1016/ j.neuroscience.2012.03.024.
14. Ko, M., Kamimura, K., Owaki, T., Nagoya, T., Sakai, N.,

Nagayama, I. & Terai, S. (2021). Modulation of serotonin in the gut-liver neural axis ameliorates the fatty and fibrotic changes in non-alcoholic fatty liver. *Disease models & mechanisms*, 14(3), 1-12. doi.org/10.1242/dmm.048922.

15. Koga, A., Murakami, M., Kurihra, Y., Ishida, T., Hosokawa, M., Tamura, N. & Kawai, K. (2021). Portal hypertension in prolonged anorexia nervosa with laxative abuse: a case report with liver and kidney biopsy data. *Eating and Weight Disorders-Studies on Anorexia, Bulimia and Obesity*, 26(2), 733–738. doi.org/10.1007/s40519-020-00902-x.
16. Koga, A., Toda, K., Tatsushima, K., Matsuubayashi, S., Tamura, N., Imamura, M. & Kawai, K. (2019). Portal hypertension in prolonged anorexia nervosa with laxative abuse: A case report of three patients. *International Journal of Eating Disorders*, 52(2), 211–215. 10.1002/eat.23007.
17. Lennerz, J. K., Hurov, J. B., White, L. S., Lewandowski, K. T., Prior, J. L., Planer, G. J. & Piwnica-worms, H. (2010). Loss of Par-1a / MARK3 / C-TAK1 Kinase Leads to Reduced Adiposity, Resistance to Hepatic Steatosis, and Defective Gluconeogenesis. *Molecular and cellular biology*, 30 (21), 5043-5056.
18. Mai-siyama, I. B., Isyaku, M. U., Atiku, I. A., Muhammad, A. S. & Onazi, H. U. (2017). The effect of starvation on blood parameters, electrolytes and liver enzymes in albino rats. *Dutse J. Pure and Appl. Sci*, 3(2), 421–427.
19. Marczuk-Krynicka, D., Hryniewiecki, T., Piątek, J. & Paluszak, J. (2003). The effect of brief food withdrawal on the level of free radicals and other parameters of oxidative status in the liver. *Medical Science Monitor*, 9(3), BR131–BR135.
20. Marks, K. A., Marvyn, P. M., Henao, J. J. A., Bradley, R. M., Stark, K. D. & Duncan, R. E. (2015). Fasting enriches liver triacylglycerol with n-3 polyunsaturated fatty acids: implications for understanding the adipose–liver axis in serum docosahexaenoic acid regulation. *Genes & Nutrition*, 10(6), 1–14. doi.org/10.1007/s12263-015-0490-2.
21. Mojahed, L. S., Mehdi, S., Mohammadi, M. M. & Nazifi, S. (2016). Short Period Starvation in Rat: The Effect of Aloe Vera Gel Extract on Oxidative Stress Status Ion. *İstanbul Üniversitesi Veteriner Fakültesi Dergisi*, 43(1), 32–38.
22. Namazi, F., Omidi, A., Abbasi, S., Afsar, M., Honarmand, M. & Nazifi, S. (2016). Starvation and refeeding in rats: effect on some parameters of energy metabolism and electrolytes and changes of hepatic tissue. *Pesquisa Veterinária Brasileira*, 36, 101–105. 10.1590/S0100-736X2016001300015.
23. Narayanan, V., Gaudiani, J. L., Harris, R. H. & Mehler, P. S. (2010). Liver function test abnormalities in anorexia nervosa—cause or effect. *International Journal of Eating Disorders*, 43(4), 378–381. doi.org/10.1002/eat.20690.
24. Oliveras-López, M.-J., Ruiz-Prieto, I., Bolaños-Ríos, P., De la Cerda, F., Martín, F. & Jáuregui-Lobera, I. (2015). Antioxidant activity and nutritional status in anorexia nervosa: effects of weight recovery. *Nutrients*, 7(4), 2193–2208. doi:10.3390/nu7042193.
25. Oudman, E., Wijnia, J. W., Oey, M. J., van Dam, M. J. & Postma, A. (2018). Preventing Wernicke's encephalopathy in anorexia nervosa: A systematic review. *Psychiatry and Clinical Neurosciences*, 72(10), 774–779. doi:10.1111/pcn.12735.
26. Rautou, P., Cazals–Hatem, D., Moreau, R., Francoz, C., Feldmann, G., Lebrec, D. & Durand, F. (2008). Acute liver cell damage in patients with anorexia nervosa: a possible role of starvation-induced hepatocyte autophagy. *Gastroenterology*, 135(3), 840–848.
27. Rosen, E., Bakshi, N., Watters, A., Rosen, H. R. & Mehler, P. S. (2017). Hepatic complications of anorexia nervosa. *Digestive Diseases and Sciences*, 62(11), 2977–2981. DOI 10.1007/s10620-017-4766-9.
28. Sadowska, J., Dudzińska, W., Skotnicka, E., Sielatycka, K. & Daniel, I.(2019). The impact of a diet containing sucrose and systematically repeated starvation on the oxidative status of the uterus and ovary of rats. *Nutrients*, 11(1544), 2 -14. doi:10.3390/nu11071544.
29. Schalla, M. A. & Stengel, A. (2019). Activity based anorexia as an animal model for anorexia nervosa–a systematic review. *Frontiers in nutrition*, 6 (69), 1-24. doi.org/10.3389/fnut.2019.00069.
30. Siegfried, Z., Berry, E. M., Hao, S. & Avraham, Y. (2003). Animal models in the investigation of anorexia. *Physiology & Behaviour* , 79(1), 39–45. doi:10.1016/S0031-9384(03)00103-3.
31. Sylvie, G., Marion, K., Yvon, L. M., Jean-Patrice, R. & Criscuolo, F. (2012). Of the importance of metabolic phases in the understanding of oxidative stress in prolonged fasting and refeeding. *Physiological and Biochemical Zoology*, 85 (4), 415–420. doi.org/10.1086/666364.
32. van den Berg, E., Houtzager, L., de Vos, J., Daemen, I., Katsaragaki, G., Karyotaki, E. & Dekker, J. (2019). Meta□analysis on the efficacy of psychological treatments for anorexia nervosa. *European Eating Disorders Review*, 27(4), 331–351. doi.org/10.1002/erv.2683.
33. Wasselin, T., Zahn, S., Maho, Y. Le, Dorsselaer, A. Van, Raclot, T. & Bertile, F. (2014). Exacerbated oxidative stress in the fasting liver according to fuel partitioning. *Proteomics*, 14(16), 1905–1921. doi.org/10.1002/pmic.201400051.
34. Wojciak, R. W. (2014). Alterations of selected iron management parameters and activity in food-restricted female Wistar rats (animal anorexia models). *Eating and Weight Disorders-Studies on Anorexia, Bulimia and Obesity*, 19(1), 61–68. doi.org/10.1007/s40519-013-0078-z.
35. Kumar, S. P., Musthafa, M. S., Sharadha, A., & Daniel, G. S. (2010). Histological changes in the hep histological changes in the hepatopancreas of tiger shrimp ancreas of tiger shrimp ancreas of tiger shrimp, penaeus monodon penaeus monodon exposed to sublea exposed to sublea exposed to subleathal concentra thal concentra thal concentrations of lead nitra lead nitrate. *Journal of Basic and Applied Biology*, 4(1&2), 120-124.
36. Yousef, M. I., Hendy, H. A. El, El-demerdash, F. M. & Elagamy, E. I. (2002). Dietary zinc deficiency induced-changes in the activity of enzymes and the levels of free radicals , lipids and protein electrophoretic behaviour in growing rats. *Toxicology*,175, 223–234.
37. Zhang, M., Song, G. & Minuk, G. Y. (1996). Effects of hepatic stimulator substance, herbal medicine, selenium/vitamin E, and ciprofloxacin on cirrhosis in the rat. *Gastroenterology*, 110(4), 1150–1155. doi.org/10.1053/gast.1996.v110.pm8613004.
38. Zhang, K., Li, X., Wang, X., Zheng, H., Tang, S., Lu, L. & Ma, X. (2020). Gut barrier proteins mediate liver regulation by the effects of serotonin on the non-alcoholic fatty liver disease. *Current Protein and Peptide Science*, 21(10), 978-84. doi.org/10.2174/1389203721666200615171928.