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The Effect of Treadmill-Based Physical Activity on Triglyceride Levels and Hepatic Histopathology in High-Fructose Diet (HFD)- Induced Obese Rats (*Rattus norvegicus)*

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Abstract

Obesity, a metabolic disorder, is characterized by excessive fat accumulation in the body. A key contributing factor to obesity is insufficient physical activity. Utilizing a treadmill for physical activity can stimulate fat metabolism, thereby reducing fat accumulation in adipose tissue. This study aimed to investigate the effect of treadmill-based physical activity on elevated triglyceride levels and the amelioration of hepatic histopathological damage in high-fructose diet (HFD)-induced obese rats (*Rattus norvegicus*). Male Wistar rats, aged 6-8 weeks and weighing 150-200g, were used as experimental animals. Obesity was induced in the rats using a 40% HFD, with an obesity condition determined by a Lee obesity index greater than 0.3. The rats were divided into four treatment groups: a control group, an obese control group, an obese group subjected to 5 minutes/day of treadmill physical activity, and an obese group subjected to 10 minutes/day of treadmill physical activity. Triglyceride levels were measured using the GPO-PAP (Enzymatic Spectrophotometry) method, and histopathological slides of liver organs were prepared using Hematoxylin-Eosin (HE) staining. Triglyceride data were analyzed using quantitative statistical methods, while liver histopathological images were analyzed descriptively and qualitatively. The results demonstrated that treadmill-based physical activity for both 5 minutes/day and 10 minutes/day significantly reduced triglyceride levels ($p<0.05$). Notably, 10 minutes/day of treadmill activity yielded the best results, decreasing triglyceride levels by 67.93%. Furthermore, the liver histopathology showed a reduction in fat accumulation. In conclusion, 10 minutes/day of treadmill-based physical activity is an effective intervention for lowering triglyceride levels and mitigating hepatic steatosis in obese rats.

Keywords*:* Obesity; HFD; Treadmill; Hepatic histopathology

1 Introduction

Obesity is an abnormal condition characterized by the accumulation of fat in the body. It is a complex disease with multiple causes, primarily occurring when physical activity is not commensurate with the very low caloric expenditure (Buchman, 2006). Both humans and animals can experience obesity. Livestock such as goats and cattle can become obese due to owners too frequently providing snacks and feed. Owners often do not realize that the negative effect of overfeeding will make their pets lazy and eventually lead to obesity (Agro Media, 2008). Commercial feeds sold in the market mostly contain carbohydrates because carbohydrates are cheaper and the easiest source of energy compared to other ingredients. While carbohydrates are the primary ingredient in commercial feeds, excessive consumption can lead to symptoms and cases of obesity and digestive disorders (Yuliarti, 2010). Fructose is one carbohydrate that can cause obesity. Excessive fructose consumption can induce metabolic disorders such as obesity (Bray et al., 2004).

According to Diez and Nguyen (2006), many factors trigger obesity in individuals, especially in pet cats and dogs. These factors include genetics, age, sex, endocrine diseases, unbalanced diets, type of feed, medications, and lack of physical activity. Individuals suffering from obesity will experience an increase in triglyceride (TG) levels due to the activity of

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lipoprotein lipase (LPL) enzyme and abdominal obesity. The LPL enzyme is a crucial enzyme that functions in the body's fat metabolism process (Dugi et al., 1997). A decrease in LPL enzyme activity will impair the hydrolysis of VLDL and chylomicron triglycerides (TG) (Goldberg, 2001).

One effective way to combat obesity is through physical activity, as a lack thereof can lead to fat accumulation and subsequent obesity. Physical activity is often more effective than dieting for fat reduction. Weight loss can be managed with physical activity because it leads to higher energy expenditure even when energy intake remains constant (Pescatello and Heest, 2000). Based on the foregoing, this study aims to determine whether physical exercise will affect triglyceride levels and the histopathology of the ovaries in an obesity model goat induced with a high-fructose diet (HFD), specifically through regular treadmill activity.

2 Material and method

2.1 Experimental Animal Preparation

Rats were divided into four treatment groups, with five rats per group: Group 1 served as the negative control (K-) and received no treatment. Group 2 consisted of obese rats and served as the positive control (K+). Group 3 comprised obese rats subjected to 5 minutes/day of physical activity on a treadmill, while Group 4 consisted of obese rats subjected to 10 minutes/day of physical activity on a treadmill.

Before treatment, the rat models were acclimatized to the laboratory environment for 7 days. During this adaptation period, they received a standard diet of 30 grams/rat/day and water ad libitum. The standard feed was a concentrate with a composition of: maximum 12% water, minimum 12% crude protein, 3-7% crude fat, 8% crude fiber, 10% ash, 0.9-1.2% calcium, and 0.6-1% phosphorus. Rats were housed according to their treatment groups and maintained in a room at 26-27°C with 83% humidity.

2.2 Preparation of Obese Animal Models

Obesity in the animal models was induced using a 40% high-fructose diet (HFD). The 40% HFD was prepared by mixing fructose and water in a 3:2 ratio, consisting of 18 grams of starter pig feed (Pokphand 551®) and 12 grams of fructose, combined to provide 30 grams per day. The starter pig feed (Pokphand 551®) is a concentrate with a composition including: 12% water, 12% crude protein, 3-7% crude fat, 8% crude fiber, 10% ash, 0.9-1.2% calcium, and 0.6-1% phosphorus. The calculation and preparation of the HFD (high-fructose diet) are detailed in Appendix 2.

The obese condition in the rat models was determined by calculating the Lee Obesity Index (Figure 4.1), with an animal model considered obese if its Lee Obesity Index was > 0.3 (Hermawan et al., 2011). The induction of obesity with the 40% HFD was carried out for 10 weeks (Modified from Zafeshani et al., 2012). Body weight measurements and Lee Obesity Index calculations were performed weekly before the rats were induced with the 40% HFD.

2.3 Treadmill-Based Physical Activity Treatment

Physical activity treatment for the obese animal models was performed using a treadmill. The duration of treadmill activity was divided into two groups: Treatment Group 1 (P1) exercised for 5 minutes/day, and Treatment Group 2 (P2) for 10 minutes/day. The determination of these durations for treadmill-based physical activity referred to Burghardt et al. (2004) for studies on the natural running ability of rats. Additionally, low-intensity physical activity typically ranges from 20 to 30 minutes (Blair, 1995), while cardiopulmonary exercise programs are usually 40 minutes long (Kraemer and Ratamess, 2004).

Physical activity treatment was conducted once daily, in the morning. Performing the treatment in the morning was chosen to avoid stress induction in the rats due to the procedure (Suckow et al., 2006).

Treatment Group 1 (P1) received treadmill physical activity for 5 minutes/day in the morning for 14 days. Meanwhile, Treatment Group 2 (P2) received treadmill physical activity for 10 minutes/day in the morning for 14 days. The treadmill physical activity was carried out alternately according to the treatment group, starting with Treatment Group 1 (P1) for 20 minutes, followed by Treatment Group 2 (P2) for 40 minutes. During physical activity, efforts were made to ensure the animal models continuously performed the activity. The placement of a needle at the back of the treadmill was expected to encourage the animal models to perform physical activity without stopping. Body weight measurements and Lee obesity index calculations were performed after each physical treatment.

2.4 Treadmill-Based Physical Activity Treatment

In this study, blood collection was performed after two weeks of treadmill exercise. Blood serum was obtained from the rats following cervical dislocation and subsequent dissection. Blood was then collected by cardiac puncture using a 3 ml syringe. The collected blood was transferred into a vacutainer, placed at a 45º angle, and allowed to clot at room temperature. Subsequently, the serum was harvested, transferred into an Eppendorf tube, and stored in a refrigerator.

2.5 Rat Liver Organ Collection

Rat liver organs were collected according to the method described by Kusnia (2014). Organ collection was performed after two weeks of treadmill-based physical activity treatment were completed. Prior to necropsy, rats were euthanized using cervical dislocation. Dissection was then performed on the abdominal cavity, with the rats positioned in dorsal recumbency on a dissecting board. The liver organ was excised and divided into two sections. The right lobe of the liver was placed in phosphate-buffered saline (PBS) for triglyceride level measurement and in 4% paraformaldehyde (PFA) for histopathological slide preparation.

2.6 Triglyceride (TG) Level Measurement

Following blood sample collection from the rats, triglyceride levels were determined using the GPO-PAP (Enzymatic-spectrophotometric) method. The triglyceride assay involved reagent preparation, spectrophotometric measurement of blank solution absorbance, and automated measurement of serum triglyceride absorbance using a Biosystem A15 analyzer.

The reagent was prepared by mixing 45 mmol/L Pipes buffer, 5 mmol/L magnesium chloride, 6 mmol/L 4-chlorophenol, >100 U/ml lipase, >1.5 U/ml glycerol kinase, >4 U/ml glycerol-3-phosphate oxidase, >0.8 U/ml peroxidase, 0.75 mmol/L 4-aminoantipyrine, and 0.9 mmol/L ATP. The blank was determined by measuring the absorbance of 300 µl of reagent using a spectrophotometer at λ 505 nm for 312 seconds. Absorbance values were measured by mixing 300 µl of reagent with 3 µl of sample, followed by washing with 12 µl of washing buffer. Absorbance was then measured using a spectrophotometer at λ 505 nm for 312 seconds, with the blank solution serving as a control.

2.7 Histopathological Preparation of Liver

Histopathological, slide preparation involved several phases: fixation, dehydration, clearing, paraffin infiltration, embedding, sectioning, mounting, and staining (Junquiera and Carneiro, 2004). Initially, the liver organ was fixed in 10% formaldehyde solution for 24 hours. Subsequently, it was sectioned into pieces measuring 2×1×0.5 cm to fit into cassettes for processing in a tissue processor. Next, the liver sections were sequentially immersed in 70% ethanol, 80% ethanol, 90% ethanol, 95% ethanol, Xylol I, and Xylol II, each for 2 hours. This was followed by infiltration in liquid paraffin at 56◦C for 2 hours. The tissue was then retrieved using forceps and embedded in a paraffin block sized to fit the microtome holder. The organ was sectioned using a microtome at a thickness of 4-5 µm. The cut tissue sections were floated on a water bath at 40◦C and then mounted onto glass slides. The slides were then dried at room temperature (26–27◦C). Finally, the preparations were stained with hematoxylin-eosin (HE) (Wati et al., 2013).

2.8 Hematoxylin-Eosin (HE) Staining of Liver Histopathology Slides

The sequence for hematoxylin-eosin (HE) staining began with deparaffinization, achieved by immersing the slides in sequential Xylol solutions (I-III) for five minutes each. This was followed by rehydration, where slides were placed in graded ethanol solutions: absolute ethanol (I-III), 95%, 90%, 80%, and 70%, each for five minutes, then rinsed in distilled water for five minutes.

Subsequently, staining commenced. Slides were immersed in hematoxylin stain for approximately 10 minutes, then rinsed under running water for 30 minutes, followed by a distilled water rinse. They were then transferred to eosin stain for 5 minutes. Afterward, the slides were immersed in distilled water to remove residual eosin stain.

The next step involved dehydration, achieved by immersing the slides in graded ethanol solutions from 70%, 80%, 90%, and 95% up to absolute ethanol (I-III). This was followed by clearing, with slides being immersed in Xylol I-II and then air-dried. Finally, mounting was performed using Entellan (Jusuf, 2009).

2.9 Histopathological Observation

Histopathological observation of the liver organs was performed using an Olympus BX 51 light microscope at 40x, 100x, and 400x magnifications. Images were captured using a digital camera. The observed features in the liver included

hepatocyte steatosis, adipose cell infiltration, endothelial damage, vacuole formation, and hypertrophy and hyperplasia of adipose cells in the liver.

2.10 Data Analysis

Quantitative analysis of triglyceride levels was performed statistically using one-way analysis of variance (ANOVA), followed by a Honestly Significant Difference (HSD) or Tukey's post-hoc test to determine significant differences at a 5% significance level. This was conducted using Microsoft Office Excel and Statistical Package for the Social Sciences (SPSS) version 16.0 for Windows. Meanwhile, the results of the liver histopathological observations were analyzed descriptively.

3 Result and discussion

3.1 Effect of Treadmill-Based Physical Activity on Body Weight of High-Fructose Diet (HFD) 40%-Induced Obese Rats (*Rattus norvegicus*)

Body weight measurements in rats (*Rattus norvegicus)* were used to assess the success of 40% High-Fructose Diet (HFD) induction in causing obesity and the effectiveness of treadmill-based physical activity in reducing obesity. The determination of weight gain and loss among treatment groups was assessed using the Lee obesity index. A decrease in body weight was observed in obese rats induced with 40% HFD that received treadmill-based physical activity (Darmono, 2015). Table 1 presents the body weight and Lee Obesity Index of rats.

Table 1 Triglyceride level measurement on each treatment groups.

Treatment	Average Triglyceride Level (µg/ml)	Triglyceride Level (%)	
		Increase from Negative Control	Decrease from Negative Control
Negative control (K-)	44,6 ± 2,073a	-	-
Positive Control (K+)	152,2 ± 3,114c	241	-
Physical activity for 5 minutes	100,2 ± 3,962b	-	34,16
Physical activity for 10 minutes (T2)	48,8 ± 0,836a	-	67,93

Note: Notations a, b, and c, indicate significant difference between treatment groups ($p<0.05$).

Treadmill-based physical activity significantly ($p<0.05$) reduced the body weight and Lee obesity index of rats with 40% High-Fructose Diet (HFD)-induced obesity. The body weight and Lee obesity index of the negative control group and the 10 minutes/day treadmill physical activity group did not differ significantly ($p<0.05$), indicating they were not obese. In contrast, these parameters did not differ significantly ($p>0.05$) when compared to the positive control group and the 5 minutes/day treadmill physical activity group, both of which remained obese. Treadmill-based physical activity for 10 minutes/day was an effective duration for reducing body weight and obesity, as indicated by a Lee obesity index below 0.3 in 40% HFD-induced obese rats (Darmono, 2015).

The negative control group (K-) exhibited a mean body weight of 235.3±18.05 grams and a Lee obesity index of 0.255±0.01, which indicates that these rats were not in an obese condition. Body weight can determine obesity by influencing the Lee obesity index calculation, with values below 0.3 signifying a non-obese state. This is supported by Hayatin (2007), who stated that the basal energy requirement for rats is 50.1598 kcal. The standard feed provided, 30 grams/rat/day, with an energy content of 38.685 kcal (Appendix 9.1), did not significantly increase body weight.

The body weight of the positive control group (K+) increased by 46.41% compared to the negative control group (K-), which directly correlated with a Lee obesity index of 0.315±0.01. This increase is attributed to the 70-day induction with a 40% HFD, which contained 81.276 kcal (Appendix 9.2) and led to an increase in triglyceride levels. The excess energy of 31.118 kcal that was not utilized for physiological needs was stored in adipose tissue as triglycerides.

A 70-day High-Fructose Diet (HFD) at 40% in rats is more efficient in lipogenesis by providing carbon atoms for glycerol and acyl-CoA for triglyceride synthesis, leading to increased fat accumulation in the liver and subsequent elevated triglyceride levels (Le et al., 2006; Stanhope & Havel, 2008). Fructose is absorbed by the intestine, primarily the jejunum, and enters the portal vein to be transported to the liver. In the liver, fructokinase phosphorylates fructose using adenosine triphosphate (ATP) to form fructose-1-phosphate. Fructose-1-phosphate is then broken down by aldolase B into glyceraldehyde and dihydroxyacetone, which is converted to glyceraldehyde-3-phosphate. Fructose is metabolized into two triose phosphate molecules, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate, serving as a primary shortcut in glycolysis. Dihydroxyacetone phosphate and glyceraldehyde-3-phosphate are precursors for the formation of glycerol-3-phosphate and acetyl-CoA. Fructose stimulates lipogenesis by providing carbon atoms for glycerol-3-phosphate and acyl-CoA to form triglycerides (Basciano et al., 2005). This aligns with Trisviana's (2012) research, which stated that increased triglycerides are directly proportional to increased body weight.

The body weight reduction of 3.37% and a decrease in Lee obesity index to 0.307±0.01 in the 5 minutes/day treadmill physical activity group (T1) indicate that 14 days of this intervention were insufficient to reverse the obese condition. This is supported by the non-significant difference ($p<0.05$) between group T1 and the positive control group, suggesting that rats in group T1 remained obese.

Breaking down stored food reserves can be achieved through physical activity to burn energy. However, excessive caloric intake coupled with insufficient physical activity will lead to obesity. According to Aminudin (2011), the energy requirement for human running activity is 10 kcal/minute. Consequently, rats in the 5 minutes/day treadmill physical activity group experienced an energy deficit of 18.882 kcal. This finding is consistent with Novitasari et al.'s (2013) research, which demonstrated that less physical activity combined with higher food intake results in fat accumulation. Therefore, the energy expenditure during 5 minutes/day of physical activity was not enough to reduce the obese condition.

The body weight of rats subjected to 10 minutes/day of treadmill-based physical activity (T2) decreased by 24.53% compared to the positive control group, with their Lee obesity index falling to 0.265±0.01. This indicates that 14 days of 10 minutes/day treadmill activity effectively reduced obese conditions. This effectiveness is further supported by the non-significant difference ($p<0.05$) in body weight and Lee obesity index when compared to the negative control group. Ten minutes of physical activity requires 100 kcal of energy, while the energy provided by the 40% High-Fructose Diet (HFD), totaling 81.276 kcal, was insufficient to meet this energy demand (Appendix 9.3). The energy deficit during physical activity was compensated by breaking down energy reserves, primarily fat. According to Jeukendrup and Gleeson (2004), rat body weight tends to decrease because triglyceride reserves in adipose tissue are broken down to form ATP during physical activity. Thus, 10 minutes of physical activity proved effective in reducing both body weight and obesity.

Therefore, treadmill-based physical activity for 10 minutes/day is effective in reducing the body weight of 40% HFD-induced obese rats. This is evidenced by the non-significant difference ($p<0.05$) in body weight and Lee obesity index compared to the negative control group. This is because 10 minutes/day of treadmill activity constitutes high-energy physical activity. The increased energy demand is met by breaking down the body's energy reserves, including fat. Fat breakdown occurs through lipolysis, involving the breakdown of triglycerides. The breakdown of triglycerides leads to a decrease in adipose tissue accumulation, which in turn results in weight loss.

3.2 Effect of Treadmill-Based Physical Activity on Triglyceride Levels in Obese Rats (*Rattus norvegicus*)

Triglyceride levels in the blood were assessed to understand the impact of treadmill-based physical activity on liver physiological function in rats (*Rattus norvegicus*) with 40% High-Fructose Diet (HFD)-induced obesity. Blood triglyceride measurements were performed spectrophotometrically using a GPO-PAP kit. The study results indicated an increase in blood triglyceride levels in groups T1 and T2.

Treadmill-based physical activity significantly ($P<0.05$) reduced triglyceride levels in *Rattus norvegicus* models with 40% HFD-induced obesity. The mean triglyceride levels of the negative control group (K-), positive control group (K+), and 5 minutes/day physical activity group (T1) differed significantly ($p<0.05$). Conversely, no significant difference ($p>0.05$) was observed between the negative control group (K-) and the 10 minutes/day treadmill physical activity group (T2). Treadmill-based physical activity for 10 minutes/day (T1) proved to be the most effective duration for lowering triglyceride levels in *Rattus norvegicus.*

The triglyceride levels in the negative control group (K-) represented normal triglyceride levels and served as a reference to determine any decrease or increase in triglyceride levels occurring in the positive control group (K+), the 5 minutes/day treadmill physical activity group (T1), and the 10 minutes/day treadmill physical activity group (T2).

The mean triglyceride level in the positive control group (K+) was 152.2±3.114 mg/dL, representing a 241% increase compared to the negative control group. This elevation in triglyceride levels in the positive control group (K+) was attributed to 70 days of 40% High-Fructose Diet (HFD) induction. The 40% HFD administered to rats leads to increased triglyceride levels, which in turn causes greater adipose tissue accumulation. Adipose tissue not only stores triglycerides but also produces bioactive substances called adipokines.

The triglyceride level in the 5 minutes/day treadmill physical activity group (T1) decreased by 34.16% due to 14 days of this intervention. However, 5 minutes/day of treadmill activity was not yet effective in significantly reducing triglyceride levels. This is evidenced by the significantly different triglyceride levels in T1 ($p<0.05$) compared to the negative control group. While 5 minutes/day of treadmill activity could lower triglyceride levels, it was not effective in reducing body weight in obese rats. The reduction in triglyceride levels is due to physical activity increasing energy demands, thereby stimulating triglyceride metabolism. Triglycerides stored in fat tissue are utilized for ATP production through β-oxidation, which can reduce Very Low Density Lipoprotein (VLDL) and fat tissue.

The mean triglyceride level in the 10 minutes/day treadmill physical activity group (T2) decreased by 67.93% due to 14 days of this intervention. This duration of treadmill-based physical activity was highly effective in reducing triglyceride levels in obese rats. This is indicated by the non-significant difference ($p>0.05$) in triglyceride levels compared to the negative control group. Treadmill-based physical activity for 10 minutes/day was the most effective duration for lowering triglyceride levels in obese rats compared to 5 minutes/day. This is shown by the lower mean triglyceride level in T2 compared to T1.

Both 5 minutes/day and 10 minutes/day treadmill-based physical activity significantly ($p<0.05$) reduced triglyceride levels in obese rats *(Rattus norvegicus).* Based on the reduction in mean triglyceride levels, 10 minutes/day of treadmill-based physical activity was the most effective duration, as it reduced triglyceride levels to nearly match those of the negative control group.

3.3 Effect of Treadmill-Based Physical Activity on Liver Histopathology in Obese Rats (*Rattus norvegicus*)

Hepatic steatosis in *Rattus norvegicus* due to fructose diet was assessed using Hematoxylin-Eosin (HE) staining. Histopathological examination revealed the presence of liver steatosis in the **positive control group (B)** as well as in **treatment groups T1 and T2**.

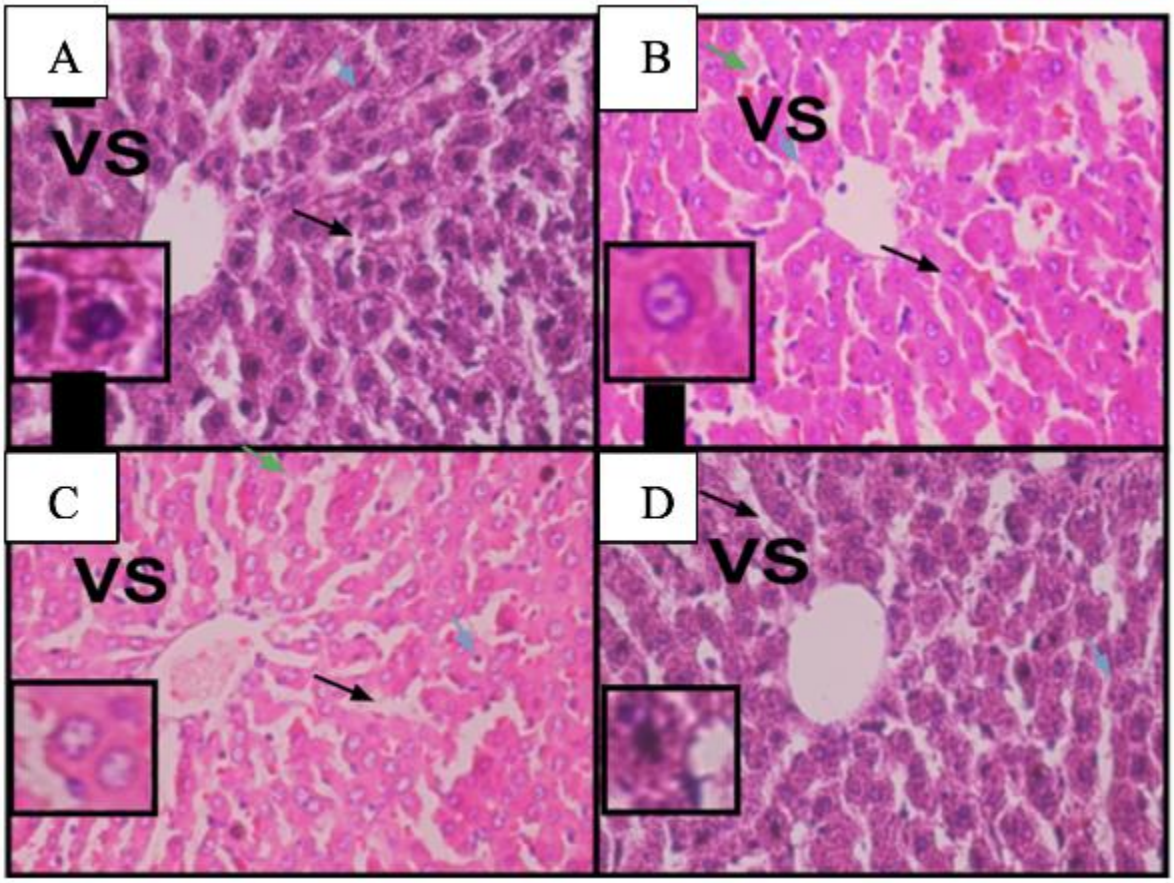

Figure 1 (A) Negative control group (K-), (B) positive control group (K+), (C) 5-minute physical activity group (T1), and (D) 10-minute physical activity group (T2). (VS) central vein, (black) hepatocyte, (blue) sinusoid, (green) hepatocyte showing steatosis

The liver tissue of rats in the negative control group (A) exhibited normal histological features. Several hepatocytes near the central vein (labeled 1) appeared normal, and the sinusoids were clearly visible. Hepatocytes were arranged radially within the hepatic lobules, forming plates that extended from the periphery to the center. The spaces between these plates contained capillaries, known as hepatic sinusoids. In the negative control liver, the sinusoids were observed to be tortuous and dilated. This observation aligns with Junqueira et al. (2007), who described the liver as being composed of anastomosing plates of hepatocytes arranged radially around the hepatic vein, with sinusoids interspersed among the hepatocyte cords. Each hepatocyte is positioned between sinusoids, receiving blood supply from both the hepatic portal vein and the hepatic artery.

In contrast, the liver tissue of rats in the positive control group (B) showed steatosis around the hepatocytes near the central vein, characterized by nuclear displacement (labeled 2) and irregular sinusoids. Steatosis was also evident in groups B, C, and D (indicated by label 2), where fat accumulation was visible around the hepatocytes. This steatosis is characterized by the accumulation of triglycerides and other lipid metabolites in the cytoplasm, appearing as clear vacuoles.

No hepatic steatosis was observed in the liver of the negative control group of rats. This is because their diet did not contain fructose, thus preventing the lipogenesis of fructose into triglycerides. In contrast, the histopathological examination of the liver in the positive control group revealed steatosis within the hepatocytes. According to Paderi (2007), fat accumulation typically begins in the portal region and extends towards the central vein. This is due to the blood supply from the intestines reaching the liver via the portal vein. The blood then flows through the sinusoids towards the central vein. Various toxins are metabolized by the liver, and their metabolic products are carried by the sinusoidal blood flow towards the central vein. Consequently, hepatocyte damage, particularly steatosis, is frequently observed in the central vein area.

Consumption of a high-fructose diet leads to excessive triglyceride formation in the liver. Fructose that is not transported accumulates in the liver, continuously resulting in steatosis. Fructose is absorbed by the intestines, primarily the jejunum, and enters the portal vein to be transported to the liver. The liver phosphorylates fructose using fructokinase and adenosine triphosphate (ATP) to form fructose-1-phosphate. Fructose-1-phosphate is then cleaved by aldolase B into glyceraldehyde and dihydroxyacetone, which is converted to glyceraldehyde-3-phosphate. Fructose is metabolized into two triose phosphate molecules, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate, serving as a major shortcut in glycolysis. Dihydroxyacetone phosphate and glyceraldehyde-3-phosphate are precursors for the formation of glycerol-3-phosphate and acetyl-CoA. Fructose stimulates lipogenesis by providing carbon atoms for glycerol-3-phosphate and acyl-CoA to form triglycerides (Basciano et al., 2005). Hepatic steatosis is characterized by the presence of spherical, clear lipid vacuoles within the hepatocytes.

In the group treated with 5 minutes of treadmill activity per day, there was a reduction in hepatic steatosis compared to the positive control group (Figure 5.1C). The decrease in intracellular fat is attributed to the high and rapid energy demand necessitated by 5 minutes of daily physical activity. To meet this energy requirement, the body utilizes energy reserves in the form of triglycerides from adipose tissue and dispersed fat infiltration throughout the body (Yuwono, 2005).

Histopathological examination of the liver in the group subjected to 10 minutes per day of treadmill-based physical activity revealed a greater reduction in fat accumulation compared to the 5-minute treadmill activity group). This enhanced reduction in intracellular hepatic steatosis is attributed to the higher and more rapid energy demands incurred by 10 minutes of treadmill exercise. Lipolysis in adipose tissue is consequently elevated to meet these energy requirements. As supported by Harikedua et al. (2012), physical activity exceeding 10 minutes per day falls into the moderate to vigorous intensity categories. Therefore, 10 minutes of daily physical activity more effectively reduces fat content within the liver.

4 Conclusion

Treadmill-based physical activity for both 5 minutes/day and 10 minutes/day significantly reduced triglyceride levels in *Rattus norvegicus* obese models induced by a 40% High-Fructose Diet (HFD) by 34.16% and 67.93%, respectively. Treadmill-based physical activity for 10 minutes/day proved to be the most effective exercise regimen.

Furthermore, treadmill-based physical activity for 10 minutes/day was the most effective duration for mitigating damage to the liver histopathology of 40% HFD-induced obese *Rattus norvegicus*. This mitigation was characterized by improved cellular arrangement and a reduction in necrotic cells, inflammatory cells, edema, and hemorrhage.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The ethical clearance was issued by the Animal Care and Use Commitee of Brawijaya University, with ethic number of No: 276-KEP-UB.

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