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 re-
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°C ±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) 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 microscopy, 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 perportal 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 behavioural 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. 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

**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, total hepatic protein, hepatic glycogen and serotonin) as shown in Table 1.

**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)

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

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.

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)

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

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 second and third groups.

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).

<table>
<thead>
<tr>
<th>Correlations</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
<th>ACP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin</td>
<td>Pearson Correlation</td>
<td>-0.579*</td>
<td>-0.690**</td>
<td>-0.623**</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>0.012</td>
<td>0.002</td>
<td>0.006</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Histopathological Lesion</th>
<th>First Group (control)</th>
<th>Second Group</th>
<th>Third Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocyte autophagy</td>
<td>0*</td>
<td>11**</td>
<td>30***</td>
</tr>
<tr>
<td>Hepatocyte swelling</td>
<td>1*</td>
<td>10**</td>
<td>22***</td>
</tr>
<tr>
<td>Hepatic fibrosis</td>
<td>0*</td>
<td>5**</td>
<td>16***</td>
</tr>
<tr>
<td>Cellular infiltration</td>
<td>1*</td>
<td>6**</td>
<td>19***</td>
</tr>
<tr>
<td>Congestion at portal area</td>
<td>1*</td>
<td>5**</td>
<td>10**</td>
</tr>
<tr>
<td>Microvesicular steatosis</td>
<td>0*</td>
<td>3**</td>
<td>5**</td>
</tr>
</tbody>
</table>

* 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
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 (Comide-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 \( \text{H}_2\text{O}_2 \) 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.

![Image A](image1.png) **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).

![Image B](image2.png) **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).
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 area showed an increase in locomotor 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.

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Conflict of interest

The authors declare that they have no conflict of interest.

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