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Experimental Research Article

Effect of Thioflavin-T on Adipokine Hormones and Fatty Liver in obese male NMRI mice fed a high-fat diet

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ABSTRACT

Introduction: The objective of the study was to find out the influence of Thioflavin-T (ThT) on obesity and fatty liver by investigating the adipokine hormones, and insulin serum level of male NMRI mice which were exposed to a high-fat diet (HFD).

Methods: 50 adult male NMRI mice were separated into five groups: n=10. The control group was given a standard diet at twelve-week intervals. The sham group was nourished with HFD that lasted for 8 weeks, afterwards, the group received a standard diet and solvent water (0.5ml) by gavage (4 weeks). The experimental groups 1-3 were nourished with HFD (4% cholesterol, 60% fat) eight-week period. Then, the treatment period started in experimental groups by receiving a normal diet in addition to ThT with three doses (5,10 and 15 mg/kg, 0.5ml), via gavage (4 weeks).

Results: HFD contributed to a substantial reduction in serum adiponectin levels and increased leptin serum in the sham group opposite to the control group (P<0.001). However, the concentration of both adipokine hormones was significantly modified under the treatment of ThT in a dose-dependent manner. Insulin serum increased in the sham group significantly (P< 0.001), meanwhile, a significant decrease was shown in experimental groups 2, and 3 than in the sham group (P<0.01). ThT also reduced HOMA-IR in experimental groups. The introduction of ThT in varying doses led to the induction of polymorphonuclear cells in the liver tissue.

Conclusion: Our findings propose that ThT can affect liver function and body weight by modulating the serum levels of adipokine hormones besides decreasing the level of insulin and HOMA-IR in mice fed with HFD.

Keywords:
Adiponectin
Leptin
Fatty liver
Obesity
Thioflavin-T

Introduction

Non-alcoholic fatty liver disease (NAFLD) is often linked to obesity, particularly increased abdominal visceral fat. NAFLD and obesity together increase the likelihood of more advanced hepatic dysfunction. (Araújo et al., 2018). Liver steatosis is a condition that is associ-

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ated with body mass index (BMI). However, it is more closely linked to the accumulation of fat in the abdomen (which is measured using waist circumference), as visceral adipose tissue (VAT) has a higher lipolytic activity per unit weight compared to subcutaneous fat (Younossi et al. 2016). NAFLD is a liver condition linked to metabolic syndrome, which is a cluster of cardiovascular risk factors related to insulin resistance, including central obesity, hypertension, dyslipidemia, and type 2 diabetes mellitus (T2DM) (Watanabe et al. 2008). Non-alcoholic fatty liver disease (NAFLD) is prevalent in 60-95% of individuals who are obese. (Watanabe et al. 2008). Obesity is closely linked to liver inflammation, which is caused by the accumulation of lipids in hepatocytes, resulting in a fatty liver. The accumulation of inflammatory mediators and liver macrophages leads to an increase in inflammatory responses. Moreover, there is a close connection between the liver and adipose tissue (Adolph et al., 2017). Adipokines affect the inflammatory response of adipose tissue and have distinct effects on NAFLD by regulating hepatic adiposity, insulin resistance, and fibrosis (Adolph et al., 2017).

Adiponectin, a mediator with anti-inflammatory properties derived from adipocytes, functions through its two binding sites (ADIPOR1 and ADIPOR2), triggering AMP-kinase signaling, which may be regulated through T-cadherin (Cui et al., 2017b). Adiponectin levels significantly decrease in cases of central obesity and insulin resistance, such as atherosclerosis, non-alcoholic steatohepatitis (NASH), and insulin-resistant diabetes (Tilg et al., 2006). Recently, lean NAFLD patients were shown to have reduced circulating adiponectin concentrations, underscoring the critical role of adiponectin in NAFLD (Hui et al., 2004). Leptin, another adipocyte hormone, regulates neuroendocrine function, energy homeostasis, hematopoiesis, and angiogenesis, while also mediating inflammatory processes and immune-mediated diseases (Obradovic et al., 2021). Furthermore, leptin plays a pro-inflammatory role in different autoinflammatory or immune-related inflammatory disease models. Leptin gene expression is regulated by insulin and glucocorticoids, primarily observed in fatty tissue, and subsequently released into the bloodstream. The main role of leptin is appetite control; however, in obesity, leptin is ineffective in correcting hyperglycemia, and metabolic dysregulation and obesity have been identified as potential causes of "leptin resistance" (Cui et al., 2017a). Elevated serum leptin levels are also linked to the severity of liver disease, including inflammation and fibrosis. Additionally, several comprehensive NAFLD research studies have reported a noticeable increase in serum leptin levels. It was found that insulin secretory dysfunction and insulin resistance act as intermediary effects to establish the observed association (Polyzos et al., 2015).

Insulin serves numerous physiological functions, with its primary role being the regulation of blood glucose levels. Additionally, insulin stimulates the synthesis of fatty acids and glycogen, enhances mitochondrial function, promotes microcirculation, and fosters cell proliferation (Tokarz et al., 2018). Insulin resistance occurs when the uptake of glucose in insulin-responsive tissues is compromised due to interference with the insulin signaling cascade. This obstruction of glucose absorption stems from the inhibition of the insulin signaling pathway. Insulin resistance can also result in hyperinsulinemia, as β-cells produce excessive amounts of insulin to regulate blood glucose levels. It has been shown that obesity-induced inflammation significantly contributes to the development of steatosis by increasing lipid utilization in hepatocytes (Ye, 2013).

Thioflavin T (ThT), a benzothiazole compound used as a fluorescent probe to detect amyloid structures (Kuznetsova et al., 2012), possesses fluorescent properties and has been shown to prolong lifespan while acting as an anti-hyperglycemic and anti-aging agent in animal models, as previously reported (Najafian et al., 2015). Thioflavin T (ThT), a benzothiazole compound used as a fluorescent probe to detect amyloid structures (Kuznetsova et al. 2012), with fluorescent properties, prolongs lifespan and acts as an anti-hyperglycemia and anti-aging in animal models as previously reported (Najafian et al. 2015). Thioflavin T is mostly used to identify amyloid fibrils, ordered protein structures related to a variety of diseases (Kuznetsova et al. 2012). Previous studies have indicated that Thioflavin-T reduces lipid profiles and fat accumulation, and regulates adipokine hormones and insulin levels in diabetic and non-diabetic animals. (Najafian et al. 2015; Jalalvand et al. 2016). Also, it has been shown that Thioflavin-T has a positive effect on diabetic rats by modifying glycemic parameters (Najafian et al. 2015; Jalalvand et al. 2016). Moreover, the positive impacts of Thioflavin-T on liver function were demonstrated by decreasing lipid

TABLE 1: High-fat emulsion compound

Corn oil	380 gr
Sucrose	165 gr
Milk powder	90gr
Cholesterol	4%
Sodium Dioxylate	10gr
Twin 80	30gr
Propylene glycol	31.1gr
Multivitamin	3 gr
Salt	2.3gr
Mixed Minerals	1.3gr
Distilled water	200ml

accumulation in the liver tissue of mice with NAFLD (Jalalvand et al. 2016). To gain a better understanding of the practical effect of Thioflavin T (ThT) on obesity and liver tissue, this study aims to evaluate the impact of Thioflavin on adiponectin, leptin, insulin, HOMA-IR, the levels of lipid profiles, glucose, and liver enzymes in the NMRI mice feeding high- fat diet.

Materials and methods

Animals

A total of 50 adult male NMRI mice, with an average weight of 25±5g, were acquired from the Pasteur Institute in Tehran. The animals were maintained in accordance with established laboratory protocols, with a consistent 12-hour light-dark cycle. Before exposure to the experimental conditions, the animals were provided with commercial pellets and tap water for a week.

The observance of animal welfare and the implementation of experimental procedures were conducted with strict adherence to the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). In addition, approval was obtained from the Animal Ethics Committee of the Science and Research Branch at Islamic Azad University. The protocol utilized in the experiment was authorized by the esteemed Research and Ethics Committee of the distinguished Science and Research University of Tehran (code: 27081).

Material

Thioflavin T (ThT) was from Sigma (USA). Commercial kits utilized for the qualification of leptin and insulin were purchased from Sunlong company, China, the adiponectin kit was purchased from R&D system company (USA), and a glucose kit was purchased from Pars Azmoon Company, Iran. All other chemicals used were from Merck (Darmstadt Germany) and analytical grade.

Experimental procedures

The overall calorie intake for HFD-receiving mice was 37610 Calories (446.20 in 100g). The animals were given a high-fat (60% of energy from fat) (Seo et al. 2021) and a high-cholesterol (4%) diet for 8 weeks (Bilal et al. 2021). High-fat emulsion compound for gavage in rats was prepared as below (Zou et al. 2006) (Table 1):

Mice were weighed once before starting the experiments, and then weekly during the 8 weeks of the experiment. The animals were randomly assigned to distinct categories: n=10

Control group: The animals received a standard diet (16 kcal/day) for 12 weeks.

Sham: The animals received HFD for 8 weeks, then they received a standard diet and 0.5 ml solvent (water) by gavage for 4 weeks.

Experimental groups (exp): exp1, exp2, and exp3: The animals were fed with HFD, for 8 weeks, then they received a normal diet in addition to ThT with 5, 10, and 15 mg/kg Doses, respectively, via daily gavage spanned 4 weeks was 0.5ml. These doses were chosen based on previous experiments (Najafian et al. 2015; Jalalvand et al. 2016).

TABLE 2: Comparison of body weight.

Groups	Control	Sham	Exp 1	Exp 2	Exp 3
Parameters					
Initial weight (g)	25.46±0.57	25.76±0.86	25.49±0.72	25.33±0.64	25.56±0.62
After 8 weeks (g)	28.83±0.57	33.57±0.83***	33.48±0.61***	33.18±0.69***	33.24±0.55***
Final weight (g)	29.02±1.37	38.98±0.33**	31.50±1.45^{++}	28.18±0.52^{+++}	26.41±0.74$^{*,+++}$
Weight gain (g)	3.56±0.8	13.22±0.53**	6.01±0.73^{++}	2.85±0.12^{+++}	0.85±0.12$^{*,+++}$

Data are expressed as mean SEM.
Exp1: received Thioflavin- T 5 mg/kg
Exp2: received Thioflavin- T 10 mg/kg
Exp3: received Thioflavin- T 15 mg/kg
***P < 0.001, **P < 0.01 and *P < 0.05 compared with control group (n=10)
$^{+++}$P < 0.001, $^{++}$P < 0.01 and $^{+}$P < 0.05 compared with sham group (n=10)

Biochemical evaluation

When the prescribed 30-day therapeutic regimen concluded, mice were fasted between 12 to 14 hours, and the animals' body weights were measured. Afterward, the animals underwent anesthesia via the inhalation of diethyl ether. Post-anesthesia, blood samples were collected from the cardiac ventricles with the use of 2.5-ml syringes and permitted to coagulate for 30 minutes at ambient temperature. Centrifugation was then carried out at 1000×g and a temperature of 37°C for 10 minutes, with the objective of separating the serum. The levels of Cholesterol, triglyceride (TG), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), blood glucose, insulin, insulin resistance (HOMA-IR[35]), leptin and adiponectin in the serum were determined with the use of enzymatic methods using commercially available kits. Because mice were small, fasting glucose was measured only once in complete anesthesia.

The insulin resistance index was assessed through the homeostasis model assessment (HOMA-IR) formula: HOMA-IR = fasting insulin (mU/L)×fasting glucose (mM)/22.5 (Barnea M et al. 2006).

Histopathological evaluation

Liver tissue from all animals was preserved at a temperature of −70 °C to determine histological alternations. To further observe the changes in liver tissue, excised liver sections were subjected to fixation in a 10% buffered formalin for a duration of 24 hours, followed by embedding in paraffin and sectioning into 5 µm cells tissues were impregnated with using Hematoxylin and Eosin (H&E) following established procedures. Light microscopy was utilized to examine the hepatic specimens (Olympus SZX10 microscope, Tokyo, Japan).

Statistical analysis

The collected data underwent One-way analysis of variance (ANOVA) afterward, Turkey's post hoc test was conducted to make multiple comparisons of the differences between experimental groups. A statistical significance level of the study was set to $P < 0.05$.

Results

Biochemical evaluation

Body weight

After 8 weeks, all groups exhibited a significant increase in body weight compared to the control group ($P < 0.001$) (Table 2). At the end of treatment (after 12 weeks), the final weight of the sham group increased in comparison to the control group ($P < 0.01$). While no significant differences were observed between experimental groups 1 and 2 and the control group, experimental group 3 demonstrated a significant decrease compared to the control group ($P < 0.05$). In the experimental groups, body weights decreased significantly compared to the sham group (exp1: $P < 0.01$, exp1,2: $P < 0.001$) (Table 2).

Lipids profile

Serum levels of cholesterol, triglycerides, LDL, and HDL for the five groups are presented in Table 3. Cholesterol and triglyceride levels in the sham group and experimental groups 1, 2, and 3 exhibited a statistically significant increase compared to the control group (sham,

TABLE 3: Effects of Thioflavin-T Treatment on Biochemical Parameters

Groups / Parameters	Control	Sham	Exp1	Exp2	Exp3
Cholesterol (mg/dl)	111±9.64	246±14.92***	199±20.91$^{++,}$***	180±15.60$^{+++,}$***	159±12.28$^{+++,}$**
Triglyceride (TG) (mg/dl)	113.33±5.77	250±19.23***	190.25±12.65$^{++,}$***	178.40±7.92$^{++,}$***	157.66±6.80$^{+++,}$**
LDL-C (mg/dl)	16.33±1.52	30±1.78***	25.25±0.95$^{+,}$***	21.20±1.30$^{++,}$**	18.33±1.15^{+++}
HDL-C (mg/dl)	101±3.60	81.16±13.06	78.50±16.54	84.20±16.46	99.66±9.50
ALT (IU/L)	44±5.76	100±12.12***	59±8.83$^{++,}$**	63±6.67$^{++,}$**	41±7.54^{+++}
ALP (IU/L)	165±19.97	253±19.21***	184±21.29^{+++}	245±22.19***	244±22.71**
AST (IU/L)	138±20.66	197.66±37.71	300±23.39$^{+++,}$***	242.40±32.42**	154.66±15.53
Fasting blood glucose (mg/dl)	96±10.14	150±8.36***	140±10.988***	130±12.62$^{+,}$*	118±10^{++}
Insulin (mU/L)	0.31±0.04	0.44±0.02***	0.39±0.008*	0.35±0.009^{++}	0.32±0.007^{++}
HOMA-IR	0.07±0.01	0.15±0.01***	0.13±0.01$^{***,+}$	0.10±0.008$^{**,+++}$	0.08±0.008^{+++}
Leptin (pg/ml)	170±11.13	228±12.83**	210±10.39*	195±14.14	182±16.97^{+}
Adiponectin (ng/ml)	0.7±0.10	0.5±0.14***	0.55±0.13***	0.6±0.14$^{++,}$**	0.66±0.12$^{+++,}$*

Exp1: received Thioflavin- T 5 mg/kg
Exp2: received Thioflavin- T 10 mg/kg
Exp3: received Thioflavin- T 15 mg/kg
Low-density lipoprotein cholesterol (LDL-C), High-density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP).
***P < 0.001, **P < 0.01 and *P < 0.05 compared with control group (n=10)
$^{+++}$P < 0.001, $^{++}$P < 0.01 and $^{+}$P < 0.05 compared with sham group (n=10)

exp1,2: P < 0.001, exp3: P < 0.01). The results indicate a significant decrease in cholesterol levels (exp1: P < 0.01, exp2,3: P < 0.001) as well as triglyceride levels (exp1,2: P < 0.01, exp3: P < 0.001) in the experimental groups, which were notably different from the sham group. Although HDL levels in the experimental groups did not display statistical significance compared to the control and sham groups, it is evident that LDL levels were notably elevated in the sham group and experimental groups 1, 2, and 3 compared to the control group (sham, exp1; P < 0.001, exp2: P < 0.01). Furthermore, the treatment groups showed a significant statistical decrease in LDL levels compared to the sham group (exp1: P < 0.05, exp2: P < 0.01, exp3: P < 0.001) (Table 3).

Liver enzymes

Hepatic enzymes such as AST, ALP, and ALT are enhanced by increased body weight and obesity, often indicating liver problems. These enzymes serve as vital markers for evaluating liver health, indicating potential damage or dysfunction within the organ during high-fat diet feeding resulting in obesity. Statistical analysis showed a considerable rise in ALT levels was identified in the sham group and experimental groups 1 and 2 compared to the control group (sham: P< 0.001, exp 1,2: P< 0.01), while this increase was insignificant in the experimental group 3. Conversely, a remarkable decrease in ALT levels was observed in the experimental groups compared to the sham group (exp1,2: P< 0.01, exp3: P < 0.001) (Table 3). Also, a marked elevation occurred in serum ALP levels in the sham group and experimental groups 2 and 3 compared to the control group (sham, exp2: P < 0.001, exp3: P < 0.01). However, experimental group 1 did not show a significant increase compared to

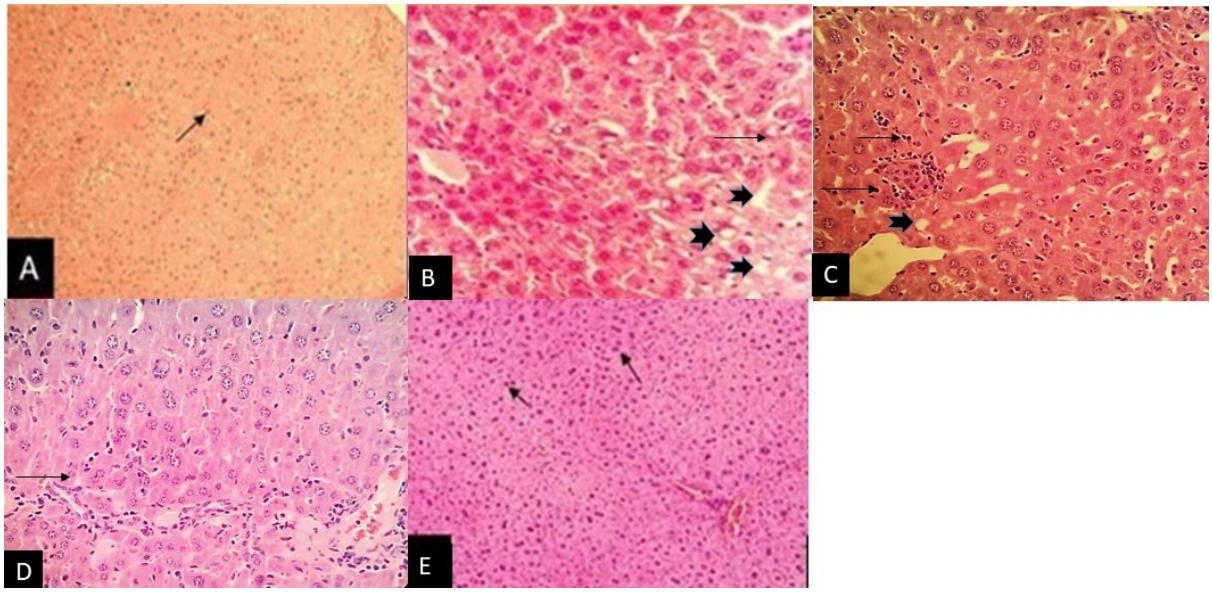

FIGURE 1. The effects of Thioflavin-T on liver tissues in mice fed HCD.
The liver sections were stained by H&E. A; Control group (×10); The arrow shows the normal structure of liver tissue. B; Sham group (×10); The arrow indicates dual-core cells and arrow icons show the lipid droplets in animals that received a high-fat diet; The arrow indicates dual-core cells and arrow icons show the lipid droplets. C; Experimental group 1(×10); the group received 5 mg/kg ThT. D, E experimental group 2,3 (×10); the groups received 10 and 15 mg/kg ThT respectively. The arrow exhibits the generation of polymorphonuclear cells, especially in exp3.
Shows the lipid droplets.
Shows polymorphonuclear cells.

the control group. Instead, experimental group 1 experienced a meaningful decline in serum ALP levels, unlike the sham group. There was no significant variation in ALP levels among experimental groups 2 and 3 and the sham group (Table 3). In addition, the serum level of AST in the sham group displayed elevated values compared to the control group, albeit not reaching statistical significance. However, the serum concentration of AST demonstrated a noteworthy increase in experimental groups 1 and 2 compared to the control group (exp1: $P < 0.001$, exp2: $P < 0.01$). On the other hand, there was no difference between experimental group 3 and the control group. The sham group presented with statistically significant alterations in levels, whereas in comparison, experimental group 1 significantly increased ($P < 0.001$), with no such alterations detected in experimental groups 2 and 3 (Table 3).

Fasting glucose, insulin levels, and insulin resistance

The results indicate a significant increase in serum glucose levels among the sham group and experimental groups 1 and 2 compared to the control group (sham, exp1: $P < 0.001$, exp2: $P < 0.05$). However, this increase was not significant in experimental group 3. Glucose concentrations observed in experimental group 1 and the sham group did not yield statistically significant outcomes. In contrast, serum glucose levels in experimental groups 2 and 3 demonstrated a significant reduction compared to the sham group ($P < 0.05$, $P < 0.01$, respectively). Furthermore, both the sham group and experimental group 1 exhibited a noteworthy elevation in insulin levels relative to the control group (sham: $P < 0.001$, exp1: $P < 0.05$), while no difference was observed in experimental groups 2 and 3. Although there was no notable contrast in insulin levels between the sham group and experimental group 1, it is evident that insulin levels were much lower in groups 2 and 3 of the experimental group compared to the sham group ($P < 0.01$). Additionally, concerning the control group, insulin resistance levels significantly increased in the sham group and experimental groups 1 and 2 (sham, exp1: $P < 0.001$, exp2: $P < 0.01$), while no significant increase was noted in experimental group 3. Conversely, experimental groups exhibited a significant reduction in the HOMA-IR index, the indicator of insulin resistance, compared to the sham group (exp1: $P < 0.05$, exp2, exp3:

P < 0.001) (Table 3) (de Almeida et al., 2014).

Leptin and Adiponectin

In comparison to the control group, both the sham group and experimental groups 1, 2, and 3 exhibited higher levels of serum leptin (sham: P < 0.01, exp1: P < 0.05). Serum leptin levels in experimental groups 1 and 2 were decreased relative to the sham group but did not reach statistical significance. However, experimental group 3 displayed a substantial reduction in serum leptin levels compared to the sham group (P < 0.05) (Table 3). Levels of adiponectin in the sham group and experimental groups 1, 2, and 3 showed a noteworthy decrease compared to the control group (sham, exp1: P < 0.001, exp2: P < 0.01, exp3: P < 0.05). There was a slight, non-significant increase in adiponectin levels in experimental group 1 compared to the sham group. A considerable rise in adiponectin levels was noted in experimental groups 2 and 3 compared to the sham group (P < 0.01, P < 0.001, respectively) (Table 3).

Histological evaluation

The hepatic tissue in the control group looked normal, however, in the subgroup following a high-fat diet adipocytes were discernible (Fig. 1 A, B). In the experimental groups, administration of ThT at varied dosages caused the generation of polymorphonuclear cells in the liver of experimental groups and a notable reduction in the adipocyte count. The amount of these cells was higher in experimental group 3 (Fig. 1 C-E).

Discussion

The present investigation aimed to explore the influences of Thioflavin-T in modifying adipokine hormones, body weight, and lipoprotein levels besides improving liver functions in mice under HFD.

The findings of the study revealed that all groups experienced significant weight gain after 8 weeks on a high-fat diet compared to the control group, consistent with prior research (Bilal et al., 2021). However, following 4 weeks of Thioflavin-T treatment, there was a notable reduction in body weight. While removing fat from the animals' diet led to weight reduction in the sham group, the experimental groups receiving varying doses of Thioflavin-T demonstrated significant weight loss. The reported weight loss associated with Thioflavin-T treatment aligns with prior research (Najafian et al., 2015; Jalalvand et al., 2016) indicating its potential benefits in weight management. This could be attributed to several factors, including appetite suppression, regulation of blood sugar levels, and improvements in lipid profiles.

Hyperlipidemia commonly occurs during the progressive development of diabetes in HFD-fed mice. The primary concern with dyslipidemia is the elevation of TC, LDL, and TG levels, along with liver lipid accumulation and damage, as well as a decrease in HDL levels, all this is the result of the effect of a high-fat diet on liver tissue (Jalalvand et al., 2016). Therefore, the effectiveness of lipid-modifying agents is typically assessed based on the reduction in TC, LDL, and TG levels, along with the increase in HDL levels (Kang et al., 2014). Mice subjected to a hypercholesterolemic diet for a 12-week duration exhibited a noticeable increase in cholesterol, LDL-C, and TG levels, while HDL-C levels notably decreased (Ale-Ebrahim et al., 2022; Karkhaneh et al., 2016). In line with prior studies such as Bilal et al. (2021), the present research indicates that mice exposed to a high-fat diet for eight weeks manifested irregularities in their plasma lipids. ThT appears to enhance the removal of LDL-C by promoting free radical production, facilitating the breakdown of LDL cholesterol. Consequently, treatment with ThT for 4 weeks led to increased HDL levels, along with decreased LDL and TG levels. ThT is suggested to possess appetite-suppressing properties through leptin hormone stimulation in rodents, which may reduce fat accumulation and promote weight loss (Najafian et al., 2015; Jalalvand et al., 2016). Our findings indicate that ThT treatment, by modulating lipid profiles and reducing fat accumulation, has the potential to improve fatty liver conditions in mice on a high-fat diet.

Elevated hepatic enzymes including AST, ALP, and ALT are often associated with increased body weight and obesity, signaling potential liver issues (Pompili et al., 2020). This phenomenon is commonly observed in patients with non-alcoholic fatty liver disease (NAFLD), with around 20% of patients displaying elevated liver enzymes (Pompili et al., 2020). Consistent with these findings, our study also identified similar outcomes. Notably, the consumption of a high-fat diet in mice led to liver fat accumulation, resulting in changes in liver function markers such as AST and ALT. However, treatment with ThT mitigated these effects by reducing fat

accumulation and liver inflammation. Our research underscores the effectiveness of ThT in reversing adverse liver function marker alterations caused by high-fat diet-induced liver fat accumulation.

Hepatic steatosis can disrupt insulin signaling in the liver, preceding systemic changes in insulin action and contributing to peripheral insulin resistance (Thorn et al., 2011). Consumption of a high-fat diet is known to increase glucose production in liver tissue, leading to hyperglycemia, hyperinsulinemia, obesity, and liver dysfunction in animal models, resembling the phenotype seen in humans with non-alcoholic fatty liver disease (Barclay et al., 2013). Rodents on a high-fat diet exhibit heightened glucose and insulin levels following a glucose challenge compared to those on a normal diet (Barazzoni et al., 2018). Moreover, decreased insulin sensitivity associated with weight gain correlates inversely with adiponectin levels, which are involved in improving fatty liver and reducing liver inflammation (Kandasamy et al., 2012). Our study findings indicate that glucose, insulin, and HOMA-IR increased in the sham group. However, administration of Thioflavin-T to high-fat diet-fed animals normalized these parameters within the normal range. ThT seems to act by regulating glucose levels through enhanced glucose uptake, thereby reducing overall glucose production in the body. This improved insulin responsiveness facilitates increased glucose uptake by cells. Consequently, ThT demonstrates potential benefits for hypoglycemic factors and insulin sensitivity, thereby influencing liver function and ameliorating NAFLD.

Plasma leptin levels significantly rise in individuals with obesity (Cui et al., 2017). Studies have demonstrated that a high-fat diet can increase serum leptin levels, potentially leading to leptin resistance (Karkhaneh et al., 2016), which exacerbates hyperinsulinemia and hyperlipidemia, with obesity itself thought to contribute to leptin resistance. Abnormal leptin levels may also be associated with obesity and sympathetic activity (Cui et al., 2017; Polyzos et al., 2015). Furthermore, circulating leptin levels correlate with body mass index (BMI) and the accumulation of visceral fat and subcutaneous fat, all of which are relevant to non-alcoholic fatty liver disease (Polyzos et al., 2015). This study reinforces these findings by demonstrating an increase in serum leptin levels due to the impact of a high-fat diet on adipocytes. Interestingly, consumption of ThT has been observed to potentially reduce leptin levels, suggesting that ThT could help reduce liver fat accumulation and improve liver function markers such as AST, ALT, and liver enzymes by lowering leptin levels and restoring leptin sensitivity. These effects have the potential to contribute to the prevention or management of NAFLD.

Consuming a high-fat diet and having a higher body weight can lead to a decrease in adiponectin levels (Kandasamy et al., 2012). It is widely acknowledged that reduced adiponectin levels increase the risk of developing diabetes and metabolic syndrome (Recinella et al., 2020). Furthermore, lower levels of circulating adiponectin, which may occur as a result of lipectomy, could negatively impact metabolic parameters, leading to increased serum levels of glucose, insulin, and TG in animals (Recinella et al., 2020). Reduced levels of adiponectin have been reported to be closely associated with non-alcoholic hepatic steatosis in healthy obese individuals. Conversely, intraperitoneal injection of adiponectin has been shown to improve insulin resistance in the muscle and hepatic tissue of obese rodents by decreasing TG concentrations (Eng et al., 2021). Adiponectin elicits various biological effects, including anti-inflammatory and anti-atherosclerotic properties, as well as enhancing insulin sensitivity while reducing insulin resistance (Barazzoni et al., 2018). In our investigation, consumption of a high-fat diet led to increased fat accumulation, inflammation, and insulin resistance, resulting in decreased adiponectin levels. However, administering ThT significantly increased adiponectin levels among the experimental groups, which could lead to enhanced insulin sensitivity and reduced inflammation. ThT's ability to increase adiponectin levels could contribute to improved insulin sensitivity, regulation of lipid metabolism, anti-inflammatory effects, and reduction of hepatic steatosis. These mechanisms collectively suggest that ThT may have therapeutic potential in managing obesity and fatty liver disease by targeting adiponectin-related pathways.

In light of the findings of the present research, it can be inferred that ThT may mitigate lipid profiles and regulate glycemic factors by augmenting adiponectin quantities, thus improving liver function and reducing NAFLD. Previous research has established that diets high in fat or hypercaloric content have the potential to induce liver lipid accumulation, resulting in liver damage, as well as an increase in both the size and quantity of liv-

er hepatocytes and dual-core cells (Chen et al., 2013; Recena et al., 2019; Jalalvand et al., 2016). Our histological observations align with these previous findings. The administration of varying doses of ThT led to a decline in lipid concentrations as well as an improvement in NAFLD (Najafian et al., 2015; Jalalvand et al., 2016). The results obtained from the current research further support this assertion. Examination of liver tissue structure and adipocyte count revealed that Thioflavin-T, by reducing inflammation and fat accumulation in the liver cells, positively impacted tissue structure, which, in turn, positively influenced the levels of liver enzymes and triglyceride reserves. This observation demonstrates a consistent reduction of fat accumulation in hepatic tissue.

While our study provides valuable insights into the potential benefits of Thioflavin-T in mitigating the effects of HFD on various physiological parameters in mice, some limitations have emerged due to constraints in time and funding. The study's primary limitation is the absence of analysis of key molecular mechanisms underlying the observed effects of ThT on liver function and lipid metabolism. This could involve investigating ThT's impact on key signaling pathways involved in lipid metabolism, such as AMP-activated protein kinase (AMPK) or peroxisome proliferator-activated receptors (PPARs). Another limitation that can be considered is ThT's interaction with specific receptors or enzymes involved in adiponectin and leptin signaling pathways to elucidate its mode of action. Our future studies will aim to overcome these limitations to gain a deeper understanding of the mechanisms of ThT on liver function.

Conclusion

The present investigation has demonstrated that the intake of a high-fat diet in mice has induced significant changes in liver enzymes, lipid profiles, leptin, adiponectin, insulin, and liver tissue. However, the administration of ThT has acted as a moderator of these factors. It appears that ThT can enhance liver function and reduce body weight by regulating lipid profiles and liver enzymes, as well as the levels of adipokine hormones. Consequently, ThT may be considered a suitable treatment option for NAFLD. Nevertheless, further research is needed to focus not only on treatment options but also on prevention approaches for NAFLD, as well as exploring other potential uses of Thioflavin-T.

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This study has been performed in the Laboratory Complex of the Science and Research Branch of Azad University.

Conflict of interest

No potential competing interests relevant to this article were reported.

Ethics approval

The experiment was authorized by the Research and Ethics Committee of Science and Research University of Tehran (code: 27081).

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