The Effect of Time-Restricted Feeding on Pathological Histological Changes in the Liver and its Relationship to Blood Lipid Disorders and Liver Function in an experimental Model of Obesity

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ABSTRACT

The metabolic liver disease known as non-alcoholic fatty liver disease (NAFLD) has a wide range of liver pathology, such as fibrosis and mild steatosis steatohepatitis (NASH). The resultant effects may be cirrhosis or hepatocarcinoma. Nonalcoholic fatty liver disease (NAFLD) has a high societal and economic burden since it is the major cause of chronic liver disease in the world. Its prevalence is increasing, as well as obesity and metabolic syndrome. objectives: to determine the lipid profile and liver enzyme levels on serum and compare it to the normal population in nonalcoholic fatty liver disease. The study involved 36 3 months of age male albino rats with a weight range of 150-200 g. The animals were selected on the basis of similarity in terms of age and weight in order to decrease the biological variability. For each kilogram of combination, the burger patties were made with 850 grams of lean ground beef, 150 grams of beef fat, 15 grams of salt, 10 grams of minced fresh garlic, and 10 grams of hamburger spice. In order to prepare the crispy chicken, the chicken pieces were initially dipped into a dry mixture of wheat, rice, corn and seasoned flour. They were then fried in hot vegetable oil (heated to 170-180 o C) until they turned golden brown and crispy, with the internal temperature maintained at 75 o C throughout the frying process. The serum ALT and AST enzyme activities were determined with the help of Randox ready-to-use assay kit (Laboratories Ltd., UK), according to the instructions given by the manufacturer. The Randox kit was used to measure both total and direct bilirubin using approved colorimetric methods. The control group's readings stayed within the range of 105-108 µL/L, while group B2 had the second highest ALT enzyme value at 167±10.2 µL/L, and group C had the lowest at 151±9.71 µL/L. Groups B and B2 had the second-highest and third-lowest AST values, respectively, at 170±11.8 µg/L and 162 ± 11.3 µg/L, whereas group A had the lowest value at 89±8.73 µg/L. The rise in A1 could be explained by a transitory shift in amino acid metabolism brought on by dietary restriction, whereas the rise in B and B2 is indicative of mitochondrial damage linked to lipid buildup. Poor hepatocyte function was evidenced by the disruption of biliary conjugation and hepatic secretion caused by the high-fat diet.

Keywords: Time-Restricted Feeding, Liver, Blood Lipid Disorders, Obesity.

Introduction

NAFLD is closely related to obesity that is experienced by more than 80 percent of adults. In example, it is suspected that severe obesity may be associated with a higher risk of fibrosis and cirrhosis. In developed countries, NAFLD has become a major health issue in the past 20 years. It can also be acquired by individuals who are not overweight but it has been associated with MetaS, T2DM, and obesity [1-3]. Organ problems that cause death to people with NAFLD are mostly cardiovascular diseases and not liver chronic disease. Besides the already known association between NAFLD and insulin resistance, the recent development of hepatic iron overload as a potential new factor in both disorders has been noted. In order to diagnose NAFLD, imaging or histology must show that there is hepatic steatosis. Another thing that needs to be checked out are secondary reasons of hepatic fat buildup, like steatogenic drugs, genetic diseases, or viral infections (like hepatitis C virus). Besides, men and women should not consume more than 20g and 30g of alcohol per day respectively. NAFLD can also proceed without being noticed until there is cirrhosis since most of the patients have no symptoms at all. Upon first diagnosis, the most commonly reported symptoms among individuals with NAFLD are right upper quadrant pain and fatigue. Ultrasonography can also present the echogenic liver in the patient with the condition or incident imaging studies may show liver fat as part of a right upper quadrant pain diagnosis [4, 5]. As part of liver-related serum tests, increased amounts of hepatocellular enzymes are common; serum alanine aminotransferase (ALT) is usually greater than serum aspartate aminotransferase (AST). Steatosis occurs in the liver soon after a high-fat diet (HFD) is taken. A recent study revealed that a diet high in saturated fat raised the risk intrahepatic TG levels among overweight persons compared to one high in free sugars. These findings lend some support to the notion that lipo-toxicity is a major contributor of NAFLD. In the liver, studies on marker levels in obese patients show that about 60 percent of the total triglyceride (TG) is made up of free fatty acids (FFAs) derived in the adipose tissue. The lipolysis of visceral adipose tissue can be accelerated in obese persons with insulin resistance (IR) and hyperinsulinemia. Continuous low-grade inflammation due to lipid toxicity is associated with NAFLD. In the last couple of years, a new term, the metabolic dyslipidemia, has been introduced to describe the dyslipidemia that arises in the combination of insulin resistance and fat. The primary cause of dyslipidemia, which is a characteristic of the contemporary epidemic of obesity, is adipokines that favor inflammation and insulin resistance. However, recent research demonstrates that dyslipidemia caused by obesity is not a single pathophysiological process, but a poly-morphous syndrome that manifests itself in many ways and is predetermined by a wide range of personal factors. This has led to mild dyslipidemia or none at all in some metabolically healthy obese (MHO) individuals. Dyslipidemia is a condition with high levels of triglycerides (TGs), low-density lipoprotein (LDL), total cholesterol, and high-density lipoprotein (HDL). Hepatocytes can also play a role in the progression of liver diseases due to the accumulation of additional fat in lipid droplets that are covered with different structural proteins. The lipid abnormalities that cause intrahepatic lipid accumulation in non-alcoholic fatty liver disease (NAFLD) are; reduced export of triglyceride (TG), augmented uptake of liver free fatty acids (FFA), augmented whole-body lipolysis, and augmented synthesis of very low-density lipoprotein (VLDL). These changes in lipid metabolism are associated with an increase in inflammation and oxidative stress, as well as abnormal production of adipokines (such as leptin, adiponectin, resisting, vastatin, and retinol-binding protein-4). Studies have indicated that ALT, AST, and γ-glutamyl-transferase (GGT) which are liver dysfunction indicators may prove useful in determining liver functioning and the indicators are linked to insulin resistance in the liver. While ALT is mostly found in the liver and is believed to be an indicator of liver damage, GGT is located on many cell surfaces and is extremely active in the kidney, pancreas, and liver [6, 7]. A possible explanation for the link between liver disease and type 2 diabetes could be hepatic enzymes, which are biological markers. The objectives of early NAFLD treatment which has gained a robust backing in the literature include aggravating factors reduction and lifestyle modifications. Various studies have revealed that this decrease in body weight, physical activity, and alcohol intake may be of great help in improving the symptoms of the disease and reversing the early fibrosis in some instances. The idea is to estimate the concentrations of lipid profile (cholesterol, HDL, VLDL, LDL, TG) and liver enzymes (AST, ALT, GGT) in the blood of individuals with nonalcoholic fatty liver disease and compare it with the healthy patients.

Materials and Methods

Experimental Animals

The study used 36 albino male rats aged between 3 months and weighted 150-200g. To minimize the biological variability, the animals were selected based on their close age and weight. The rats were confined in separate cages to eliminate any possible tendency of competitive food consumption and to ensure accurate intake of food. The entire experiment was also given to them with full access to clean water. The common animal housing conditions were a temperature of 22-25 o C and 12 hrs light/12 hrs dark. To aid them adapt to the experimental treatments all the animals were fed and given their usual food and drink over a period of 7 days. All protocols were followed to house, feed, anesthetize, sample, and sacrifice animals.

Experimental Design

This biochemical experiment was conducted during 28 days, to investigate the effects on the body weight, metabolic rate, and histological changes of diverse types of diets and time-restricted feeding schedule, where calorie consumption was different in the morning and evening. The acclimatization period was followed by the random allocation of six groups of six rats each to the animals. The task was performed in the following way:

First: Standard Food Groups

- Control Group A: Standard food without time restrictions.
- A1 Group: Standard food with time restrictions, distributing calories 25% in the morning and 75% in the evening.
- A2 Group: Standard food with time restrictions, distributing calories 75% in the morning and 25% in the evening.

Second: High-Calorie Food Groups

- B Group: High-calorie food without time restrictions.

- B1 Group: High-calorie food with time restrictions, distributing calories 25% in the morning and 75% in the evening.
- B2 Group: High-calorie food with time restrictions, distributing calories 75% in the morning and 25% in the evening.

Eating and Timing of Meals

A constant 25 g of food was given to each rat every day. The daily ration among the time-restricted groups was divided into two portions 6.25 g of 25% of the participants and 18.75 g of 75% according to the group design. The group A and group B were not timed meaning that they could have meals whenever they wished. Time-restricted groups A1, A2, B1 and B2 were fed twice daily at 10:00 AM and 10:00 PM to standardize feeding schedules and to reduce the variances in circadian rhythms.

Making High-Calorie Food

Burgers, French fries, and crispy chicken were cooked and served in the required time proportions of each group of people, and was a high-calorie meal.

Burger Preparation

For each kilogram of combination, the burger patties were made with 850 grams of lean ground beef, 150 grams of beef fat, 15 grams of salt, 10 grams of minced fresh garlic, and 10 grams of hamburger spice. A 4 mm fines grinder was used to grind the beef at a temperature of less than 4 o C. The well mixed materials were used to form patties that were 1-1.5 cm thick and had a weight of 80-100 g. The cooking of the patties was done at an altitude of between 180 and 200 degrees Celsius until an internal temperature of 72 degrees Celsius was achieved, which is in line with the standards of preparing burger patties.

Preparing Crispy Chicken

In order to prepare the crispy chicken, the chicken pieces were first dipped in a dry mixture of wheat, rice, corn and seasoned flour. Then they were cooked in vegetable oil which was heated up to 170-180 C till golden brown and crispy, ensuring the internal temperature remained at 75 C throughout the cooking process.

Preparation of Potato Fingers

The fresh potatoes used to make the potato fingers were all of the same size. The potatoes were washed, peeled and cut in fingers of similar sizes. Then they were pre-fried and rinsed to get rid of any extra starch. They were then dried, salted and cooked in sunflower oil using an electric fryer until crispy and golden brown.

Body weight and Feed intake measurements

The weight of each animal was measured with an electronic digital scale at the beginning of the experiment and weekly after that. To as much as possible we attempted to consider circadian rhythms by ensuring that we took our measurements at the same time each day. The following equation was used to determine weight gain:

Weight Gain = Final Weight − Initial Weight

Mice were placed in a state of fasting as per the accepted guidelines on the anesthesia of rodents by administering a 10 per cent solution of ketamine and 2 per cent xylazine. After the establishment of deep anesthesia, rodents were subjected to routine procedures in taking blood samples: plasma and serum samples in EDTA and anticoagulant-free tubes, respectively. Sterile syringes were used to collect the samples.

Serum and Plasma Separation and Sample Preservation

The serum and plasma were separated by centrifugation at 3000 rpm at room temperature using 10 minutes. In the laboratory, samples were stored in Eppendorf tubes and stored in the fridge until analysis of the chemical.

Biochemical Assays

ALT and AST activities in serum were determined with the help of Randox ready-to-use enzyme assay kit (Laboratories Ltd., UK), according to the procedure as described by the manufacturer. Total and direct bilirubin levels were measured by Randox kit with approved colorimetric techniques.

The blood was collected, the animals were cut open, and the intestines and liver taken out, and the rest of the blood rinsed out of the tissues with 0.9% physiological saline. The samples were fixed in a 10 percent neutral formalin solution during 24 hours according to the conventional protocols of histology, rinsed in the running water, dehydrated in an increased series of ethyl alcohols, cleared in xylene, saturated in paraffin wax, and embedded in wax molds. After the usual histology protocols, the histological sections were cut with a rotary dissection instrument to a depth of 5-7 µm. They were then placed on glass slides, dewaxed, rehydrated and stained with hematoxylin and eosin (H&E). Sections were mounted in DPX media and examined under various magnifications microscopically. Histological changes were recorded through taking representative digital photographs through the camera attached to the microscope.

Statistical Analysis

A totally random design (CRD) was used for statistical analysis of the data. To investigate the difference in the means we used the multiple range test of Duncan. The level of significance of $P < 0.05$ was determined. All of our analyses were performed on SAS 2012 software.

RESULTS AND DISCUSSION

Because of the reciprocal influence of food time and type on metabolic efficiency and hepatocyte integrity, the present investigation found that the rat groups' liver enzyme and bilirubin levels were significantly different. A study conducted by the Azemi et al. (2025) [10] revealed that the obese rats fed a high-fat diet experienced a reduction in the initial structural abnormalities in their liver when they were put on time-restricted feeding (TRF). Compared to the high-fat free-feeding group, TRF had a significant effect in the reduction of the hepatic fat accumulation and the histological organization of the liver. This effect is due to reduced hepatic metabolic load, increased lipid metabolism, and consequently, decreases the rate of fatty degeneration linked to obesity. These findings give support to the notion that the timing of intake of food at specific times that are in tandem with the natural circadian cycle of the body can make the liver stay healthy and can also slow the structural harm that high-calorie diets may inflict.

Table 1. Liver Functions.

Test	ALT u/L	AST u/L	Bilt mg/dL	Bilc mg/dL
A	105	89	0.18	0.09
A1	108	192	0.20	0.10
A2	106	150	0.21	0.09
B	167	170	0.28	0.19

B1	120	148	0.25	0.14
B2	151	162	0.27	0.12

Bilc: Conjugated bilitubin Bilt: Total bilirubin

ALT (Alanine Aminotransferase) Enzyme

The control group recorded readings of 105-108 μL/L and the second highest was group B2 with 167±10.2 μL/L and the lowest was group C with 151±9.71 μL/L. This provided more evidence that hepatic stress due to fat buildup within hepatocytes and changed mitochondrial enzyme activity was induced by the high-fat diet. This validates the protective effect of meal timing on liver damage as evidenced in a study by Feng et al. (2024) [11], who found that time-restricted feeding lower ALT in fatty liver mice than random fed groups. Moreover, Báez-Ruiz et al. (2025) [12] discovered that the genes in the liver concerned with oxidation and antioxidant activity can be restructured with a change in the timing of feedings. This, in turn, has the potential of reducing oxidative stress and enhancing enzyme indicators.

The same results were demonstrated by Wang et al. (2025) [13] who provided a high-fat diet model that enhanced liver functioning and decreased fatty degeneration when mice were permitted to eat only during the day. The current values showed a significant difference between group B1 (75% in the morning) and group B2 (75% in the evening), with the group with a higher percentage of morning consumption (group B1) having a higher level of ALT. This confirms the theory that consuming the majority of food in the morning enables the liver to function as per its circadian pattern and reduce the amount of fat-related damage. Jakubowicz et al. (2021) [14] showed that a high-fat meal consumed in the morning was better than that consumed in the evening in terms of metabolic response and lowering the increase in ALT. Subsequent studies [15, 16] compared nighttime feeding to time-restricted feeding in the early part of the day, which enhanced liver functioning and decreased inflammation- this was the case even in the presence of a high-fat diet.

AST (Aspartate Aminotransferase) Enzyme

AST was the highest in group A1 with a value of 192±12.2 μg/L, B and B2 had a value of 170±11.8 μg/L and 162±11.3 μg/L, respectively. The lowest value was in Group A at 89±8.73 μg/L. The increase in A1 might be due to a temporary change in the metabolism of amino acids induced by dietary restrictive effect and an increase in B and B2 suggests mitochondrial damage due to lipid accumulation. Even in rather balanced diets, the timing of meals can disrupt the hepatic circadian rhythm and decrease the activity of oxidative enzymes, as demonstrated by Guo et al. (2023) [17] and Liu et al. (2024) [18]. Feng et al. (2024) [11] discovered that models of fatty liver decreased the levels of ALT and AST when time-restricted feeding was applied, compared to the free-diet groups. This gives further support to the fact that restricted eating in the daytime alleviates liver damage caused by fat.

Total and Conjugated Bilirubin

When compared to the control groups, which had total bilirubin levels ranging from 0.18 to 0.21 mg/dL, groups B and B1–B2 had higher amounts (0.28±0.02 mg/dL and 0.25-0.27 mg/dL, respectively). The conjugated bilirubin levels in group B were also significantly higher (0.19 ± 0.03 mg/dL). This implies that the functioning of the hepatocytes was impaired because of the disruption of biliary conjugation and hepatic secretion by the high-fat diet. This observation is supported by the findings of both Cui et al. (2022) [19] and Chiang (2020) [20], who determined that a high-fat diet with the delayed time of meals influences gene expression that affects the bile acid metabolism and increases bilirubin levels. Although bilirubin values can range in some cases without a negative trend of progress, time-restrained feeding enhances liver functioning parameters and decreases inflammation compared to free-range diets, as indicated by Deng et al. (2025) [21] and Emara et al. (2024) [22]. We found that, B1 (75% in the morning) reduced biliary atresia, and preserved hepatocyte integrity, as B1 (75% in the morning) caused a relative decrease in bilirubin relative to B2 (75% in the evening). All things considered, the data show that enzyme and biliary marker levels are drastically affected by a high-fat diet, but that the timing of meals is a key regulator; feeding in the morning at the circadian rhythm had a substantial protective impact. This validates the idea that timing meals is a possible treatment to improve liver functioning and reduce the risk of fatty liver disease which is consistent with other studies [23] that established that time-restricted feeding restores liver gene expression and reduces inflammation and fat buildup despite daily calorie intake being the same.

Table 2. Blood Lipid Profiles mg/dL.

Test	**TC**	**TG**	**LDL**	**HDL**	**AIP TG/HDL**

A	**131.2**	**119.5**	**65.2**	**47.3**	**2.52**
A1	**132.3**	**117.8**	**66.8**	**51.1**	**2.30**
A2	**140.2**	**122.4**	**68.3**	**42.3**	**2.89**
B	**205.5**	**190.7**	**163.6**	**32.5**	**5.86**
B1	**172.4**	**169.3**	**120.4**	**40.2**	**4.21**
B2	**206.4**	**180.2**	**160.7**	**32.1**	**5.63**

Total Cholesterol (TC)

The results demonstrated that there were substantial differences across the groups ($p<0.05$). Group B and B2 had the highest levels (205.5±12.5 and 206.4±13.1 mg/dL) in comparison to the control groups A, A1, and A2, which had levels ranging from 131.2 to 140.2 mg/dL. The sudden increase in cholesterol production in hepatocytes caused by the high saturated fat and high simple carb content in fast food likely led to this sharp rise in cholesterol in blood through the downregulation of LDL receptor gene expression and upregulation of HMG-CoA reductase activity. Newer research, such as those of Qiu et al. (2024) and Habe et al. (2025) [24, 25], lend credence to this conclusion by showing that meals eaten at times not in sync with the circadian cycle enhance cholesterol buildup and hinder the elimination of low-density lipoproteins (LDL). Chaix et al. (2024) affirmed that early time-restricted feeding triggers the process of lipid oxidation and lowers cholesterol even without a calorie reduction. Group B1 (75% of meals in the morning) had a much lower level of cholesterol (mg/dL 172.4±11.4) than group B and B2. This indicates that breakfast meals were better metabolized and cholesterol levels decreased.

Triglycerides (TG)

There were notable variations among the groups, as indicated by statistical analysis ($p<0.05$). Groups B and B2 had much higher triglyceride values (190.7±12.9) and (180.2±12.2) compared to the control groups A, A1 and A2. Eating huge amounts of carbs and animal fats leads to the increased activity of liver enzymes that take part in de novo lipogenesis, or the creation of new fats. Conversely, comparing B1 (169.3±13.4) with B and B2, the relational decrease indicates that morning feeding facilitated the fatty acid oxidation and minimized the unnecessary lipogenesis at the period of high liver metabolic rates. Despite the identical intake of calories, a study by Petridi et al. (2024) and Xie et al. (2022) [26, 27] demonstrated that first-thing breakfast lowers triglycerides and increases insulin sensitivity. Daytime feeding decreases hepatic lipogenesis and switches on lipid oxidation genes, which are confirmed by Guo et al. (2023) [17]. A longitudinal study by Yoshida et al. (2018) found that night eating, particularly, eating dinner near bedtime and multiple meals following dinner, is associated with increased levels of triglycerides (TG) and total cholesterol (TC) and low-density lipoprotein (LDL), and an increased risk of dyslipidemia [28]. These findings offer further support that the timing of meals is a unique determinant that influences lipid homeostasis, contributing to the explanation of the fact that groups with members who consumed more in the evening had a higher index of blood lipids than those with members that consumed more during the day.

Low-Density lipoprotein cholesterol (LDL-C)

The findings showed that there were significant differences between the categories. Groups B and B2 were a little bit higher in LDL (163.6±11.73) and 160.745) compared to the rest of the groups, particularly the control (65-68 mg/dL). Factors behind this increase are an increase in the production of low-density lipoproteins due to excessive consumption of saturated fats and a reduction in liver-expression of LDL receptors, which results in lower levels of LDL being cleared out of the blood. The significant decrease in the LDL levels (Group B1 120.4±9.91) compared to Groups B and B2 suggests that the capacity of the liver receptors to eliminate LDL was enhanced and that accumulation was reduced when the feeding was recorded at the time of daytime metabolic activity. This lines up with the results of the study by Dote-Montero et al. (2023) [29], which shown that consuming a high-fat meal first thing in the morning reduces LDL levels, in contrast to doing so in the evening. The study conducted by Manoogian and Panda (2022) indicates that eating according to the biological clock improves the balance between the formation and excretion of cholesterol.

High-Density lipoprotein cholesterol (HDL-C)

Also, significant differences among the groups were observed ($p<0.05$). The HDL levels of the groups B and B2 were much lower (32.5±4.92 and 32.1±4.61 respectively) than the control groups (47-51 mg/dL). This decrease is due to impaired cholesterol reverse transport by decreased activity of liver enzyme LCAT, which forms HDL and inhibition of ApoA1 protein synthesis. The morning feeding increased the cholesterol reverse transport and enzyme activity that produces HDL as was seen by significantly higher levels of HDL in A1 (51.1±5.88) than in the other groups. Our findings are in line with findings by Habe et al., 2025 [25], who discovered that morning consumption enhances the activity of the genes that are relevant in the production of HDL and functional flexibility and that our findings are corroborative.

Cardiovascular Risk Index (AIP = TG/HDL)

The analysis revealed that there were significant differences between the groups ($p < 0.05$). Group B (5.86±0.82) and group B2 (5.63±0.99) had the highest values and indicate a greater risk of atherosclerosis and metabolic syndrome. The Atherogenic Index of Plasma is given by the following formula: AIP = TG/HDL. Despite the fact that the A1 and A values were not out of the normal range (<3), physiologically, high TG and low HDL leads to the creation of small dense LDL (sd-LDL), which is more likely to penetrate the vascular endothelium and induce chronic inflammation. This result is consistent with the results of Dobiášova and Fröhlich (2001) [30] who employed AIP as an indicator of atherosclerosis, and with Lumu et al. (2023) [31], who wrote that an AIP of more than 0.24 is a strong sign of metabolic risk. Sutton et al. (2018) state that breakfast has such an effect as raising the level of lipid oxidation activity and enhancing insulin sensitivity, which explains why B1 (4.21±0.90) is better than B and B2. The fact that fast food was found to significantly increase TC, TG, and LDL levels and significantly decreased HDL and significantly elevated AIP was supported by the statistical and physiological analysis of poor lipid metabolism and the high risk of liver disease and atherosclerosis. In comparison, lipid profiles were very much better when A1 and B1 were consumed simultaneously every morning. This was due to the fact that the meals were timed with the normal metabolic activity of the liver that minimized the oxidative stress and cellular inflammation. Such findings are consistent with other recent studies (Zhang et al., 2025) [15, 23, 27, 32] that espouse the notion that timing your foods is a key physiological factor in optimizing cholesterol metabolism, and maintaining the well-being of your liver and blood vessels.

Table 3. Average weight gain over 3 weeks.

B2	B1	B	A2	A1	A
226.6	248.5	236.7	170.5	204.5	227.6
Weight gain in 3 weeks			Weight gain in 3 weeks		

The change in weight during the four weeks of the experiment was significantly different ($p<0.05$) among the six groups as indicated in the table. This is indicative of the fact that the dietary type and the time of consumption have a dual effect on the energy balance and metabolic processes. Between week one and week four, the control group (A) had a steady 227.6 kg, which showed normal physiological development and energy balance. Physiological studies suggest that this trend can be attributed to a balance between the quantity of the energy intake and the quantity of the energy to repair and grow the proteins without excess fat storage. Metabolic obesity is a consequence of the overactivation of the mTOR pathway; a balanced diet maintains the feed conversion ratio of rats at normal level according to the research of Bensalem et al. (2023) [33]. Group A1 had a significant amount of weight (204.5 kg) than the control, indicating that the metabolic efficiency was enhanced by the time-limited morning feeding. Research indicates that breakfast enhances insulin and blood sugar levels, and increases fatty acid oxidation since it coincides with mitochondrial activity. The findings agree with the results of the Gong et al. (2025) [16] experiment which demonstrated that consuming breakfast raises the expression of proteins related to thermoregulation and lipid oxidation, resulting in reduced accumulation of adipose tissue, despite remaining calorie intake remaining constant.

Group A2 had a reduced weight gain of 170.5. This pattern is also followed by reduced metabolic efficiency at night due to the decreased nightly mitochondrial activity and insulin resistance to the uptake of glucose. Long-term effects of consuming at the time when the circadian clock between the muscle and the liver is out of sync are the accumulation of visceral fat and resistance to insulin, as demonstrated by a study by Gong et al. (2025) [16] and Garaulet et al. (2013) [34]. Mild inflammation was more evident in the night groups than in the morning groups and this is why. The third week had the highest average weight gain of 236.7 for the high-calorie, unrestricted group (B), which meant that they gained a lot of weight. This is supported by the adverse effects of fast food on the satiety and energy regulation. The fast food is rich in saturated fats and fried foods. Oxidized fat diets impair the signals sent by the hormones leptin and ghrelin, which stimulate people to eat more food when they are already full, as shown in studies by Cai et al. (2012) and Netam (2024) [35, 36]. Oxidized lipids also contribute to increase the hypothalamus production of reactive oxygen species (ROS) in addition to disrupting the neurological pathways that regulate hunger.

A high-fat meal was associated with poor glucose tolerance, increased LEAP2 gene expression in the liver, and decreased levels of active and total ghrelin, according to research by Holá et al. (2023) [37]. With the return to regular diet, LEAP2 gene expression returned to normal, and active ghrelin levels returned to normal, despite overall low levels of ghrelin. The action of ghrelin, which is mimicked by the stable analog: Dpr3 Ghrelin, is inhibited by a high fat diet, but this inhibition is reversible on returning to normal diet. Group B1 only put on an average of 248.5 pounds, which was not as physiologically damageable as group B, despite having higher amounts of high-energy fast food (75% in the morning and 25% in the evening). Exercising during the day with synchronization with feeding enhanced the weight gain pattern as compared to other methods as it maximized energy expenditure on growth and not fat storage. Recent studies indicate that the CD36 protein is a dual lipid metabolic regulator (Li et al., 2019; Wang et al., 2025) [13, 38]. It has the ability to inhibit lipid droplet autolysis via the AMPK controlled pathway, and it regulates hepatocyte metabolism by activating AMPK by phosphorylation, enhancing fatty acid oxidation and reducing their synthesis.

Studies have found that a high-fat diet in the daytime induces this metabolic pathway leading to a reduction in visceral fat storage of 18-25% and the efficiency of fatty acid oxidation in the liver. Along with reducing the risk of fatty liver disease and enhancing metabolic performance, time alignment with the body internal circadian rhythm reduces low-grade inflammation inhibiting normal metabolism. Group B2 that consumed 25 percent calories in the morning and 75 percent in the evening showed a significant but uneven weight gain of .226.6 indicating more fat mass rather than lean mass gain

The SREBP-1c pathway, which is involved in fatty acid production and storage in tissues, is more active throughout the night, which is associated with this. Digestion, absorption, and oxidation are influenced by the circadian cycle, according to Gu et al. (2020) [39] and Reytor-Gonzalez et al. (2025) [40]. Symptoms of late-night eating include triglyceride accumulation and insulin and cortisol insensitivity of tissues, which interferes with the production of insulin and cortisol. The decrease of metabolic rate and shift of preferences towards nutrition use are also two other consequences of daily metabolism control. A recent study by Vujović et al. (2022) demonstrated that late-night eating disrupts the ghrelin-to-leptin ratio that controls hunger and significantly increases hunger levels. It decreased the basal metabolic rate, caused the core body temperature to be low and less energy would be .used when awake

The results can be understood by considering the points made by Sa'ari et al. (2024) [41] and Davis et al. (2022) [42]. During their study, they discovered that individuals who have later meals in the day consume more sugary and fatty foods and their energy expenditure efficiency declines during the night as both their basal metabolic rate and thermic effect of food are lower. A positive energy balance, which is created due to the interaction of excess energy intake and circadian rhythm disruption, even with relatively constant food intake, encourages the growth of adipose tissue and gain of weight. Therefore, compared to eating during the day, eating at night is a source of weight gain and obesity on its own. The hypothalamic-pituitary axis governs hunger, fat catabolism, and metabolic efficiency in the body; it is provided with information by the periphery, such as leptin and GH, and combines it with the circadian rhythm in a complex mechanism that balances energy and body mass .[43] (Panda, 2016)

The researchers concluded that fast food which had high calories was the leading cause of weight gain in this physiological condition. The reason is that the fat oxidation is suppressed, the hormones which govern hunger are disturbed, and the fat accumulation is enhanced. This is in line with the results of Hall et al. (2019) [44] concerning the effects of ultra-processed foods on regulating hunger and increasing energy expenditure. Conversely, it was also evident that meals time had a significant impact on metabolic response. Groups A1 and B1 were more efficient in metabolism and less accumulated fats when they consumed the majority of their food in the morning, and Groups A2 and B2 consumed the majority of their food in the evening, was slower in metabolism and accumulated more fats. This can be attributed to the fact that metabolic enzyme activity and insulin sensitivity are best considered relative to the time of the day which individuals eat, which is evening (Garaulet et al., 2013).

The results support the hypothesis that, despite the high-energy diets, a circadian-timed diet can contribute to a more balanced energy state and reduce metabolic obesity. This lends credence to the idea of chrononutrition as a physiological strategy forwarding against metabolic diseases.

Table 4. Liver Functions

Conj.Bil. mg/dL	T.Bil. mg/dL	AST u/L	ALT u/L	Test
0.09 ± 0.01 c	0.18 ± 0.03 b	89 ± 8.73 e	105 ± 8.83 d	A
0.10 ± 0.02 bc	0.20 ± 0.01 b	192 ± 12.2 a	108 ± 9.11 d	A1
0.09 ± 0.02 c	0.21 ± 0.02 b	150 ± 10.3 d	106 ± 8.20 d	A2
0.19 ± 0.03 a	0.28 ± 0.02 a	170 ± 11.8 b	167 ± 10.2 a	B
0.14 ±0.03 b	0.25 ± 0.01 a	148 ± 10.6 d	120 ± 8.29 c	B1
0.12 ± 0.01 b	0.27 ± 0.03 a	162 ± 11.3 c	151 ± 9.71 b	B2

Conj. Bil.: Conjugated bilitubin T.Bil: Total bilirubin

Table 5. Blood Lipid Images mg/dL

AIP TG/HDL	HDL	LDL	T.G	T.Ch	Test
2.52 ± 0.68 b	47.3 ± 6.91 b	65.2 ± 7.47 c	119.5 ± 10.7 c	131.2 ± 10.3 d	A
2.30 ± 0.88 b	51.1 ± 5.88 a	66.8 ± 6.73 c	117.8 ± 10.2 c	132.3 ± 12.1 d	A1

2.89 ± 0.49 b	42.3 ± 7.01 c	68.3 ± 8.11 c	122.4 ± 12.7 c	140.2 ± 11.6 c	A2
5.86 ± 0.82 a	32.5 ± 4.92 d	163.6 ± 11.73 a	190.7 ± 12.9 a	205.5 ± 12.5 a	B
4.21 ± 0.90 a	40.2 ± 5.83 c	120.4 ± 9.91 b	169.3 ± 13.4 b	172.4 ± 11.4 b	B1
5.63 ± 0.99 a	32.1 ± 4.61 d	160.7 ± 10.45 a	180.2 ± 12.2 a	206.4 ± 13.1 a	B2

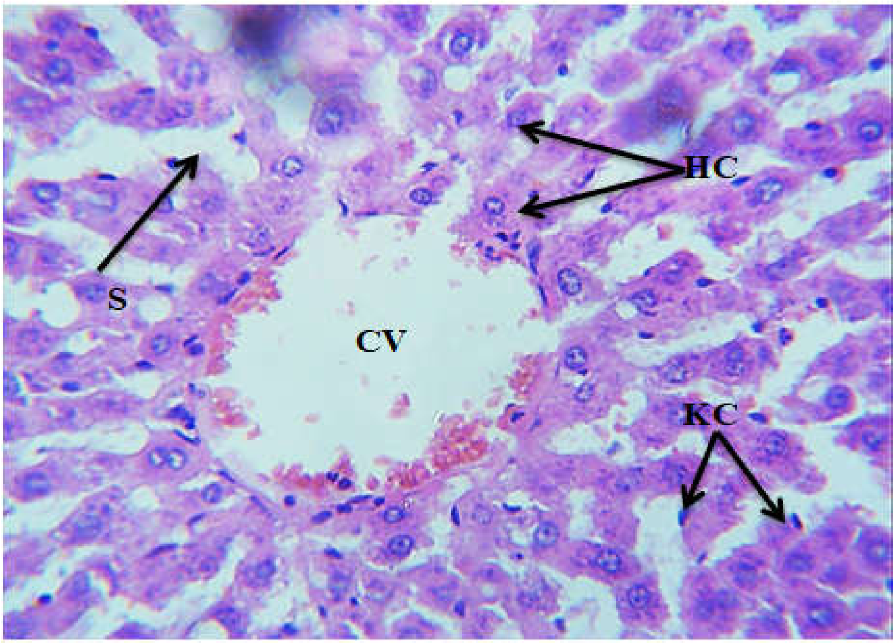

Image 1. Liver section from control group (A) showing the central vein (CV), hepatocytes (HC), and sinusoids (S). Kover cells (KC) can also be observed. H&E 4000X.

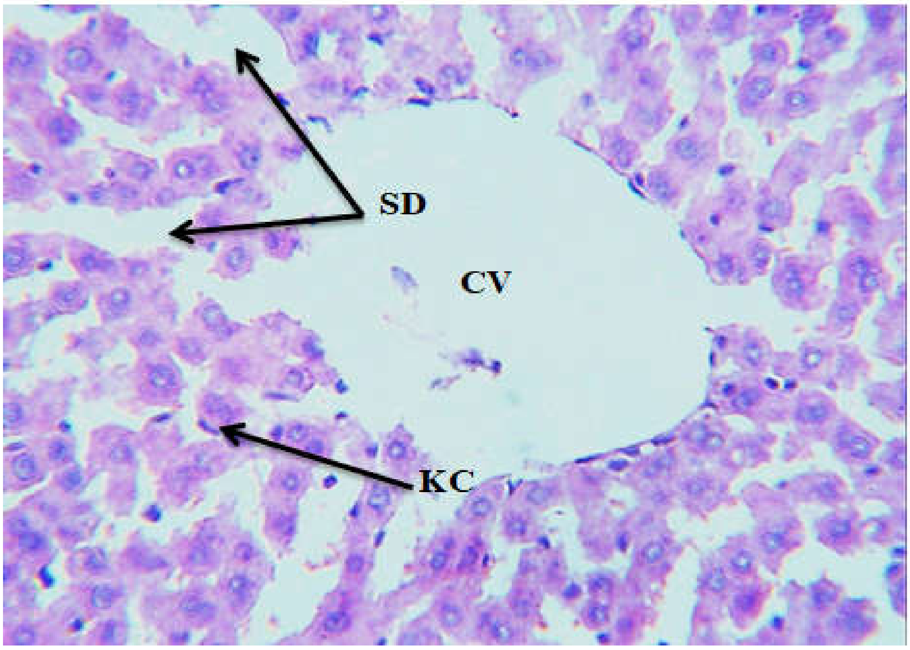

Image 2. Liver section of group (A1) showing the central vein (CV) and dilated sinusoids (SD). Kover cells (KC) can also be observed. H&E 4000X.

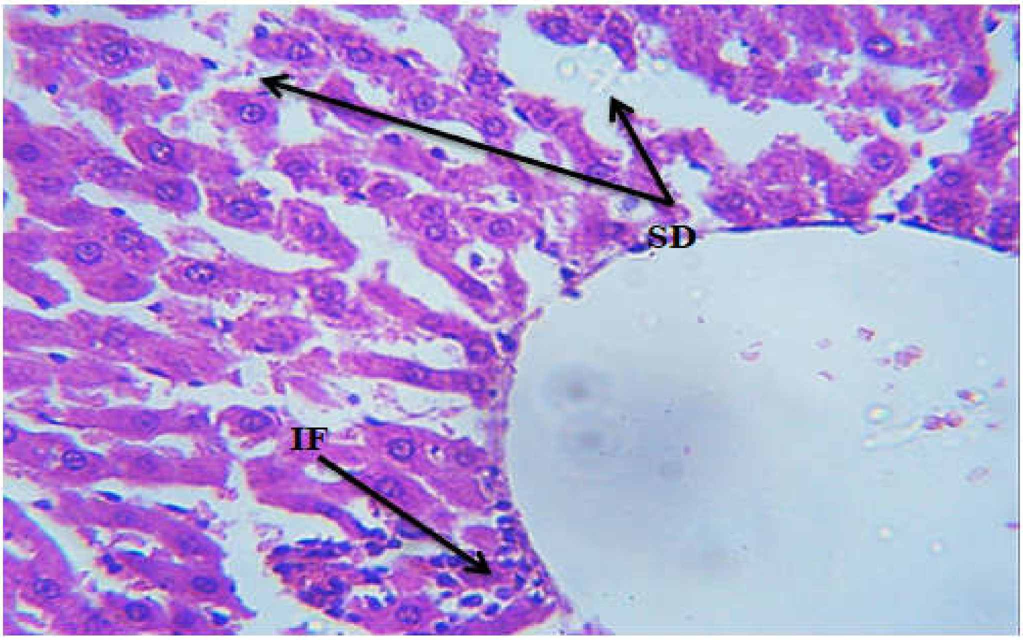

Image 3. Liver section of group A2 showing dilated sinusoids (SD). Inflammatory cell clusters (IF) can also be observed. H&e 4000X.

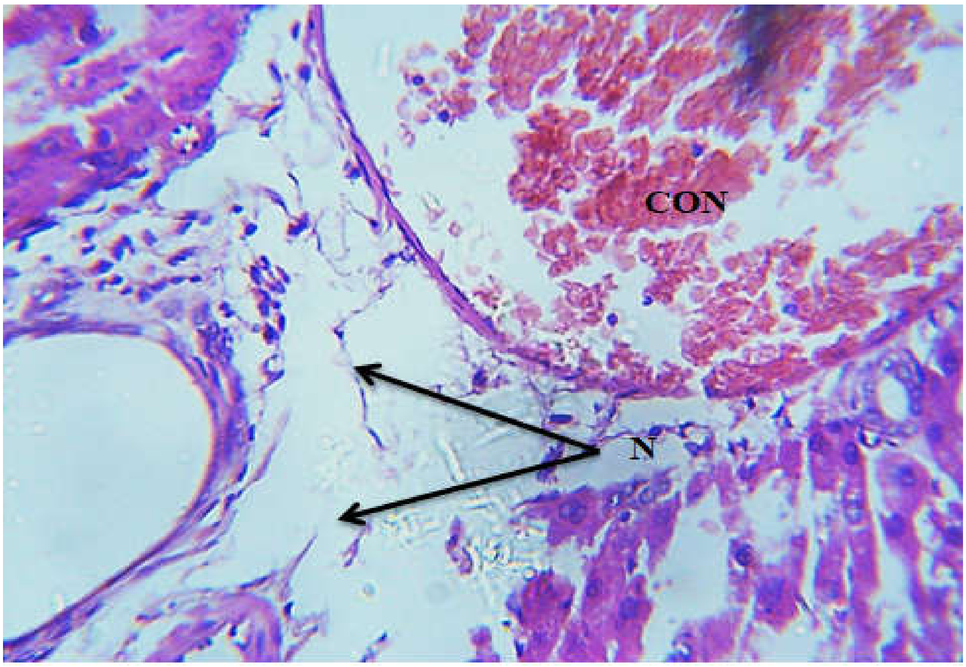

Image 4. Section of liver, group A2, showing vascular congestion (SD). Tissue necrosis (N) can also be observed in the liver tissue of the triad region. H & E 400X.

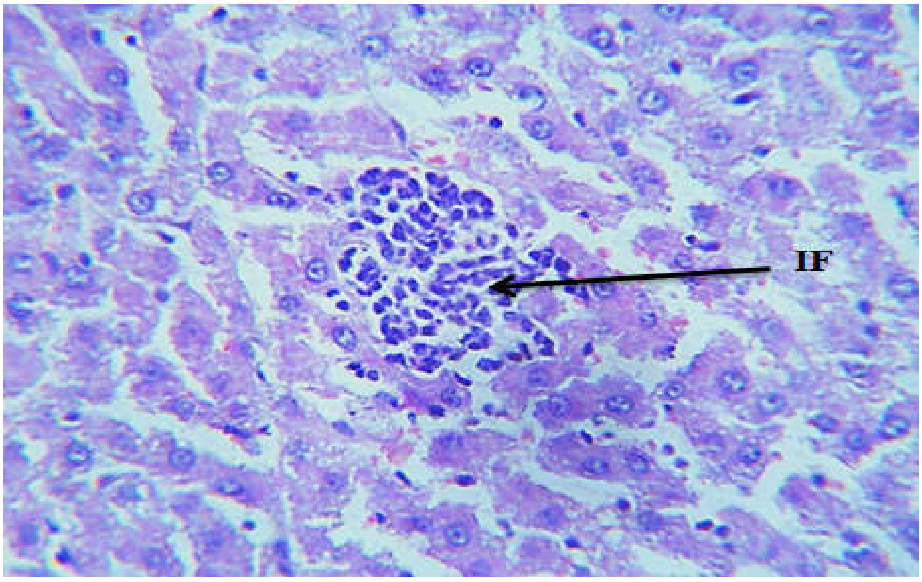

Image 5. Liver section (Group B) showing focal infiltration of inflammatory cells (IF) in the liver tissue. H & E 400X.

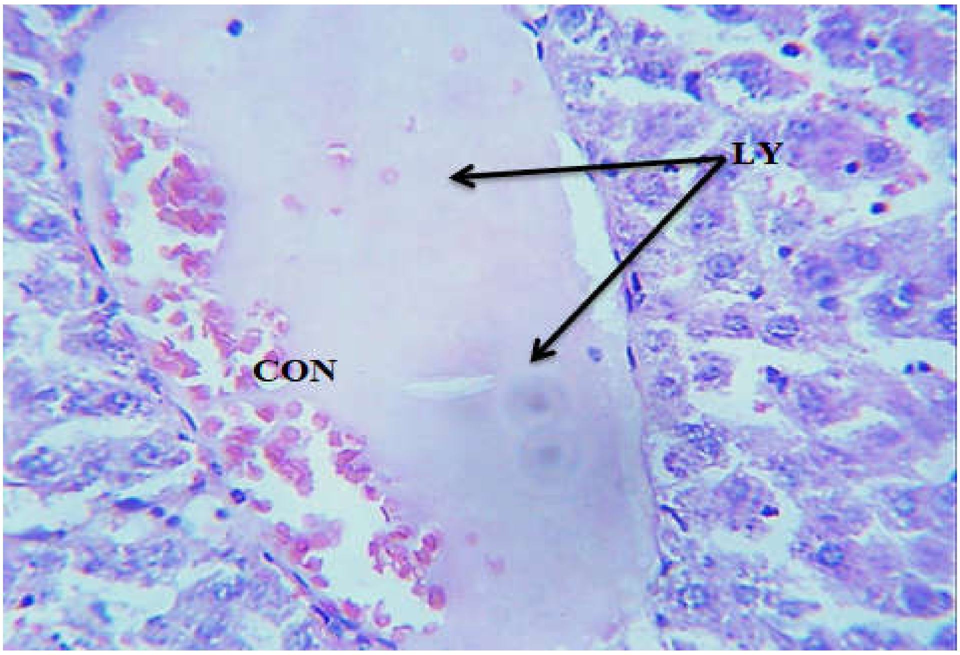

Image 6. Liver section (Group B) showing vascular congestion (CON) with hemolysis (LY). H & E 400X

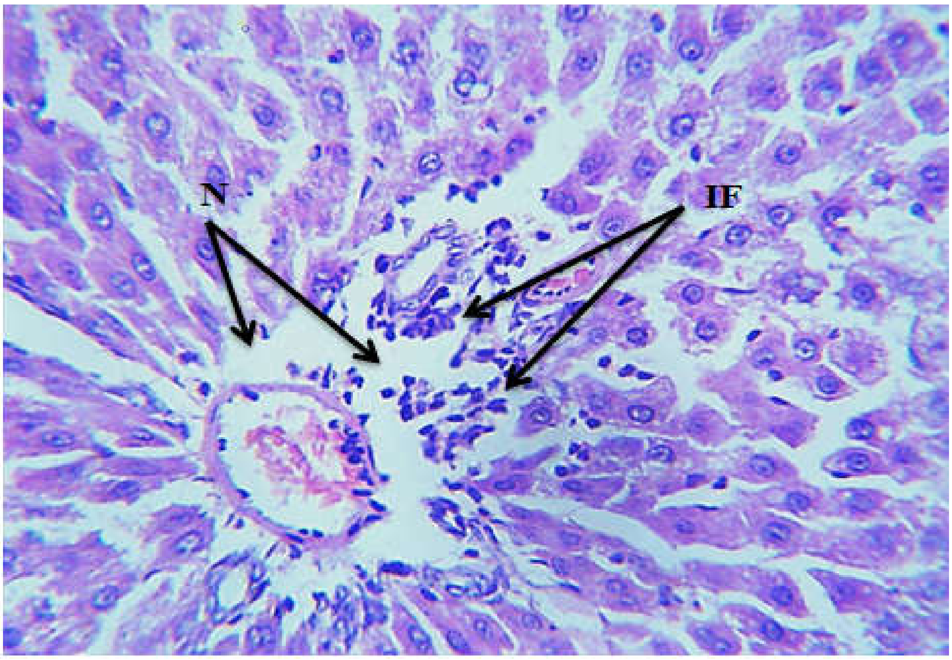

Image 7. Liver section (Group B) showing inflammatory cell infiltration (IF) with tissue necrosis (N) in the triad region. H & E 400X.

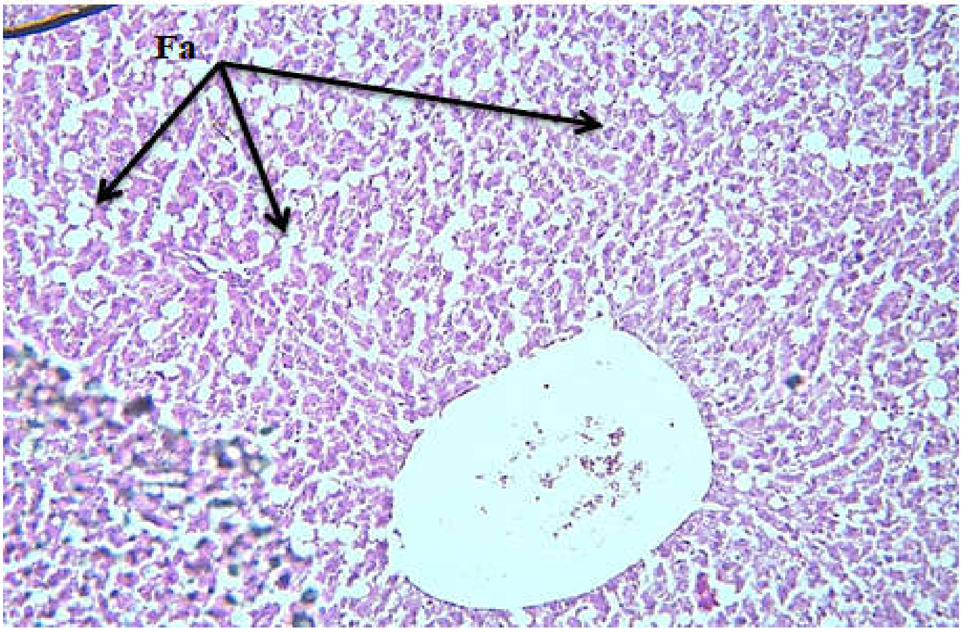

Image 8. Liver section (group B1) showing clear fatty liver (Fa).H & E 100X.

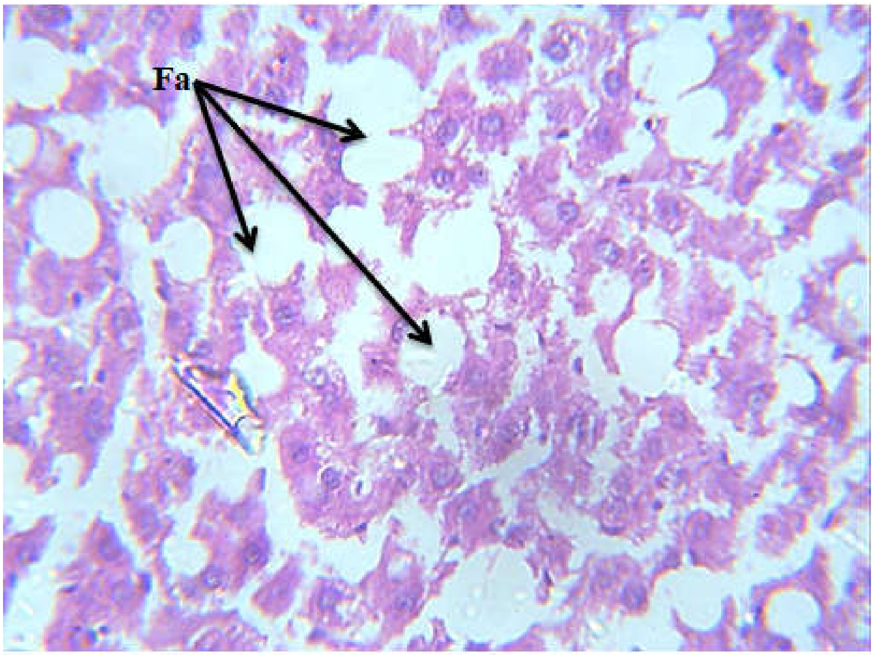

Image 9. Magnified section of the liver in group B1 showing clear fatty liver disease (Fa).H & E 400X.

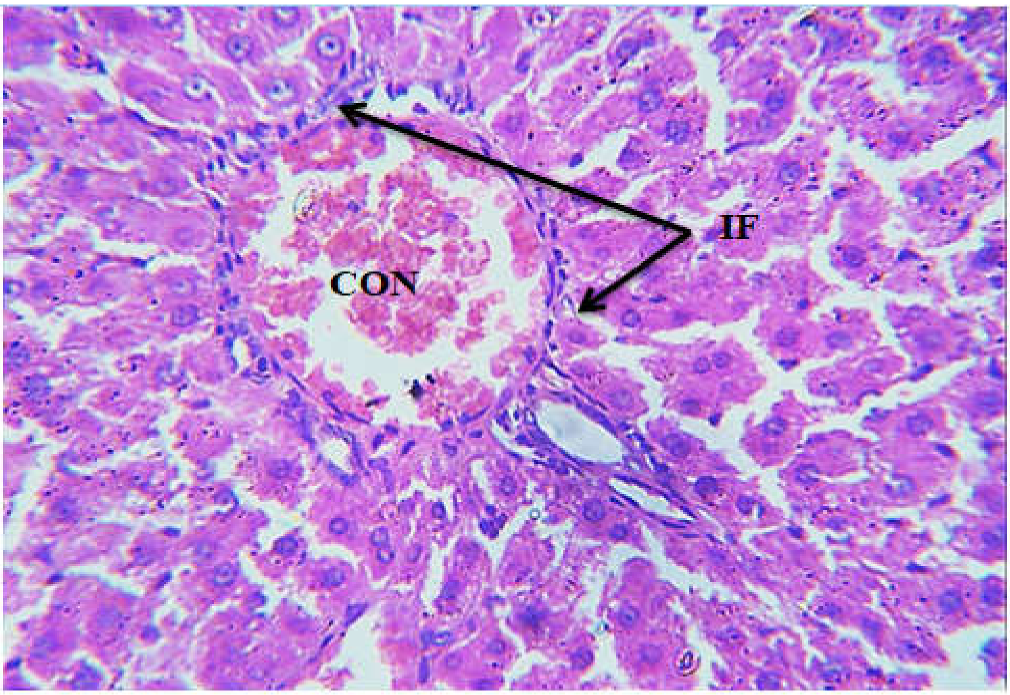

Image 10. Liver section (group B2) showing vascular congestion (CON) and inflammatory cell accumulation around it (IF). H & E 400X.

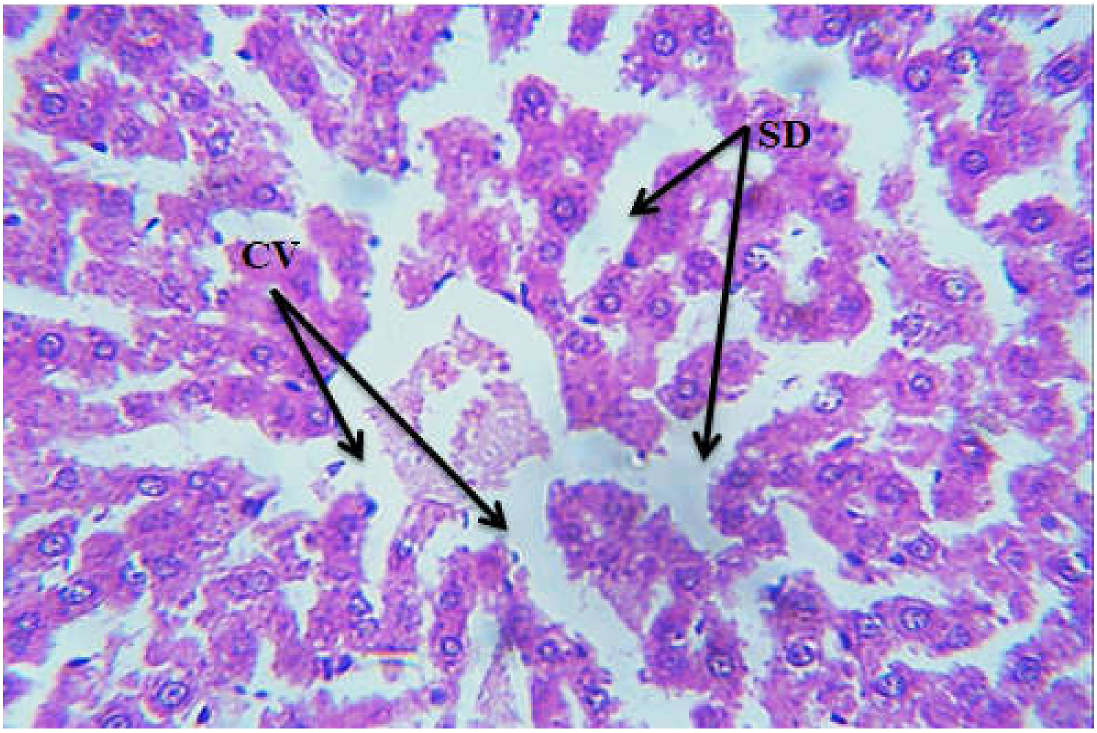

Image 11. Liver section (group B2) showing dilation of the sinuses (SD) and rupture of the central vein wall (CV). H & E 400X.

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