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RESEARCH ARTICLE

Exploring the Anti-obesity Properties of Corn Silk Extract (CornFit®) in C57BL/6 Mice Induced with Obesity via a High-Fat Diet

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Abstract

Objective: The impact of CornFit®, an extract derived from corn silk was assessed on obesity-induced mice subjected to a High-Fat Diet (HFD). **Methods:** C57BL/6 mice were assigned to seven groups: normal control, HFD control, reference standard and four CornFit® dosage groups. Body weight, food intake, fat mass and metabolic parameters were assessed throughout the study. Biochemical markers, gene expression in liver tissue and histopathological analysis were also performed. **Results:** Supplementation with CornFit® notably decreased body weight gain, fat mass and enhanced lipid profile compared to the HFD group. It also decreased pro-inflammatory markers and insulin levels while increasing adiponectin and leptin, indicating anti-inflammatory and metabolic benefits. RT-PCR analysis revealed downregulation of lipogenic genes and upregulation of genes involved in fatty acid metabolism and energy expenditure. Liver function tests and organ weights improved in treated groups. **Conclusion:** CornFit® demonstrates promising anti-obesity potential by reducing fat accumulation, improving metabolic health and modulating related gene expression.

Keywords: Anti-adipogenic, Inflammation, Lipogenic, Metabolic Health, *Zea mays*

1. Introduction

Obesity is a global health challenge with far-reaching implications, demanding innovative prevention and management strategies[1-3]. Unhealthy habits, poor dietary choices and sedentary lifestyles significantly increase physiological risks, contributing to obesity. Behaviours like tobacco and excessive alcohol consumption impact metabolism, inducing hormonal imbalances and heightened stress, leading to weight gain[4-6] supermarket growth, fast unplanned urbanization, sedentary lifestyle, economy, and social position slowly develop behavioral risk factors in humans. Behavioral risk factors such as unhealthy habits, improper diet, and physical inactivity lead to physiological risks, and "obesity/overweight" is one of the consequences. "Obesity and overweight" are one of the major lifestyle diseases that leads to other health conditions, such as cardiovascular diseases (CVDs. Consuming energy-dense foods disrupts the balance between intake and expenditure, increasing the risk. Insufficient physical activity disrupts energy equilibrium, fostering excess body fat accumulation. These factors collectively disturb energy homeostasis, heightening obesity and overweight risks, which in turn contribute to health issues like cardiovascular diseases and type II diabetes. Preventive measures involve promoting healthy lifestyles, including regular physical activity, balanced diets and weight management, along with creating supportive environments for healthy living through accessible nutritious foods and opportunities for physical activity[7-11]. The study estimated that in 2019, the economic costs of obesity ranged from 0.8% to 2.4% of GDP across the eight countries studied, with

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the total obesity costs per capita ranging from US$17 in India to US$940 in Australia[12-16]. Current findings suggest the safety of various herbal extracts, such as those derived from *Paullinia cupana, Plantago psyllium, Garcinia cambogia* and *Ilex paraguariensis*, in weight management by facilitating lipid and carbohydrate metabolism. Herbal extracts sourced from natural origins present promising and secure alternatives for addressing obesity. Corn silk has a history of utilisation across various regions worldwide for addressing conditions such as oedema, cystitis, gout, kidney stones, nephritis and prostatitis. According to traditional practices, corn silk has been employed in China for several decades as an oral antidiabetic agent. The quest for natural remedies has led to the investigation of Corn silk extract, Corn Silk (CS), which comprises yellow hairy-like strands, derived from *Zea mays* L. and has been traditionally employed to address diverse health issues. It encompasses a range of active components, including tartaric acid, isoquercitrin, allantoin, stigmasterol, hordenine, maysin, resin, cryptoxanthin and anthocyanins. Notably, CS extract is rich in maysin, a prominent flavonoid within CS[17-21].

2. Materials and Methods

2.1 Materials

Carboxy Methyl Cellulose (CMC) sodium obtained from SDFCL, India, Picric acid procured from Himedia, India, Isoflurane from Raman and Well Pvt. Ltd, India, Glucose, Triglycerides, Cholesterol, Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline Phosphatase (ALP) were obtained from Agape Diagnostics, India., Mouse Igf 1, Mouse Insulin, Mouse TNF-α, Mouse Leptin, Mouse Adiponectin and Mouse Interleukin 6 were procured from BT LAB Bioassay Technology Laboratory, China Corn Silk Extract/ Cornfit® (sample no: RR231168) from M/s. Botanic Healthcare Pvt. Ltd.

2.2 Methods

2.2.1 Animal Studies

Animal experiments were conducted adhering strictly to the guidelines set forth by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), with a registration number of 1803/PO/RcBi/S/2015/CCSEA. There were no amendments or deviations from the approved procedure. For the experiment, 56 C57BL/6 mice were acclimatised for one week. Forty-eight of them were subjected to an HFD for 2 weeks to induce obesity, while the remaining 8 animals in the normal control group received a standard chow diet. After this acclimatisation period, animals were randomised into 7 groups, each comprising 8 animals. The experimental groups were categorised as follows: Group 1 (Normal control, normal chow diet), Group 2 (HFD control), Group 3 (Reference standard, 100mg/kg *Garcinia cambogia* extract) and Group 4 to Group 7 (Test substance treated groups with HFD) receiving the test substance at dose levels of 100mg/kg/b.wt, 200mg/kg/b.wt, 400mg/kg/b.wt, and 600mg/kg/b.wt, respectively. This treatment was administered once daily for 8 weeks. The test formulations, consisting of Corn silk extract (Cornfit®), were orally administered once each day at dose levels of 100mg/kg, 200mg/kg, 400mg/kg, and 600mg/kg/b.wt/day to corresponding groups of mice for 8 weeks. The dose volume given to each animal was 10mL/kg/b.wt/day. In parallel, the normal control group received the vehicle orally at the same dose volume for the 8-week duration[22].

2.2.2 Measurement of Bodyweight and Feed Weight

To accurately track the impact of the intervention, several key measurements were taken regularly. Weights of each individual were documented at various time points: upon arrival, on the randomisation day, on Day 1 of treatment prior to dosing, weekly thereafter (within a 2-day window) and on the final day of treatment. Changes in body weight for all animals were calculated and reported along with the raw data. Food intake was monitored weekly, coinciding with body weight measurements. This assessment started at the beginning of treatment and continued until the end of the study. Both the amount of food provided and the amount consumed were measured weekly. This comprehensive approach to monitoring weight and diet allows for a detailed understanding of the intervention's effects on both overall body composition and food intake habits.

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2.2.3 Necropsy and Gross Pathology

On the 57th day, all the animals from every group were humanely sacrificed using Isoflurane and underwent a comprehensive gross pathological examination. Vital organs and tissues, including the liver, kidney, heart, spleen, pancreas, subcutaneous fat, retroperitoneal fat and epididymis fat, were harvested for subsequent analysis.

2.2.4 Biochemical Analysis

Two weeks after completing the treatment period, blood samples were collected and serum was separated by centrifugation at 10000rpm (for 10 minutes) followed by the analysis of the samples. Liver function tests, lipid profiles and glucose levels were determined using appropriate kits. Additionally, serum biomarkers including TNF-α, IL-6, Adiponectin, Leptin, IGF-1 and Insulin were assessed using ELISA kits to evaluate the treatment's impact. Furthermore, gene expression analysis of PPARγ, C/EBPα, SREBP1c, FAS, aP2, PGC-1α, DGAT1, UCP1, UCP2, adiponectin, and leptin in liver tissues of treated animals was performed using Reverse Transcription Polymerase Chain Reaction (RT-PCR).

2.2.5 RT-PCR Analysis

Liver samples from the control and treatment groups underwent total RNA extraction using the Invitrogen Pure LinkTM RNA Mini kit as per the manufacturer's protocol. Following RNA isolation, quantification was performed at 260nm. The isolated total RNA was utilised for cDNA synthesis with oligo dT primers and reverse transcriptase enzyme treatment (Bio-Rad). Real-time quantitative RT-PCR measured the mRNA expression of genes such as PPARγ, C/EBPα, SREBP, FAS, aP2, PGC-1a, DGAT1, UCP1, UCP2, adiponectin and leptin. The CFX Opus 96 Real-time PCR system (BIO-RAD) facilitated detection without additional processing, utilising a dye-labelled DNA probe specific for the gene of interest and another for GAPDH, serving as an endogenous control. In a 20µL reaction mixture with designed primers, PCR amplification with 2-minute incubation at 50°C, with 30-minute reverse transcription at 60°C. Subsequent 40 cycles of two-step PCR reactions comprised a 5-minute period at 95°C, denaturation at 95°C for 30 seconds and annealing at the designated melting Temperature (Tm) was determined for 30 seconds, followed by an extension at 72°C for 45 seconds, concluding with a final extension at 72°C for 10 minutes. Gene-specific forward and reverse primers were procured from Eurofins, India and designed via NCBI Primer Blast.

2.2.6 Histopathological Analysis

Livers and adipose tissues from all the mice were carefully collected and immersed in 10% formalin (pH 6.8) for overnight fixation. Following fixation, the liver tissues were dehydrated and fixed in paraffin wax for sectioning. Next, thin sections (4µm) of both liver and adipose tissues were precisely prepared using a Leica microtome (model DM500). These sections were then systematically processed to remove the paraffin wax (deparaffinisation using xylene) and rehydrated through decreasing concentrations of ethanol (down to 70%). Finally, they were rinsed with distilled water to ensure a clean surface for staining followed by staining with hematoxylin and eosin (H and E), a standard histological staining technique that allows for clear visualisation of cellular structures. Pathological changes in the tissues were meticulously evaluated using the Leica Application Suite software, enabling a comprehensive analysis of potential treatment-related effects.

2.2.7 Statistical Analysis

Data analysis, including body weight, clinical chemistry and organ weights, was conducted using Graph Pad Prism Software, version 5.01. Results were expressed as Mean ± SD. To assess significant differences between treatment and control groups, one-way ANOVA with Dunnett's test was utilised. A summary of all statistical analyses was compiled in separate tables.

3. Results

In this study, the investigation of potential weight-reducing effects of CORNFIT® in mice with diet-induced obesity was observed.

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3.1 Body Weight and Body Weight Changes

Table1. Effect of corn extract on high-fat diet-induced mice body weight

Body Weight(gm.)					
Group	**Basal**	**1stWeek**	**2ndWeek**	**3rdWeek**	**4thWeek**
Group 1	22.0±0.51***	23.2±0.42***	24.6±0.30***	26.5±0.46***	27.5±0.47***
Group 2	24.2±0.50	26.5±0.35	28.3±0.55	32.0±0.89	34.3±0.64
Group 3	24.5±0.47	26.2±0.34	28.1±0.34	30.6±0.67***	31.0±0.55***
Group 4	24.4±0.40	26.7±0.34	29.1±0.58	31.3±0.67	32.7±0.50
Group 5	24.8±0.35	26.8±0.34	29.1±0.34	31.6±0.41	33.3±0.67
Group 6	24.7±0.25	26.7±0.27	29.1±0.29	31.3±0.31	30.1±1.20***
Group 7	24.8±0.54	26.4±0.49	28.9±0.38*	31.0±0.35**	31.2±1.22***
	Body Weight(gm.)				
	5thWeek	**6thWeek**	**7thWeek**	**8thWeek**	
Group 1	28.4±0.57***	29.1±0.60***	30.2±1.00***	31.9±1.12***	
Group 2	35.5±0.69	36.5±0.73	37.8±0.85	40.0±1.03	
Group 3	31.7±0.75***	31.7±1.18***	30.4±1.13***	30.0±1.21***	
Group 4	32.8±1.03	33.2±1.10	33.0±2.27***	34.0±1.21***	
Group 5	32.8±0.63	33.3±0.61	32.7±1.63***	33.4±1.41***	
Group 6	31.3±1.55***	32.0±0.61***	31.2±1.11***	31.1±0.74***	
Group 7	31.3±1.32***	31.7±0.95***	31.0±0.99***	30.8±1.12***	

The data was presented as Mean ± SD (n = 8). Statistical significance was assessed between the Normal control (Group 1) and the other HFD groups (G2, G3, G4, G5, G6 and G7) (* P Value < 0.05; ** P Value < 0.001; *** P Value < 0.0001).

Table 2. Summary of weekly mean feed consumption of mice

	Groups						
	G1	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**
Weeks	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Week: 01	42.10 ± 1.13	42.17 ± 1.13	42.87 ± 1.46	41.91 ± 1.31	43.03 ± 1.50	43.11±0.86	43.38±0.93
Week: 02	42.41 ± 1.43	42.90 ± 0.85	41.89 ± 1.20	42.54 ± 1.42	43.06 ± 1.32	43.27±0.93	43.00±0.77
Week: 03	43.16 ± 1.33	44.04 ± 1.22	44.40 ± 1.26	44.10 ± 0.98	43.86 ± 1.73	43.94±1.63	44.66±1.08
Week: 04	44.54 ± 2.03	44.33 ± 1.28	43.97 ± 1.63	44.11 ± 1.35	44.56 ± 1.34	44.40±1.07	44.06±1.54
Week: 05	45.70 ± 1.12	45.84 ± 1.31	46.33 ± 1.13	45.90 ± 1.31	45.99 ± 0.80	45.70±1.01	45.90±1.30
Week: 06	46.34 ± 0.74	46.09 ± 1.18	45.93 ± 1.18	46.16 ± 0.62	46.17 ± 1.28	46.01±1.36	45.94±1.28
Week: 07	47.16 ± 1.51	48.00 ± 1.17	48.09 ± 1.12	47.81 ± 1.10	47.77 ± 1.27	48.14±1.25	47.41±1.04
Week: 08	47.81 ± 0.67	48.20 ± 1.19	47.16 ± 1.73	47.73 ± 1.21	47.56 ± 1.29	48.13±1.25	47.57±1.28

Values were expressed as Mean ± SD (n = 8), Statistical significances are compared betweenHFD control (Group 2) versus other groups (G1, G3, G4, G5, G6 and G7) (* P Value < 0.05; ** P Value < 0.001; *** P Value < 0.0001).

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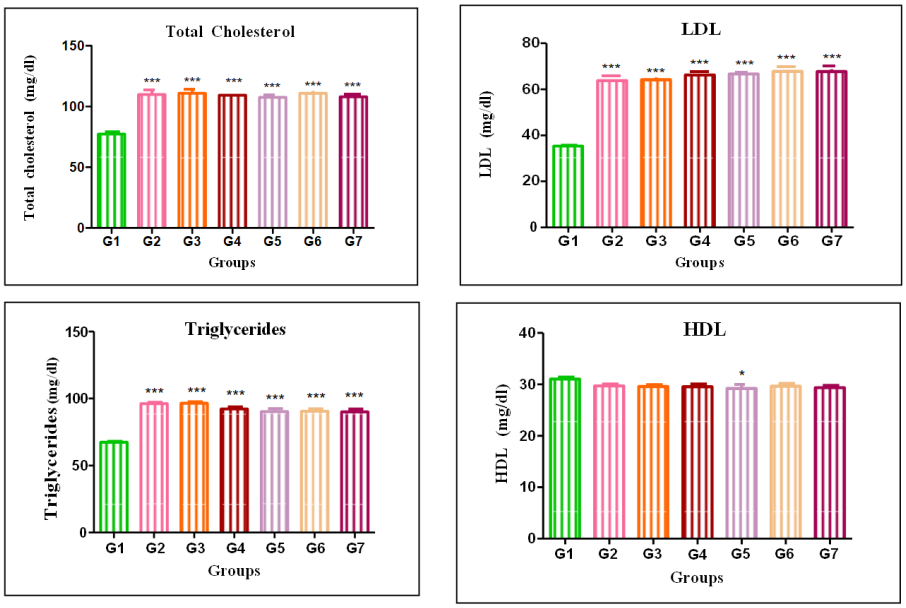


Figure 1. Effect of HFD on lipid profile test in mice on day 14.

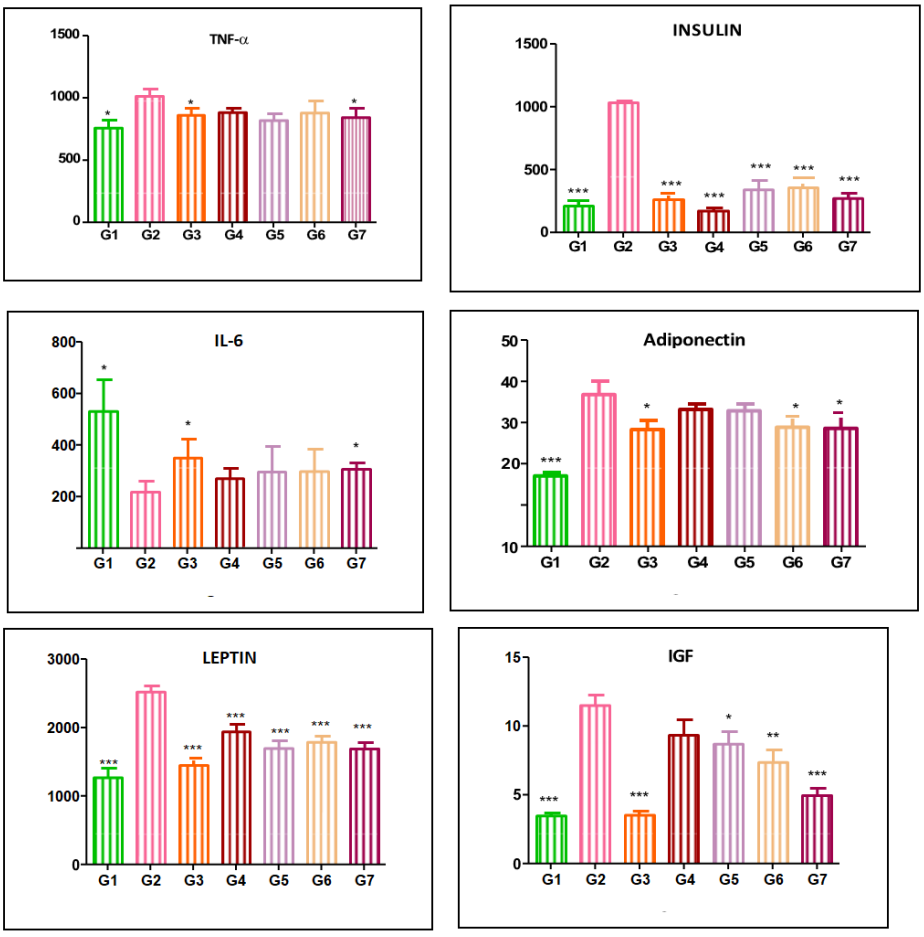


Figure 2. Impact of test substance on serum biomarker levels.

3.2 Biochemical Parameters

The study data reveals alkaline phosphatase, SGOT and SGPT levels showed a significant decrease in the test substance-treated groups when compared with the HFD Control group (G2).

3.3 RT-PCR Results

This study investigates the anti-obesity potential of a test product at various concentrations (100mg, 200mg, 400mg and 600mg) by analysing the messenger RNA (mRNA) expression of key genes involved in the lipid metabolic pathway. Mice which are induced with a high-fat diet- served as controls, allowing comparisons against normal, standard *G. cambogia* extract, and test product groups (Figure 3).

3.4 Histopathology

Liver and adipose tissue samples were fixed, processed using standard techniques, and subjected to staining with Haematoxylin and Eosin (H and E).

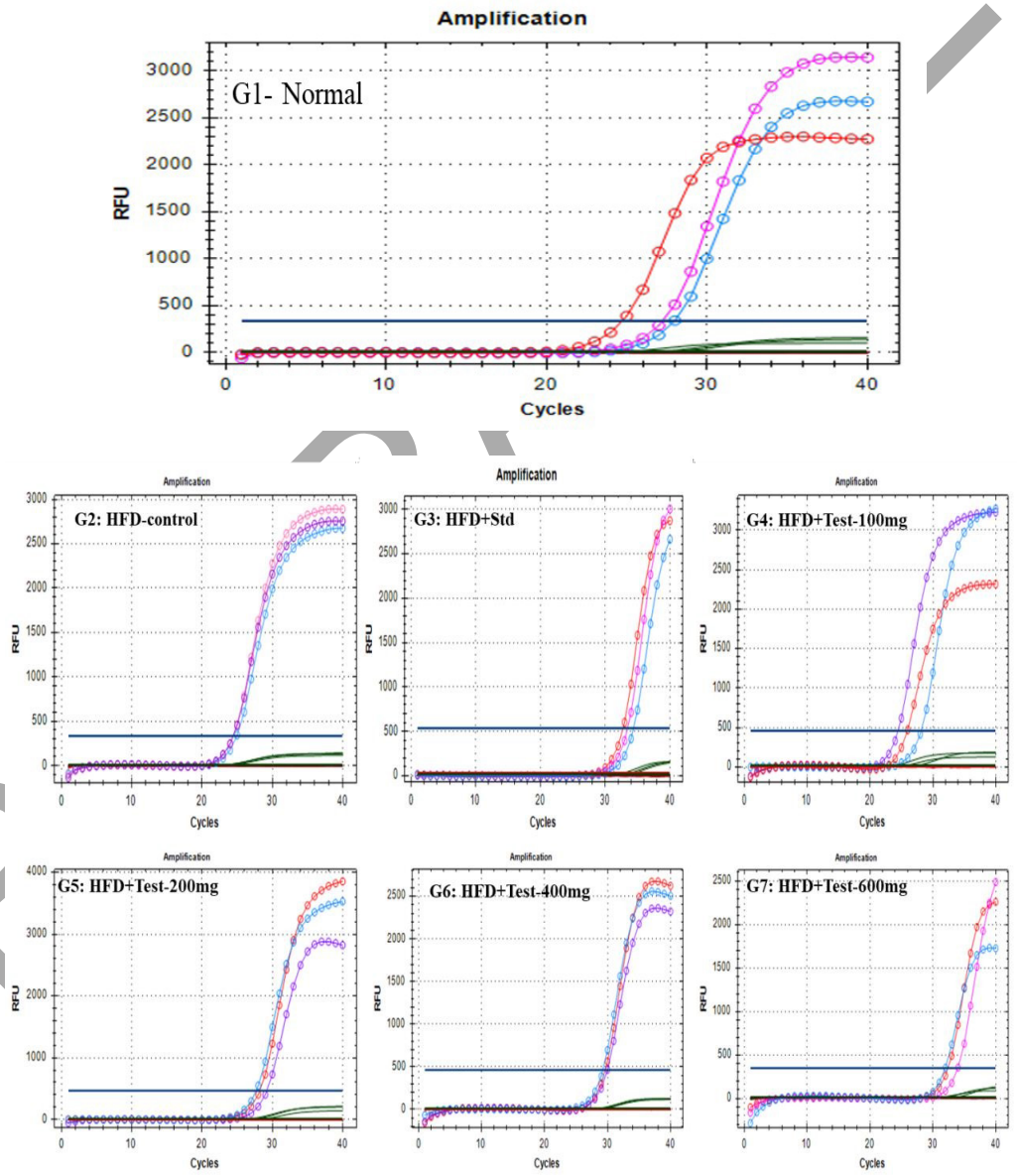


Figure 3. Relative expression of PPARγ, C/EBPα and SREBP genes in normal (G1) and treated groups of animals (G2 – G7) modulated by the application of HFD, standard and test product.

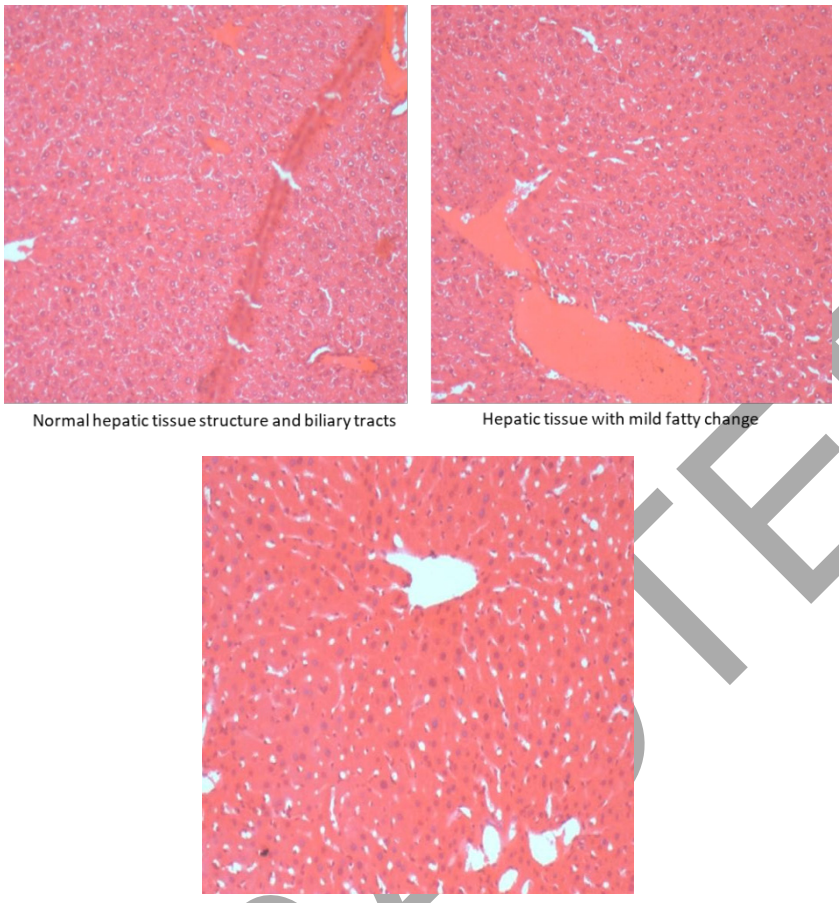


Figure 4. Histopathological evaluations of the liver in mice.

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4. Discussion

Obesity leads to a disruption in metabolic balance, causing issues such as insulin resistance, dyslipidemia, abnormal blood pressure regulation and heightened susceptibility to conditions like diabetes, cardiovascular disease and chronic kidney disease. The current study was investigated with Forty-eight animals which underwent a HFD regimen for 8 weeks to induce obesity, while an additional eight animals were fed a standard diet in the normal control group for the same duration. The HFD-fed groups demonstrated a marked increase in body weight (Table 1). Administration of the test substance and the reference standard (groups G3 to G7) led to a significant reduction in body weight by the 7th and 8th week in contrast to the HFD control group (G2). Feed consumption did not exhibit significant differences among all groups (G1, G3 G7) compared to the HFD control group (G2) (refer to Table 2 and Figure 1). No instances of mortality, clinical symptoms, or changes in behaviour were noted in any of the treated groups throughout the treatment period. Post the 2-week induction with the HFD, lipid profile parameters displayed a notable increase in Cholesterol, Triglycerides and LDL levels in all groups compared to the Normal control group (G1). Conversely, HDL levels notably decreased in the groups subjected to the HFD compared to the Normal Control group (G1). Subsequent to the treatment with the test substance, lipid profile parameters demonstrated a significant decrease in Cholesterol, Triglycerides and LDL levels in all treated groups compared to the HFD control group (G2). Moreover, HDL levels exhibited a significant increase in the treatment groups compared to the HFD Control group (G2) (Figure 1). The findings of the study suggest that treatment with the test substance impeded the accumulation of visceral (Epididymal and Subcutaneous) adipose tissue. Given that liver weight changes and hepatic steatosis are commonly associated with obesity, alterations in liver

weight and fat accumulation were assessed in mice with diet-induced obesity. The animals in the HFD control group demonstrated an escalation in liver weight. Nonetheless, the treated groups with diet-induced obesity showed a notable decrease in liver weight compared to the control group (G2). Biochemical parameter estimation showed a significant decrease in the test substance-treated groups when compared with the HFD Control group (G2). It also reveals that TNF-α, Insulin, Adiponectin, Leptin and IGF levels were significantly decreased in treated groups but IL-6 levels showed a significant increase in the treatment groups when compared with the HFD control Group (G2) (Figure2). Histopathology estimation has shown Normal (control) group organ's section shows normal hepatic tissue structure and biliary tracts. The treatment group organ's section showed hepatic tissue with mild fatty change. The HFD group organ's section showed hepatic tissue with moderate fatty change (Figure 4).

5. Conclusion

In conclusion, the study suggests that administering Corn silk extract/Cornfit® has beneficial effects on adipose tissue accumulation and lipid profile in mice with diet-induced obesity. Notably, the treatment led to reduced levels of cholesterol, triglycerides and LDL, while increasing HDL levels, indicating its potential for lowering lipid levels. Moreover, the observed reduction in pro-inflammatory markers such as TNF-α and IL-6, along with changes in insulin levels and modulation of adiponectin, leptin and IGF levels, indicates anti-inflammatory and metabolic benefits associated with the treatment. Additionally, decreases in Liver Function Tests (LFT) and organ weights suggest an overall improvement in metabolic health. Molecular analysis via RT-PCR shed light on the underlying mechanisms, showing downregulation of lipogenic gene expression (PPARγ, C/EBPα, SREBP, FAS, aP2 and DGAT1), potentially inhibiting lipid synthesis. Meanwhile, upregulation of UCP1, UCP2, Adiponectin, PGC-1a and Leptin levels suggests enhanced fatty acid metabolism and inhibited lipid synthesis, consistent with effects observed in groups treated with the reference standard (*G. cambogia* extract). These multifaceted findings collectively indicate the promising therapeutic potential of Corn silk extract/Cornfit® in addressing lipid-related disorders, inflammation and overall metabolic health. Further research and clinical investigations are warranted to validate and expand upon these promising results.

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