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Original Article

Melatonin and its Agonist Meliorate Anthropometric and Biochemical Variables in High-Fat Plus High-Fructose Fed Rats

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ABSTRACT

Background: High-fat and fructose-rich diets are known to trigger obesity, lipid abnormalities, and organ damage in rats, closely resembling the features of human metabolic syndrome. Melatonin and its synthetic agonists have been suggested as possible protective agents, but their effects in this context remain unclear. Aims: This study set out to assess the protective role of melatonin and its agonist (agomelatine) against the negative impacts of a high fat and high fructose diet (HFHFD) diet in rats, focusing on body measurements, biochemical markers, adipokines (omentin and chemerin), oxidative stress, and tissue health. Materials and Methods: Thirty-two male Wistar rats were randomly assigned to four groups (n=8 each): control, HFHFD, HFHFD + melatonin, and HFHFD + agomelatine. The treatments continued for 10 weeks. Anthropometric parameters were monitored. Serum biochemical parameters (lipid profile, glucose, MDA, liver and kidney function tests) and adipokine levels were measured using ELISA and standard assays. Histological examination of liver and adipose tissues was performed with hematoxylin–eosin staining. Data were analyzed using ANOVA followed by post hoc testing. Results: Rats fed HFHFD showed increases in cholesterol, triglycerides, LDL, ALP, fat mass, adiposity index, and marked liver and adipose damage. Melatonin and agomelatine reduced weight gain, fat accumulation, and adiposity index, confirming anti-obesity effects. Melatonin further improved cholesterol, LDL, ALP, and preserved tissue by reducing fat cell enlargement, steatosis, and inflammation. Both agents affected adipokines, with melatonin tending to raise omentin (not significant). Agomelatine lowered weight more strongly but offered less liver protection, with persistent steatosis and necrosis. Conclusions: Melatonin and its agonist countered obesity-related changes in HFHFD-fed rats, but melatonin provided broader benefits by improving lipid profile, lowering ALP, and preserving tissue integrity. Agomelatine reduced body weight more effectively yet offered less liver protection. Overall, melatonin appears the more promising candidate against diet-induced metabolic and structural damage.

Keywords: Melatonin, Chemerin, Omentin, High fat and high fructose diet (HFHFD), Rats.

1 Introduction

The global burden of obesity and metabolic syndrome has risen dramatically in recent decades, largely driven by dietary patterns rich in saturated fats and refined sugars, particularly fructose [1]. These unhealthy nutritional habits promote central obesity, insulin resistance, dyslipidemia, oxidative stress, and organ dysfunction, which collectively mimic the pathophysiology of metabolic syndrome and non-alcoholic fatty liver disease [2]. Experimental rodent models employing high fat and high fructose diet (HFHFD) diets reliably reproduce the anthropometric, biochemical, and hormonal disturbances observed in humans, making them valuable for mechanistic studies and therapeutic exploration [3].

HFHFD consumption strongly impacts anthropometric parameters by driving excessive weight gain, fat accumulation, liver weight, and lees index, which are key indicators of adiposity and ectopic lipid deposition [4].The rise in anthropometric indices under HFHFD feeding is closely associated with the development of metabolic complications, including systemic inflammation, oxidative stress, and insulin resistance, making them important early indicators of obesity-related metabolic dysfunction [5]. HFHFD feeding significantly alters adipokine secretion, particularly affecting omentin and chemerin, two key regulators of metabolic homeostasis. Omentin, an anti-inflammatory and insulin-sensitising adipokine, is often reduced under HFHFD conditions, reflecting impaired adipose tissue function and

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diminished protective signalling against obesity-related complications [6]. In contrast, chemerin, which is associated with adipogenesis, inflammation, and metabolic dysfunction, tends to be elevated with HFHFD intake, contributing to insulin resistance and systemic low-grade inflammation [7]. These reciprocal changes in omentin and chemerin highlight how HFHFD disrupts the adipokine network, promoting a pro-inflammatory and metabolically unfavourable profile that accelerates the progression toward obesity and metabolic syndrome.

Atherogenic dyslipidemia—characterized by elevated triglycerides (TG), total cholesterol (TC), and low-density lipoprotein (LDL), alongside reduced high-density lipoprotein (HDL)—is a hallmark of metabolic syndrome and strongly linked to cardiovascular risk [8]. High-fat and high-fructose diets (HFHFD) disrupt glycemic regulation, leading to hyperglycemia and insulin resistance [9]. Oxidative stress is another central mediator of diet-induced metabolic dysfunction, often assessed via malondialdehyde (MDA), a marker of lipid peroxidation. HFHFD feeding increases MDA and depletes antioxidant defenses, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) [10]. In the liver, HFHFD feeding disrupts normal metabolic regulation and induces hepatocellular stress, which is reflected in elevated serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) [11]. These enzymes are widely recognized as biochemical indicators of hepatic dysfunction, and their elevation correlates with impaired lipid and glucose handling, oxidative stress, and altered hepatic metabolism. Beyond hepatic injury, fructose-rich diets also compromise renal function, reflected in elevated serum urea, creatinine, and uric acid [12]. Hyperuricemia itself contributes to insulin resistance and renal dysfunction in metabolic syndrome.

High fat and high fructose diet (HFHFD) feeding induces marked pathological changes in both adipose and liver tissues, reflecting its strong obesogenic and steatogenic properties. In adipose tissue, HFHFD promotes adipocyte hypertrophy, increased fat pad mass, and inflammatory cell infiltration, often accompanied by fibrosis and disrupted tissue architecture, which together impair normal adipose function [13]. Parallel alterations occur in the liver, where excess nutrient load drives lipid accumulation, hepatocellular ballooning, and micro- to macrovesicular steatosis, frequently associated with oxidative stress and inflammatory responses. Over time, these changes compromise hepatic function, predisposing to nonalcoholic fatty liver disease and related metabolic disorders [14]. Collectively, the structural and cellular disturbances in adipose and liver tissues under HFHFD feeding illustrate the interconnected pathophysiology of obesity and metabolic syndrome.

Melatonin, a pineal indoleamine known for its chronobiotic properties, plays a significant role in metabolic regulation, especially in the context of high fat and high fructose diet. It has been reported to attenuate body weight gain and reduce adiposity by stimulating mitochondrial biogenesis, enhancing brown adipose tissue thermogenesis, and suppressing the expression of adipogenic genes [15]. About biochemical variables, melatonin supplementation reduces circulating TG, TC and free fatty acids, while elevating HDL and adiponectin in fructose-fed rats [16]. Similarly, in diet-induce obesity, it improved insulin sensitivity and normalized fasting glucose [17].with potent free radical scavenging capacity, melatonin counteracts MDA effects by lowering its levels, upregulating antioxidant enzymes, and preventing oxidative damage [18]. In liver, Melatonin supplementation has been reported to significantly reduce ALT, AST, and ALP activities, thereby improving overall liver biochemical function. This effect is attributed to melatonin's ability to modulate hepatic enzyme activity, enhance antioxidant defenses, and stabilize cellular homeostasis, ultimately preventing the metabolic disturbances induced by excessive fat and fructose intake [19]. Beyond hepatic effect, melatonin administration in fructose-fed rats reduced serum uric acid and improved renal biochemical parameters, highlighting its protective role against diet-induced renal injury [20]. Although studies on melatonin's influence on omentin and chemerin adipokines remain limited, evidence suggests that it modulates adipokine secretion, lowering leptin and inflammatory mediators while improving adiponectin levels[21].

Melatonin treatment exerts notable protective effects on the histopathological alterations induced by HFHFD feeding in adipose and liver tissues. In adipose tissue, melatonin reduces adipocyte hypertrophy, restores more uniform cell morphology, and decreases inflammatory cell infiltration, reflecting its anti-inflammatory and antioxidant actions. These improvements are often accompanied by attenuation of fibrosis and preservation of normal adipose architecture, supporting healthier adipose tissue function [13]. In the liver, melatonin limits excessive lipid accumulation, mitigates hepatocellular ballooning, and reduces steatosis severity, while also counteracting oxidative stress and inflammatory changes that drive disease progression. Through these effects, melatonin not only alleviates tissue injury but also helps preserve organ integrity, highlighting its therapeutic potential in preventing or reversing obesity-related adipose and hepatic damage [22].

While melatonin is recognized for its protective role against diet-induced obesity and metabolic derangements, its efficacy within the context of a combined high fat and high fructose diet (HFHFD) model remains insufficiently characterized, particularly in relation to adipokine modulation (omentin, chemerin), redox balance, and tissue integrity. Furthermore, the relative effectiveness of synthetic melatonergic agonists has not been conclusively established. Therefore, the present study investigates the influence of melatonin and its agonist on anthropometric, biochemical, and histopathological parameters in HFHFD fed rats, with particular focus on metabolic indices, adipokine dynamics, and organ preservation.

2 Methods and Materials

2.1 Animals

Thirty-two male Wistar rats (initial body weight 190–200 g) were obtained from the Animal House. All animal handling and experimental procedures were performed with the ethical guidelines for animal research. The study protocol was reviewed and granted full ethical approval by the Animal Ethics Committee of the University of Raparin .Animals were housed 4/cage (sterilized plastic cages bedded with soft wood chips) under controlled conditions (22 ± 2 °C; 55–60% humidity; 12-h light/dark cycle) with ad libitum access to food and water[23]. Rats were acclimatized for 7 days before starting the experiment [24].

2.2 Experimental design

After acclimatization, rats were randomly allocated to four groups (n = 8/group) each group receive different diet for 10 weeks: Group 1, receive Standard diet (Control): a balanced formulation designed to provide essential nutrients for normal growth, physiological functions. Group 2, receive high fat and high fructose diet (HFHFD): (Rendered adipose tissue from large animals was incorporated into the rats' standard diet 50%) + (20% dissolved in tap water every two days). Group 3, receive HFHFD + melatonin: 12.5mg of melatonin /500ml of tap water (in dark bottle) dissolved in 0.1% Ethanol every two days. Group 4, receive HFHFD + agomelatine: 12.5mg of Agomelatine/500ml of tap water (in dark bottle) dissolved in 0.1% Ethanol every two days.

2.3 Anthropometric measurements

Body weight, body length (nose-to-anus), and tail length were systematically recorded at two-week intervals across the 10-week experimental period (weeks 0, 2, 4, 6, 8, and 10). Body weight was determined by using a digital balance with a precision of ±0.1 g. Body length and tail length were obtained using a flexible measuring tape while the animals were gently held without the use of anesthesia [25].

2.4 Blood Sampling Procedure

At week 10, rats were fasted for 8–10 hours with free access to water [26], then anesthetized using a ketamine (100 mg/ml) and xylazine (20 mg/ml) mixture [27]. Blood samples were obtained via cardiac puncture [28], transferred into gel tubes, and allowed to clot at room temperature for 30 minutes. The samples were then centrifuged at 3,000 rpm for 10 minutes to separate serum. Resulting serum aliquots were stored at −18 °C until biochemical analysis [29]. following exsanguination, the liver and visceral adipose tissues were dissected, weighed, and processed for subsequent analyses.

2.5 ELISA-Based Determination of Chemerin and Omentin

Chemerin and omentin concentrations were quantified using a commercial rat ELISA kit (Sunlong Biotech, China), following the manufacturer's protocol. Standards were prepared through serial dilution to yield concentrations ranging from 7.5 to 180 pg/ml, while samples were diluted 1:5 prior to assay. In brief, diluted standards and samples were loaded into the wells and incubated at 37 °C for 30 minutes. Wells were then washed and treated with an HRP-conjugated reagent, followed by a second incubation and wash cycle. Chromogenic substrates (chromogen solutions A and B) were subsequently added, and color development was allowed to proceed for 15 minutes under dark conditions. The reaction was terminated using a stop solution, and absorbance was recorded at 450 nm with a microplate reader. Finally, chemerin and omentin concentrations were calculated from the standard curve and adjusted for the dilution factor. This methodological approach ensured reliable detection within the assay's dynamic range.

2.6 Measurement of Serum MDA via TBA–TCA Method

Serum malondialdehyde (MDA) concentrations were determined using the thiobarbituric acid–trichloroacetic acid (TBA–TCA) method [30], which detects thiobarbituric acid reactive substances (TBARS). In brief, serum samples were mixed with TCA and TBA reagents, vortexed, and incubated in a boiling water bath at 95 °C for 45 minutes. Following cooling to room temperature, 70% TCA was added, and the mixture was centrifuged at 1000 rpm for 15 minutes. The resulting pink-colored supernatant was carefully collected, and absorbance was recorded at 532 nm using a spectrophotometer. MDA concentrations (µmol/L) were calculated from the absorbance values using an extinction coefficient of 1.56×10^5 $M^{-1} \cdot cm^{-1}$, with results adjusted for the sample dilution factor [31].

2.7 Serum Biochemistry Evaluation

Serum glucose, lipid profile tests (triglyceride, total cholesterol, HDL and LDL), liver function tests (ALT, AST, ALP, and GGT), renal function tests (Creatinine, urea and uric acid) are determined by a fully automated biochemical analyzer (Cobas 311).

2.8 Histological Analysis

2.8.1 Adipose tissue and liver, dissection and weighing

At the time of sacrifice, rats were anesthetized and dissected to obtain visceral white adipose tissue (WAT) depots and liver samples following blood collection. Epididymal, perirenal, and omental fat pads were carefully dissected, freed from surrounding connective tissue, and weighed. The total WAT mass was calculated as the sum of these three depots. The liver was isolated, gently blotted dry, and weighed to determine relative liver mass. Representative portions of both adipose tissue and liver were subsequently fixed in 10% neutral-buffered formalin for subsequent histological examination [32]. Histological examination of adipose tissue was performed to assess adipocyte size and macrophage infiltration using hematoxylin and eosin (H&E) staining. Liver sections were examined for signs of steatosis and inflammation using H&E staining and Oil Red O staining for lipid accumulation.

2.9 Data analysis

All of the obtained data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple

comparison test. Normality and homogeneity tests also applied to the data using GraphPad Prism (version 9), and the data are expressed as mean ± SEM.

3 Results

Serum chemerin and omentin levels are presented Figure 1. Chemerin (Figure 1A) concentrations did not differ significantly among all groups (ns). The numerical values range around (161 pg/ml) between Control, high fat and high fructose diet (HFHFD) and HFHFD + melatonin groups, whereas a slight, non-significant reduction was observed in the HFHFD + agomelatine group (145.7 ± 8.5 pg/ml). Similarly, serum Omentin (Figure 1B) shows that concentrations were not significantly altered between groups. The tabulated data demonstrate a modest numerical increase in omentin with HFHFD (135.9 ± 6.4 pg/ml) then melatonin continue to this elevation (146.9 ± 10.8 pg/ml), in contrast agomelatine cause to decrease omentin level (125.1 ± 10.9 pg/ml). Overall, although no statistically significant differences were detected, melatonin supplementation tended to increase serum omentin, whereas agomelatine showed a strong lowering effect on Omentin level.

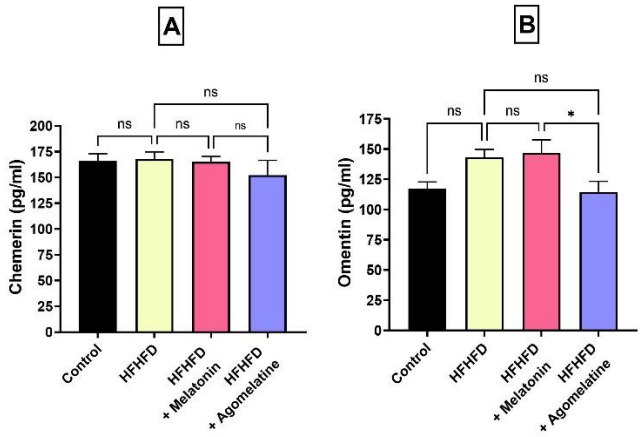

Figure 1: Serum levels of (A) chemerin and (B) omentin (pg/ml) in control and experimental groups. Data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA followed by post hoc comparisons. ns=non significance.

Table 1 summarizes the key biochemical variables measured in the different experimental groups, providing an overview of metabolic and organ function changes. cholesterol levels significantly increase in the high fat and high fructose diet (HFHFD) group compared to the control. In contrast both treatment groups (HFHFD + melatonin and HFHFD + agomelatine) substantially reduces cholesterol and bringing it close to control levels. These results suggest that melatonin and its agonist have beneficial roles in controlling cholesterol levels. a significance elevation of triglycerides in the HFHFD group and actually continue to rise in the HFHFD + melatonin and HFHFD + agomelatine groups which exceeding the levels in the HFHFD group but not significantly detected. High-Density Lipoprotein levels are slightly elevated in the HFHFD group compared to control and remain modestly increased across the melatonin-treated groups. This suggests that HDL is not adversely affected by high-fat and fructose intake and is relatively remain stable under melatonin treatment. Low-Density Lipoprotein (LDL) increased with the HFHFD diet in compare to control, indicating metabolic effect. Melatonin and its agonist (agomelatine) appears to reverse this effect.

Glucose levels in the HFHFD group, which aligns with the table value slightly higher than control group. While decreases slightly with melatonin treatments, this reduction is not statistically significant. This suggests that while melatonin and agomelatine may have some influence on glucose levels, it is not strong enough to be considered a consistent therapeutic effect in this setup. Malondialdehyde (MDA) – Oxidative Stress, appears to decrease across all HFHFD and melatonin groups compared to control. However, the differences are not statistically significant. The table supports this trend with generally lower MDA values in the treated groups. This indicates a possible mild antioxidant effect of melatonin, though not at a level of strong statistical confidence in this study.

A strong and significant increase in ALP activity detected in the HFHFD group compared to control. In melatonin + HFHFD group decreased but dramatic increase in agomelatine. Results illustrated that HFHFD cause to elevation of ALP and agomelatine further elevate it but in contrast melatonin decrease this elevation. This suggests that melatonin may protect against this elevation and might even reduce ALP elevation, particularly when used with HFHFD. the ALT levels appear relatively unchanged across all groups, with no significant differences. all groups result remained closely around control. AST shows a clear decrease in the HFHFD group compared to control. Interestingly, AST level increased in melatonin also in agomelatine compared to HFHFD group. This indicates that melatonin and its agonist decrease the effect of HFHFD and return the enzyme's level close to its original point. GGT levels remain relatively unchanged across all groups. However, HFHFD and other treatment groups have slightly elevated GGT levels, the variation is not statistically meaningful. Therefore, GGT appears unaffected by diet or treatment.

Creatinine levels remained relatively stable across all groups, with no statistically significant differences. Urea level significantly decreased in the HFHFD group. As well as in treatment groups continue to decrease especially in agomelatine + HFHFD group. Uric acid concentration remained statistically unchanged across all experimental groups.

Table 1: Lipid, Oxidative Stress, Hepatic, and Renal Profiles in Rats Across Experimental Groups.

Groups / Biochemistry tests	Control	High fat and high fructose diet (HFHFD)	FHFD + melatoni	HFHFD + agomelatine
Cholesterol (mg/dl)	58.13±4.043	72.65±3.367**	61.13±2.064##	69.50±7.114#
Triglyceride (mg/dl)	31.70±4.419	51.62±7.352*	71.90±5.189	77.73±9.602
Low density lipoprotein (LDL) (mg/dl)	13.73±0.8864	19.03±0.6354*	15.78±1.004	17.90±2.416
High density lipoprotein (HDL) (mg/dl)	39.25±3.541	50.70±3.630	39.32±1.806	43.45±4.309
Glucose (mg/dl)	204.5±22.26	233.7±20.39	191.0±19.59	223.3±15.85
Malondialdehyde (MDA) (µmol/L)	5.293±0.2867	4.605±0.5008	4.055±0.1544	4.435±0.4469
Alkaline phosphatase (ALP) (IU/L)	145.7±13.97	320.3±27.77****	275.8±19.35	411.2±20.49#
Alanine Transaminase (ALT) (IU/L)	50.37±12.55	48.17±2.611	44.83±1.707	58.93±4.367
Aspartate aminotransferase (AST) (IU/L)	124.6±22.15	76.75±3.391*	133.7±6.520#	140.80±7.971##
Gamma-glutamyl transferase (GGT) (IU/L)	1.667±0.4944	2.217±0.2023	4.500±0.7638	3.000±0.5774
Urea (mg/dl)	44.35±2.193	27.45±1.393***	26.28±0.6284	20.87±1.253#
Creatinine (mg/dl)	0.3700±0.0073	0.3750±0.0092	0.3517±0.0104	0.3600±0.0152
Uric acid (mg/dl)	1.433±0.2362	1.217±0.1014	1.717±0.1701	1.367±0.1054

*Indicate significance differences between HFHFD and control group
indicate significance difference between treatment groups and HFHFD group

Anthropometric parameters are illustrated in Figure 2 and Table 2. Fat Tissue Weight (Figure 2A) in HFHFD group showed a slight increase (25.37±0.1626) in comparison to control group (24.78±0.2358). Treatment with melatonin caused a sharp reduction and similar values were seen with agomelatine. Despite a significant rise in fat tissue with HFHFD compared to Control, both melatonin and agomelatine interventions caused highly significant decreases, nearly halving fat tissue weight. This demonstrates strong anti-adiposity effects of melatonin and its agonist when administered with HFHFD. Liver Tissue Weight (Figure 2B) showed modest changes across groups. while the HFHFD diet slightly increased liver mass but it is statistically significant. Neither melatonin nor its agonist (agomelatine) had a notable effect on liver tissue weight in this model. Adipose Tissue Index % (Figure 2C) significantly arised in HFHFD group, then Melatonin and agomelatine treatment reduced this markedly. This present a strong protective impact against diet-induced adiposity by both melatonin and agomelatine, which exceeding baseline (control) values. Initial Body Weight (Figure 2D) were nearly identical across groups with slight increases in the melatonin groups but not statistically significant. This demonstrates that all groups started at comparable weights, ensuring the validity of subsequent weight gain comparisons. Final Body Weight (Figure 2E) as expected, the HFHFD group showed significantly lower final body weight (396.5±1.973) compared with the control group (412.9±2.065). in melatonin treated group, the result continues to deacrese (302.3±0.8399), then agomelatine further decrease final body weight. The decrease was highly significant (****$p < 0.0001$), indicating powerful weight-reducing effects of melatonin and agomelatine. Body Weight Gain (Figure 2F) prove righted data on Graph E, that Body weight gain is highest in control group then followed lower weight gain in HFHFD group. about HFHFD + melatonin and HFHFD + agomelatine, both treatments sharply reduced weight gain compared to the HFHFD group, confirming their anti-obesogenic effect.

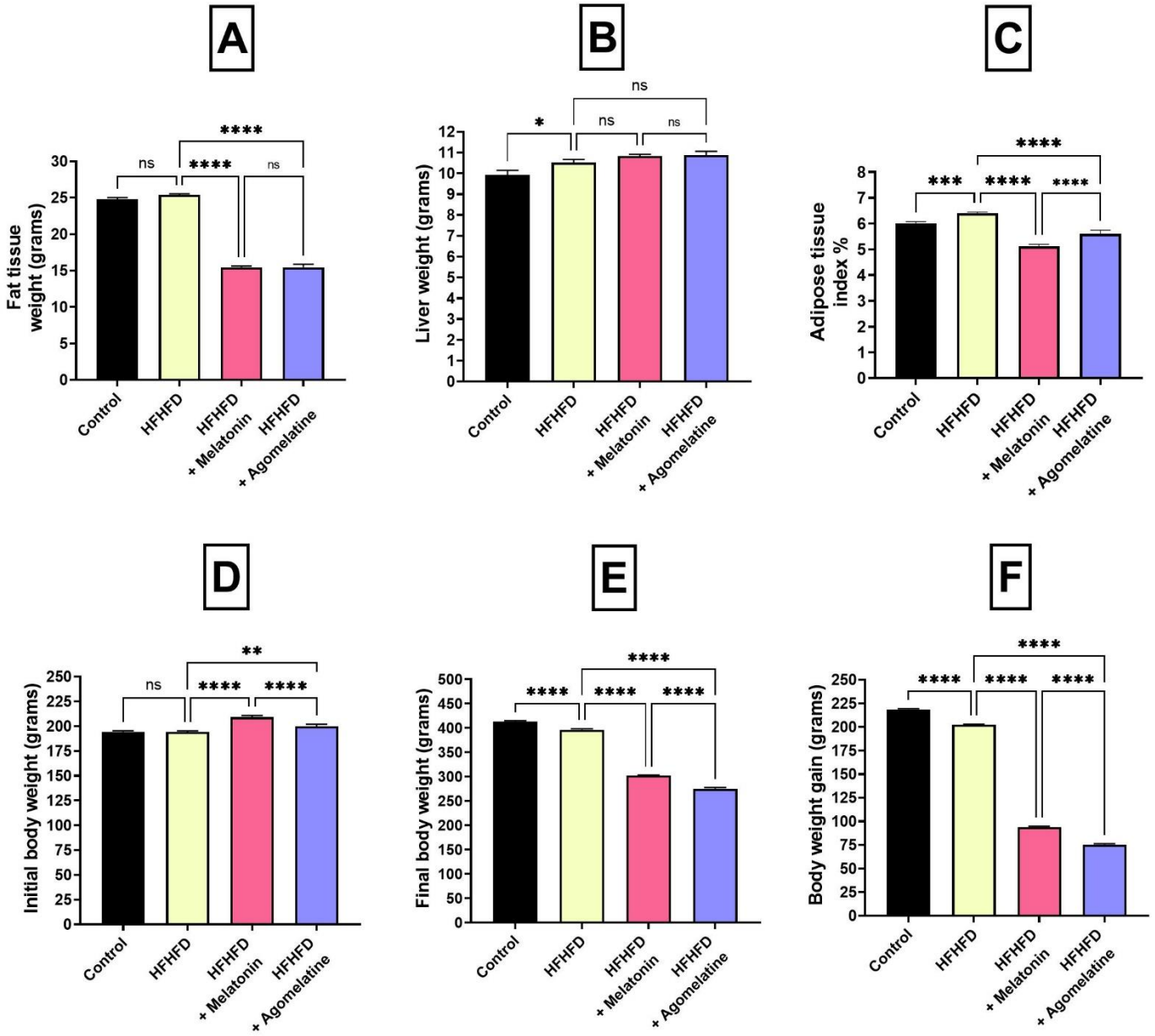

Figure 2: Effects of treatments on body and tissue weight parameters. (A) Fat tissue weight, (B) Liver weight, (C) Adipose tissue index, (D) Initial body weight, (E) Final body weight, and (F) Body weight gain in control and experimental groups. Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by post hoc tests; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns = not significant.

Table 2: Comparison tissue weight (fat and liver), adipose tissue index, initial body weight, final body weight and body weight gain. body weight, among different rat groups.

Groups / Variables	Control	High fat and high fructose diet (HFHFD)	HFHFD+melatonin	HFHFD + agomelatine
Fat tissue weight (grams)	24.78±0.2358	25.37±0.1626	15.45±0.1727####	15.45±0.1727####

Liver tissue weight (grams)	9.933±0.2171	10.52±0.1537*	10.83±0.08819	10.88±0.07719
Adipose tissue index %	6.006±0.06607	6.401±0.04870***	5.123±0.07084####	5.601±0.05948####
initial body weight (grams)	194.4±1.194	194.3±1.161	209.1±1.630####	200.0±0.7071##
Final body weight (grams)	412.9±2.065	396.5±1.973****	302.3±0.8399####	275.1±0.8543####
Body weight gain (grams)	218.5±0.8466	202.3±0.5578****	94.00±0.8165####	75.33±0.4216####
*Indicate significance differences between HFHFD and control group # indicate significance difference between treatment groups and HFHFD group				

Body Weight (Figure 3A) This graph shows a significant increase in the high fat and high fructose diet (HFHFD) group similar to Control group untill week 10. But oppositely Both Melatonin and agomelatine groups showed a significant reduction in body weight compared to the HFHFD group. This difference became highly significant (****) for the agomelatine group starting from week 2 and for the Melatonin group from week 4. Body Mass Index(BMI) (Figure 3B) Similar to body weight, the HFHFD group's BMI increased significantly same with Control group, untill week 10 which become lower than control (indicated by four asterisks). Focus on Both melatonin treatment groups showed a significant reduction in BMI relative to the HFHFD group, from week 4 onward. Body Length (Figure 3C) & Tail Length (Figure 3D) in both graphs, The HFHFD group showed a slight significant difference in both body and tail length comapared to Control group. In contrast, both the HFHFD + melatonin and HFHFD + agomelatine groups had significantly reduced lengths compared to the HFHFD group, especially detected in weeks 6,8,10 (****). Body Weight/Tail Length Ratio (Figure 3E) displays the ratio of body weight to tail length, which is a key indicator of obesity. The HFHFD group showed a steady and slight significant differenece compared to Control group throughout the experiment. Both HFHFD + melatonin and HFHFD + agomelatine groups demonstrated a significant reduction in this ratio compared to the HFHFD group, especially HFHFD + agomelatine group. Lees Index (Figure 3F) , another metric for obesity. It is critical to note that there are no asterisks comparing the HFHFD group to the Control group, meaning a statistically significant difference between these two groups is not shown on this graph. However, clearly shows that both the melatonin and agomelatine treatments led to a significant decrease in the Lees Index compared to the HFHFD group wich strongly can seen after week four. Finally, note that all graphs strong their data by each other, that illustrate effect of melatonin and its agonist can work as antiobestic treatment.

The provided Figure 4 displays hematoxylin and eosin (20µm H&E) stained histopathological sections of adipose tissue from different treatment groups. The staining highlights cell nuclei in purple and the cytoplasm and extracellular matrix in pink. The figure uses black and blue arrows to point out specific features.

The images from the Control group (A, B, C) display normal adipose tissue morphology. The blue arrows point to typical adipocytes, characterized by their consistent polygonal shape. The black arrows mark denotes normal nuclei, which appear small and are displaced toward the periphery of the cells by the large central lipid droplet. In contrast, the high fat and high fructose diet (HFHFD) group (D, E, F) exhibits marked pathological alterations. Here, the black arrows indicate enlarged adipocytes with irregular morphology, a condition referred to as hypertrophy, reflecting excessive lipid accumulation. The blue arrows emphasize the presence of fat deposition with a heterogeneous irregular pattern, suggesting that adipocytes are not only hypertrophic but also structurally disrupted.

The melatonin (G, H, I) and agomelatine (J, K, L) treated groups demonstrate significant histological improvements. The blue arrows denote adipocytes of smaller and more uniform dimensions compared with those in the HFHFD group, while the black arrows reveal nuclei in their normal peripheral position and an overall more organized tissue architecture. Collectively, these findings indicate that both melatonin and its synthetic agonist effectively counteracted adipocyte hypertrophy and preserved the normal histological features of adipose tissue, despite exposure to an obesogenic diet.

After adipose tissue, histopathology of liver known as another indicator of melabolic disorder (Figure 5). In Control group (A, B, C), the liver retains its normal architecture. Black arrows denote the portal vein in its physiological state, blue arrows identify healthy hepatocytes, and yellow arrows indicate intact Kupffer cells. Red arrows highlight well-preserved sinusoids, while green arrows in images (A) and (C) mark minor bleeding, a normal incidental finding. Collectively, these observations confirm a healthy hepatic baseline in the absence of high fat and high fructose diet (HFHFD) feeding. In contrast, the HFHFD group (D, E, F) demonstrates pronounced pathology. Black arrows identify large macrovesicular steatosis, while blue arrows

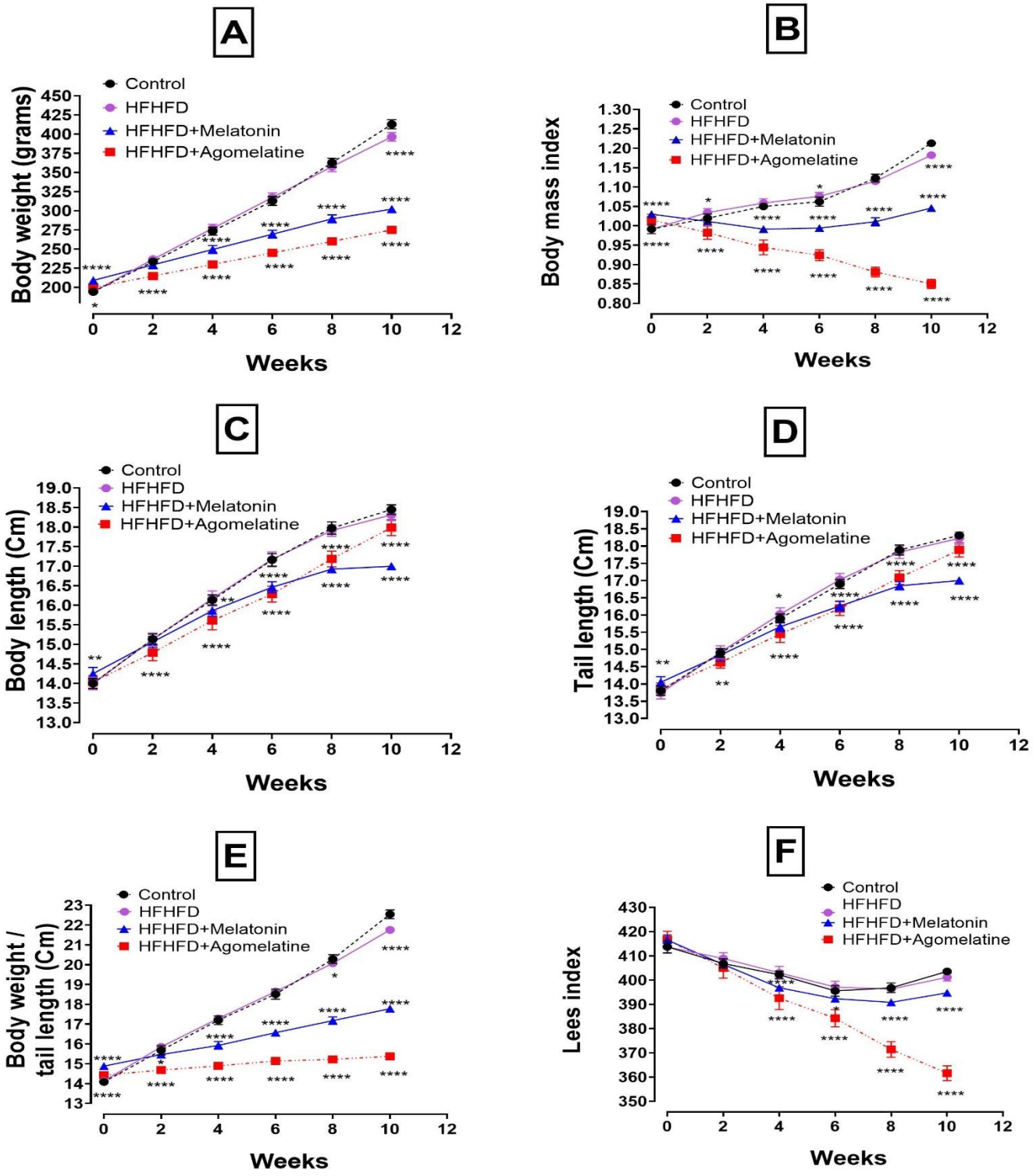

Figure 3: Effects of melatonin and agomelatine treatments on anthropometric and metabolic parameters over 10 weeks. Graph A: Body Weight, Graph B: Body Mass Index, Graph C: Body Length, Graph D: Tail Length, Graph E: Body weight to tail length ratio, and Graph F: The Lees index. statistical significance indicated by asterisks: *** and **** denote highly significant differences ($p<0.001$ and $p<0.0001$, respectively).

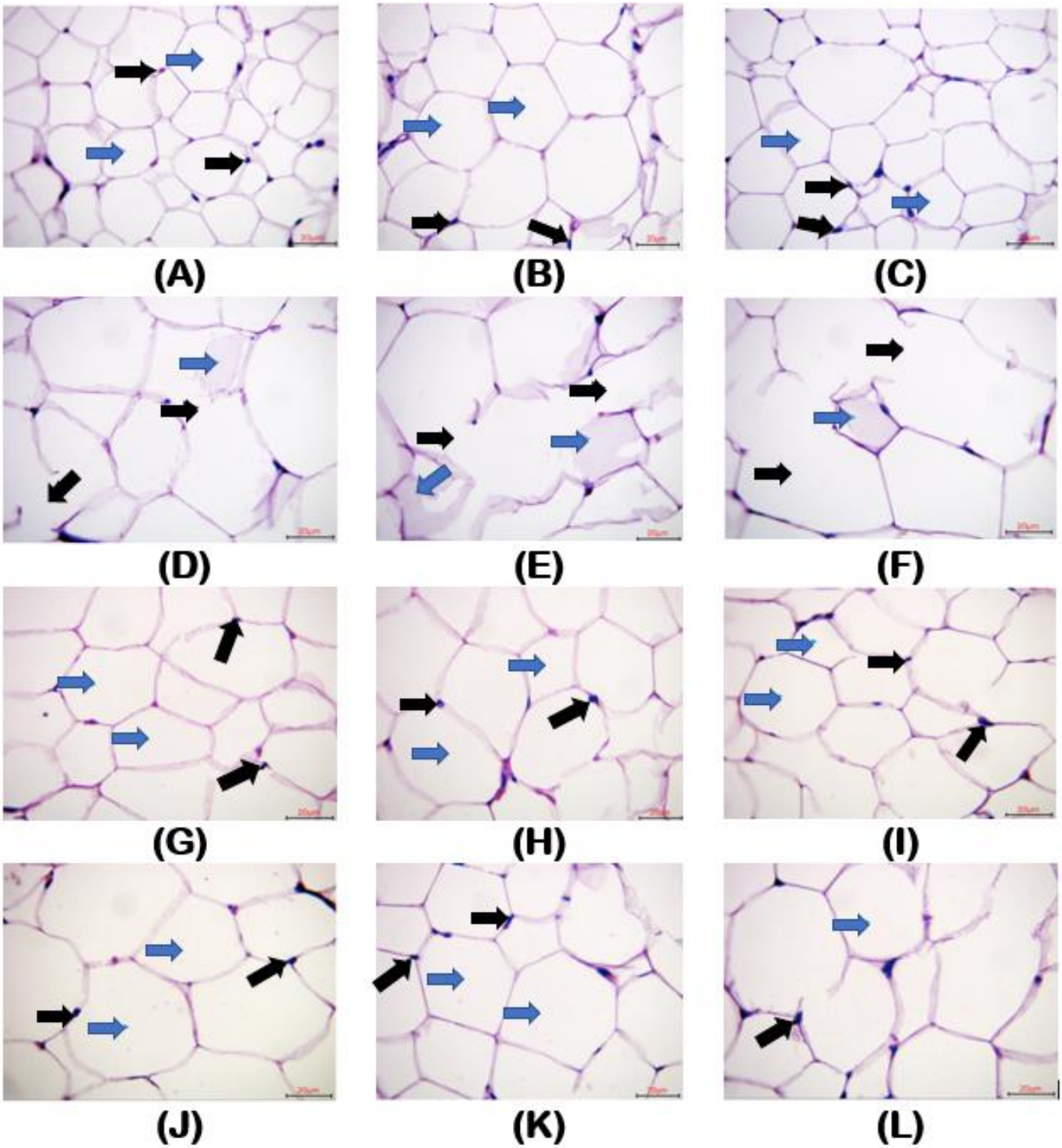

Figure 4: Histological section of adipose tissue of rats in different groups. (A, B, C) normal rats (standard diet in normal environment). (D, E, F) rats fed with high fat and high fructose diet (HFHFD) at normal environment. (G,H,I) rats fed with HFHFD and treated with melatonin (J,K,L) HFHFD and treated with agomelatine.

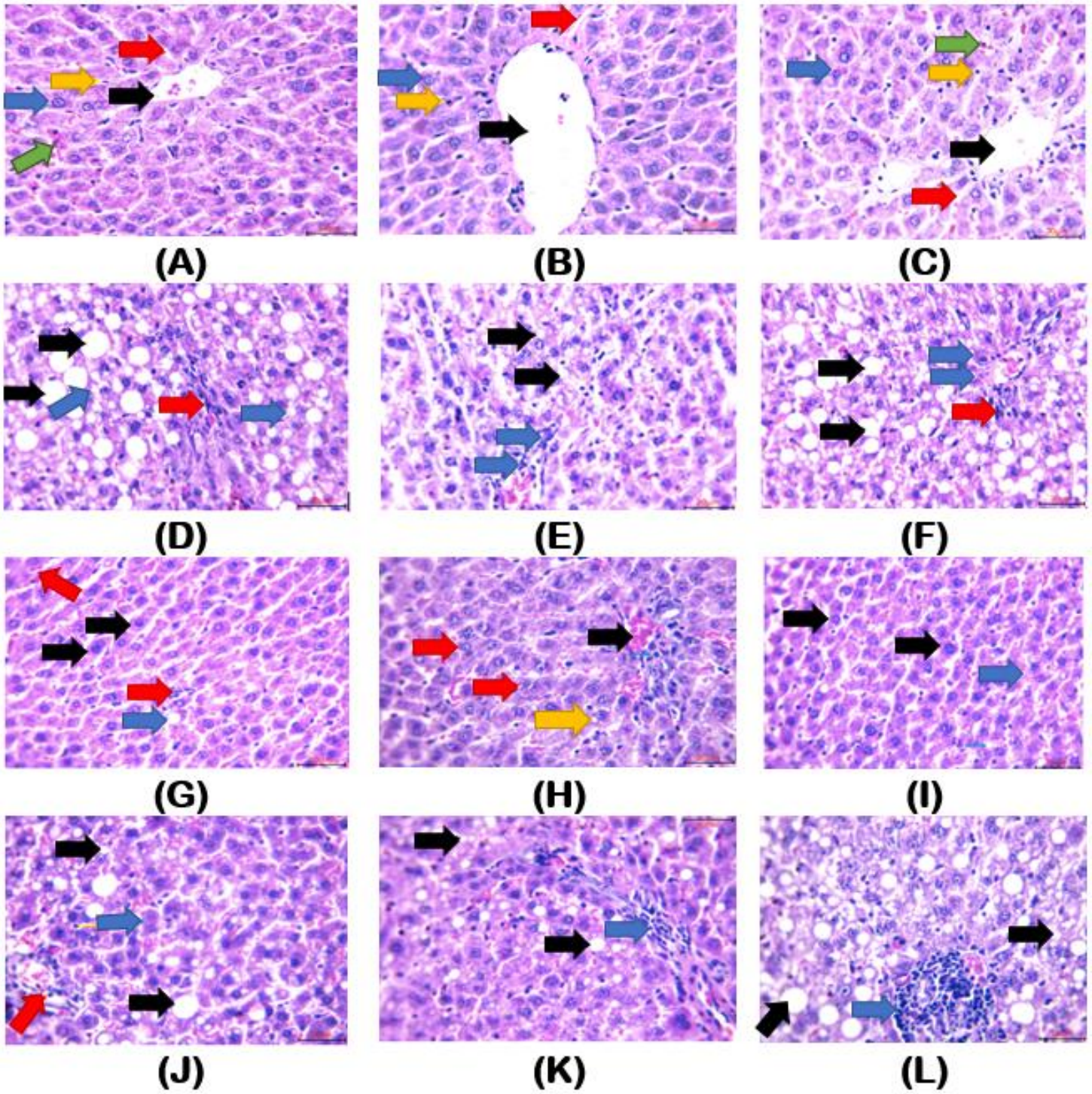

Figure 5: Histological section of rat's liver in different groups (20µm H&E) (A, B, C) normal rats (standard diet in normal environment). (D, E, F) rats fed with HFHFD. (G, H, I) rats fed on HFHFD and treated with melatonin. (J, K, L) rats fed on HFHFD and treated with agomelatine.

in (D) and (F) denote microvesicular steatosis, both indicating substantial lipid accumulation within hepatocytes. Red arrows in (D) and (F) highlight focal inflammatory cell infiltration, and the blue arrow in (E) shows periportal inflammation surrounding the portal vein, reflecting a strong inflammatory response characteristic of diet-induced hepatic injury. The HFHFD and Melatonin-treated group (G, H, I) exhibits evident improvement. Black arrows in (G) and (I) indicate the presence of normal hepatocytes, suggesting a protective effect. Blue arrows in these images point to reduced macrovesicular steatosis, implying partial prevention of lipid accumulation. Although the black arrow in (H) shows portal vein congestion, the red arrows (H) and blue arrows (H, I) reveal markedly reduced inflammatory cell infiltration, confirming the anti-inflammatory and

hepatoprotective properties of melatonin. Conversely, the HFHFD and agomelatine-treated group (J, K, L) displays a more complex and injurious profile. Black arrows demonstrate the persistence of both macrovesicular and microvesicular steatosis, while additional features indicate advanced hepatic injury. The blue arrow in (J) marks hepatocyte necrosis, and blue arrows in (K) and (L) highlight extensive inflammatory cell infiltration. Furthermore, red and yellow arrows in (J) reveal inter-hepatocytic bleeding and inflammatory infiltration, respectively, suggesting that while the agonist modulated fat deposition, it concurrently provoked severe hepatocellular damage and inflammation.

4 Discussion

This study demonstrates that melatonin and its synthetic agonist (agomelatine), provide multifaceted protective effects in rats subjected to a high fat and high fructose diet (HFHFD) diet, for produce metabolic disorder even in the absence of overt obesity. Both compounds elicited significant reductions in fat tissue mass, adiposity index, and final body weight compared with the HFHFD group, confirming their efficacy in limiting excessive lipid accumulation. Notably, the beneficial effects extended beyond body weight and adiposity, encompassing adipokine modulation, improvements in serum lipid profiles, reduction of glucose and Malondialdehyde (MDA), hepatic enzyme homeostasis (liver enzyme balance) and histological preservation of critical tissues. These results underscore the potential of melatonergic pathways in mitigating metabolic complications associated with unhealthy dietary intake (high fat and high fructose).

Chemerin, which is commonly associated with pro-inflammatory signaling and adipogenesis, showed modest fluctuations but no pronounced increase under melatonergic intervention. This stability of chemerin levels despite high fat and high fructose diet intervention is consistent with some studies suggesting that chemerin may not always increase dramatically in such metabolic stress models without additional factors [33]. As well as, the modest changes observed in chemerin with melatonergic intervention highlight a complex interplay where melatonin may not strongly upregulate chemerin but could modulate its levels indirectly through anti-inflammatory and metabolic pathways. overall, these data underscore the complex regulation of chemerin in diet-induced metabolic dysfunctions and melatonergic interventions. Melatonin treatment tends to elevated omentin levels is well supported by previous research demonstrating melatonin's role in increasing omentin and other beneficial adipokines, contributing to improved insulin sensitivity and reduced inflammation in metabolic disorders [34]. However, the observed slight reduction of omentin with agomelatine contrasts with the typical melatonin effect, indicating a divergence from established findings. This discrepancy may be explained by agomelatine's unique pharmacological profile as both a melatonin receptor agonist and serotonin receptor antagonist, which might influence adipokine regulation differently [35].

The biochemical findings further reinforce these protective effects. As expected, the HFHFD exposed rats have elevated total cholesterol and LDL levels, whereas melatonin and its agonist treatments effectively normalized these alterations. These observations are consistent with prior reports demonstrating that melatonin reduces cholesterol absorption and enhances lipid metabolism in experimental models [36]. Interestingly, while melatonin treatment lowered cholesterol and adiposity, triglyceride levels continued to rise, even exceeding those of the HFHFD group. Although this increase was not statistically significant, it may reflect enhanced lipolysis and hepatic re-esterification of free fatty acids into triglycerides for VLDL export [37]. Glucose levels are elevated in the HFHFD group compared to controls, and melatonin treatment only slightly reduces glucose without statistical significance. This indicates that melatonin's effect on lowering glucose in this model is limited and inconsistent, supporting evidence that melatonin more reliably improves long-term glycemic control and insulin sensitivity rather than acutely lowering blood glucose. Factors like treatment duration, dosage, and model specifics likely influence its efficacy, highlighting that melatonin alone may not provide strong therapeutic glucose-lowering in this setup [38]. The observed decrease in malondialdehyde (MDA) levels across all HFHFD and melatonin groups compared to controls suggests a reduction in oxidative stress, as MDA is a key biomarker of lipid peroxidation and cellular damage caused by reactive oxygen species. This decrease may reflect the antioxidant effects of melatonin, which can reduce lipid peroxidation and oxidative damage [39]. However, the finding that MDA is lower in the HFHFD group than in controls is somewhat unusual because HFHFD typically increase oxidative stress and MDA levels. This could indicate an adaptive antioxidant response, experimental variability, or metabolic changes that reduce detectable lipid peroxidation products temporarily. Such results highlight the complexity of oxidative stress mechanisms and suggest that factors like antioxidant defences or measurement timing might influence MDA levels in these models, requiring further investigation to clarify this unexpected decrease despite a high-fat/fructose diet.

The observed increase in ALP activity with HFHFD suggests liver stress, likely involving the bile ducts or cholestasis. This aligns with findings from other studies showing that such diets cause oxidative stress, inflammation, and liver injury, reflected by elevated ALP levels [40]. Melatonin appears to reduce this elevation, highlighting its antioxidant and anti-inflammatory protective roles in the liver [41]. Conversely, the melatonin agonist (agomelatine) unexpectedly caused a further increase in ALP, which may point to distinctive effects depending on the specific compound and dosage. The observation that ALT levels remained stable across all experimental groups suggests that neither the dietary intervention nor the treatments induced overt hepatocellular injury. ALT is a sensitive marker of acute liver cell damage, yet its stability indicates that the extent of liver stress or fat accumulation was insufficient to trigger significant hepatocyte leakage. This finding aligns with previous reports demonstrating that HFHFD can lead to hepatic steatosis and oxidative stress

without necessarily elevating serum ALT, particularly in the early or moderate stages of metabolic dysfunction. Thus, unchanged ALT levels in the present study likely reflect subclinical liver alterations rather than acute hepatocellular necrosis [42]. AST decreased with HFHFD but was restored toward normal levels by melatonin and agomelatine. This offer that melatonin can help reverse or mitigate liver metabolic dysfunction or mitochondrial impairment caused by the diet, supporting its hepatoprotective properties observed in prior studies [43]. GGT levels stayed consistent amongst all groups. GGT acts as a sensitive indicator for bile duct-related liver injury [44], and its lack of change in this context points to an absence of substantial bile duct damage or cholestasis despite other indicators of liver stress. Overall in conventional liver enzymes such as ALT, AST, and GGT remained within normal ranges, indicating preserved hepatocellular function. However, alkaline phosphatase (ALP) was notably elevated, suggesting early or subclinical liver dysfunction, particularly related to cholestasis or bile duct involvement. This isolated rise in ALP, despite normal other enzymes, underscores its sensitivity as an early marker of metabolic liver disturbances and highlights the need for further evaluation of biliary or infiltrative processes when ALP is elevated [45].

Creatinine levels remained stable across all groups, with no significant differences from controls, indicating preserved glomerular filtration and unaltered renal clearance however administrating high fat diet and fructose [46]. In contrast, urea concentrations were significantly reduced in the HFHFD group compared with controls and declined further in treatment groups, particularly in the agomelatine + HFHFD group. Rather than reflecting improved kidney function, this reduction, more likely indicates altered hepatic nitrogen metabolism, since HFHFD feeding has been reported to impair urea-cycle activity and reduce serum urea in experimental models of metabolic dysfunction [47]. Similarly, melatonin and related agents have been suggested to no influence amino acid and nitrogen metabolism, then reducing urea generation [48]. Meanwhile, uric acid levels remained unchanged across all groups, consistent with previous studies showing that moderate HFHFD exposure does not necessarily alter purine catabolism or uric acid homeostasis, This supports the idea that uric acid alterations depend on exposure level and are not automatic in all HFHFD, especially moderate or shorter duration exposures [49].

The findings on fat tissue weight and final body weight collectively demonstrate that HFHFD slightly increased fat accumulation while producing only a modest decrease in body weight compared to controls. On the other hand, melatonin and agomelatine treatments led to a marked reduction in both fat tissue weight and overall body weight, confirming their potent anti-obesity actions in diet-induced obese rats. These effects are consistent with previous evidence showing that melatonin reduces fat mass and body weight by promoting the browning of white adipose tissue, enhancing energy expenditure, improving mitochondrial function, and suppressing inflammation [50]. Agomelatine elicited comparable reductions, suggesting it shares these beneficial metabolic effects, likely via melatonin receptor activation and pathways regulating adipogenesis and energy balance. Notably, the even greater weight reduction observed with agomelatine indicates potentially stronger melatonergic activity in metabolic regulation. Together, these results strengthen the growing body of research supporting melatonin and its analogs as promising therapeutic agents for managing obesity and preventing related metabolic dysfunctions [51]. Liver tissue weight in the Control group showed modest changes compared to the experimental groups, where it was slightly higher. The HFHFD caused a small increase in liver mass, but neither melatonin nor its agonist produced a noticeable effect on liver tissue weight in this model. This suggests that while the diet influences liver size to some extent, the treatments likely do not affect overall liver mass, possibly acting through other mechanisms such as biochemical or functional modulation.

The histological evaluation of adipose and liver tissues further supports the biochemical and anthropometric outcomes of this study. In adipose tissue, the HFHFD groups preserve induced classic features of obesity-related remodeling, including adipocyte hypertrophy and irregular lipid deposition, consistent with excessive fat storage and tissue dysfunction [52]. These pathological changes were markedly attenuated by melatonin and agomelatine, which preserved normal adipocyte morphology, reduced cell enlargement, and maintained nuclei in their peripheral positions. Such improvements confirm the anti-hypertrophic role of melatonergic signaling and parallel previous reports that melatonin restores adipose tissue integrity under obesogenic conditions [53].

In the liver, Melatonin treatment did not produce significant changes in biochemical and anthropometric liver parameters; however, histological examination revealed marked alterations in liver tissue structure, indicating that melatonin's effects may be more apparent at the cellular or tissue level rather than in standard biochemical markers. The HFHFD group exhibited pronounced macrovesicular and microvesicular steatosis, periportal inflammation, and leukocyte infiltration, hallmarks of diet-induced hepatic injury. Melatonin treatment conferred substantial protection, reducing steatosis and inflammatory foci while preserving overall hepatocyte integrity, thereby corroborating its recognized hepatoprotective actions [54]. In contrast, agomelatine, despite improving adipose tissue architecture, failed to replicate these hepatic benefits. Persistent steatosis, inflammatory infiltration, and even necrotic changes were evident, indicating a more complex pharmacodynamic profile. These findings suggest that while both agents counteracted adipocyte hypertrophy, only melatonin exerted consistent protection across both metabolic and hepatic endpoints.

Conclusions

Melatonin and its agonist (agomelatine) both protected rats against the harmful effects of high fat and high fructose diet (HFHFD), mainly by reducing fat mass, adiposity index, body weight gain, and BMI. Melatonin showed additional benefits by lowering cholesterol, LDL, and ALP activity, while also

improving adipose and liver tissue structure, indicating stronger metabolic and hepatoprotective effects. It also tended to raise omentin levels, suggesting better adipokine regulation, whereas agomelatonin slightly reduced omentin and was less effective for the liver, showing residual steatosis and necrosis. Overall, melatonin provided broader protective actions, combining anti-obesity, antioxidant, and hepatoprotective effects. Agomelatine, although effective in reducing body weight, may pose risks for liver health. Thus, melatonin appears to be the safer and more promising therapeutic option for diet-induced obesity and metabolic complications, while synthetic agonists require further evaluation.

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