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"Ficus Krishnae Extract: A Promising Natural Therapeutic for High-Fat Diet-Induced Obesity in Rats"

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ABSTRACT

Objective: *The present study aimed to examine the anti-obesity effects of the hydroethanolic extract of Ficus Krishnae (HELEFK) in a high-fat diet (HFD)-induced obesity in rats.*

Methods: *HELEFK was administered to rats categorized into six groups: normal control, HFD control, orlistat-treated, and HELEFK-treated (100, 200, 400 mg/kg). Over eight weeks, an HFD-induced obesity model was developed and treatments were administered. In addition, measurements were made of the body weight, BMI, Lee index, feed intake, lipid profiles, antioxidants, and liver indicators. Histological analysis of adipose tissue assessed morphological changes.*

Results*:* ***HFD-induced obesity*** *was evidenced by significant increases in body weight, BMI, Lee index, feed intake, fat depot content, and elevated triglycerides, total cholesterol, and LDL levels, along with decreased HDL levels. Oxidative stress was also elevated, as shown by increased TBARS levels and compromised antioxidant and liver markers, with enlarged adipocytes indicating fat accumulation. Treatment with HELEFK at 200mg/kg, and 400 mg/kg significantly decreased body weight, BMI, Lee index, feed intake, and fat depot content in a dose-dependent manner. Furthermore, treatment with HELEFK led to significant decreases in LDL, total cholesterol, and triglycerides, along with increased HDL levels. HELEFK also lowered TBARS levels and improved antioxidant markers and liver function, and decreased adipocyte sizes in the HELEFK-treated groups in comparison to the HFD control, indicating a favourable impact on the shape of fat tissue.*

Conclusion*: HELEFK has the potential to reduce HFD-induced obesity and oxidative stress. Its anti-obesity effects may be mediated through its antioxidant properties and potential direct actions on fat tissue morphology.*

Keywords*: Ficus Krishnae, anti-obesity, hydroethanolic extract, high-fat diet, oxidative stress, Wistar rats*

Introduction

High-fat diets (HFDs) have become a leading factor in the global obesity epidemic, primarily due to

their profound impact on lipid metabolism and their contribution to the progression of insulin resistance. These diets, typically high in saturated fats and refined sugars, disrupt normal metabolic functions, leading to increased body weight and excessive fat accumulation. Metabolic disturbances caused by HFDs include elevated serum cholesterol and triglyceride levels, along with impaired glucose regulation—key markers of metabolic syndrome. (i)

Obesity, characterized by the atypical accumulation of body fat in both central (visceral) and subcutaneous regions, poses significant health risks. Over recent decades, its prevalence has increased at an alarming rate, placing a substantial burden on global healthcare systems because of its strong correlation with long-term illnesses such as Type II diabetes and cardiovascular diseases. Obesity results from a complicated interplay between environmental, behavioral, and hereditary variables.

This growing issue is largely driven by excessive caloric intake and sedentary lifestyles. Current anti-obesity and anti-hyperlipidemic drug therapies, though available, have alleviated the burden, highlighting the need for more effective interventions. (ii)

Oxidative stress disrupts lipid metabolism by promoting lipid peroxidation, which leads to an Inequality between HDL and LDL (low- and high-density lipoproteins). This imbalance causes dangerous cholesterol levels to rise in the blood, which exacerbates insulin resistance, fat accumulation in tissues, and chronic inflammation, which are key drivers in the development of obesity.(iii)The Sterol Regulatory Element-Binding Protein 1c (SREBP1c) further regulates lipid metabolism, which stimulates the Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) gene by producing endogenous ligands. This, in turn, controls the expression of fatty acid synthase (FAS), promoting lipid synthesis and storage, thereby exacerbating metabolic imbalances commonly seen in obesity.(iv)
Studies using rat models have demonstrated that high-fat diets significantly alter lipid profiles and induce insulin resistance, contributing to metabolic dysfunction. For example, obesity induced by high-fat diets in rats has been associated with increased serum fatty acids, leading to lipotoxicity in various organs. This underscores the intricate connection between dietary fat, lipid metabolism, and the development of obesity-related metabolic disorders. (1)

Ficus krishnae (FK), a member of the Ficus genus is one of the most ancient folk medicines. The potential for its medicinal values has been studied via physicochemical characteristics and fluorescence analysis. The plant can also be used to treat various types of mineral deficiencies and can be utilized in the Ayurvedic system of medicines to cure diseases. The plant possesses potent pharmacological properties, including anti-inflammatory, antioxidant, and anti-diabetic. Its bioactive constituents, such as terpenoids, phenolic acids, and flavonoids (Quercetin, Rutin), have been shown to modulate metabolic disorders, supporting its potential role in obesity management.(v,vi,vii)

The current study investigates the hydroethanolic extract of FK for its potential ameliorative effects on obesity markers in rats subjected to a high-fat diet. This research aims to evaluate the efficacy of FK as a therapeutic agent for managing obesity and associated metabolic disorders.

Methodology:

Collection and extraction of botanical specimens

The leaves of the FK plant were procured from the Sarangpur Botanical Garden in Chandigarh, India.

The plant specimens were taxonomically recognized and authenticated by Dr. Sunita Garg of the Raw Materials Herbarium and Museum (RHMD), NISCAIR, New Delhi, India (NISCAIR/RHMD/consult/2021/3828-29-2).

After authentication, the leaves were separated from the plants manually and then cut, dried, powdered, sieved, and weighed. A hydroalcoholic solvent (ethanol and water, 80:20 v/v)) was used for the extraction. The extract was concentrated utilizing a rotating evaporator under reduced pressure at 50-60°C. After the concentration, the crude extract was stored properly. [viii]

Assessment of Raw Material Quality Control Parameters

The plant material's purity was assessed through foreign organic matter analysis, and physicochemical constants such as extractive values (alcohol and water-soluble) and ash values (total, water-soluble, and acid-insoluble) were determined following WHO guidelines. [ix]

Characterization and Analysis of FK Extracts Using Spectroscopic Techniques:

The hydroalcoholic extract of *Ficus krishnae* (FK) leaves was first isolated through column chromatography using an ethyl acetate-methanol solvent system, which yielded fractions rich in bioactive compounds. These fractions were then characterized and quantified by High-Performance Thin Layer Chromatography (HPTLC), focusing on key flavonoids such as rutin and quercetin, with densitometric scanning at 254 nm and 366 nm. ^{1}H-NMR and MS/MS spectroscopy provided structural validation of the isolated compounds, establishing a comprehensive profile of FK's bioactive constituents for further pharmacological evaluation.This comprehensive methodology ensured the thorough isolation, analysis, and characterization of the extracts. [x]

***In-vitro* antioxidant assay:**

Assessment of DPPH's (2, 2-diphenyl-1-picrylhydrazyl) capacity to scavenge radicals:

The antioxidant activity was assessed using the DPPH free radical scavenging test. A solution of 0.1 mM DPPH in ethanol was mixed with extract solutions (1–5 µg/ml) and allowed to incubate for half an hour without light. Ascorbic acid was used as the reference chemical for the absorbance measurement at 517 nm.[xi]

Assay of hydrogen peroxide (H_2O_2) scavenging activity:

A modified approach was used to evaluate the H_2O_2 scavenging activity. Following a 10-minute incubation period at room temperature, a sample containing 10–320 µg/ml was combined with phosphate buffer (pH 7.4) and H_2O_2 (40 mM As a positive control, ascorbic acid was used to assess absorbance at 230 nm.[xii]

Reducing power assay for antioxidant activity:

To assess the reducing power, extract (10–320 µg/ml) was combined with potassium ferricyanide and sodium phosphate buffer, then incubated at 50°C for 20 minutes. After adding trichloroacetic acid, the mixture was centrifuged. After adding ferric chloride and deionized water to the top layer, At 700 nm, absorbance was measured. After three iterations of the process, EC_{50} was computed using the concentration-absorbance graph. The standard utilized was ascorbic acid.[xiii]

***In vitro* anti-obesity study**

Assay for Pancreatic Lipase Inhibition:

An ELISA (Biotech) plate reader was used to measure the hydrolysis of p-nitrophenyl palmitate (PNPP) into p-nitrophenol at λ 400 nm in order to determine lipase activity. [xiv]

Experimental animals:

Healthy adult male Wistar rats with a weight of 250 and 300 grams were raised at the Chandigarh College of Pharmacy in Punjab, India, and kept under conventional settings, which included a 12:12 light-dark cycle, 23 ± 2°C, and 55 ± 10% humidity. They were given free access to water and a typical pellet diet. The Institutional Animal Ethics Committee (IAEC) accepted the study protocol (IAEC/CCP/Feb 2023/13) by CPCSEA norms under animal house reg. number. 1201/PO/Re/S/08/CPCSEA.

Standard drugs and chemicals used:

Standard drugs and chemicals for in vivo testing are primarily sourced from Sigma-Aldrich Ltd., and sodium nitroprusside are supplied by Molyhem Pvt. Ltd., India, while Nicotinamide comes from Finar India Ltd. 1,1,3,3-Tetramethoxypropane is provided by V.K. Chemicals, India, and carboxymethyl cellulose is available from various suppliers. lipid profile estimation kits were procured from Erba Diagnostics Pvt. Ltd. and additional compounds of analytical purity were employed in this investigation. Before use, each medication solution was made from scratch.

Experimental obesity:

Obesity was brought on by a diet heavy in fat., and the HFD's composition (g/kg diet) followed Srinivasan et al.'s formula. [xv]with some modifications.

Table 1. Comprehensive composition of a High-fat diet (HFD) used for inducing obesity and metabolic dysfunction in rats

S.N.	Components	Diet(g/kg)
1	Normal powdered diet	375
2	Lard	290
3	Milk casein	265
4	Vitamin and mineral mix	45
5	Yeast powder	01
6	Corn oil	10
7	DL-Methionine	03
8	Cholesterol	10
9	Sodium chloride	01

Experimental design:

The research utilised six groups, each comprising six animals. As the Normal Control (NC), Group I was given untreated, regular food and water. For eight weeks, Group II, The HFD Control was administered a HFD, Group III comprised HFD-fed rats administered Orlistat at a dosage of 30 mg/kg

by intraperitoneal injection. Groups IV, V, and VI were provided a high-fat diet alongside Hydroethanolic extract of Ficus krishnae (HELEFK) at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively, via oral administration. This design aimed to test the therapeutic potential of FK in managing HFD-induced obesity.

During the study duration, body weight, BMI, Lee index, and feed intake were assessed in the 4th and 8th weeks. Following the experiment, the animals were given 2% inhalational anesthesia and their blood was extracted via retro orbital puncture for biochemical analysis. Adipose tissues (epididymal, retroperitoneal, and mesenteric) were collected, weighed, analyzed, and excised for histological analysis with H&E staining images were visualized and captured at 100X magnification while the remaining tissues were used for antioxidant assays (TBARS, SOD, GSH) and plasma liver markers (AST, ALT, ALP) following assay kit guidelines.

Statistical analysis:

The data for continuous variables were presented as mean ± S.E.M., and a t-test was used to assess the mean difference. Data from more than two groups were examined using one-way analysis of variance (ANOVA), and the results were then assessed using Tukey's multiple comparison test. A P-value below 0.05 was considered statistically significant. The statistical analysis was performed using SPSS version 21.

Results:

Physicochemical Evaluation of *Ficus krishnae* (FK) Leaves

The physicochemical parameters of *Ficus krishnae* (FK) leaves were evaluated to assess purity and quality. The total ash content was 14.29%, acid-insoluble ash 3.38%, water-soluble ash 8.88%, and sulfated ash 1.52%, while the loss on drying was 2.72%. These findings provide essential data for standardizing FK leaf extracts, ensuring consistency in further phytochemical and pharmacological studies.

HPTLC profiling of FK plant extract:

From the table, it has been found that out of 5 components in FK, Given that the percentage area is 34.08%, the component with Rf value 0.31 was more prevalent. (Table: 2).

Table 2.: HPTLC profile of the HELEFK scanned at 254 nm

Track	Peak	Rf	Height(mm)	Area(mm²)	Assigned substance
Sample A	1	0.12	3.2	897.1	Unk.
A	2	0.31	98.7	3778.3	Quercetin
A	3	0.42	50.1	1464.5	Unk.
A	4	0.50	63.5	1762.5	Unk.
A	5	0.55	109.3	3184.7	Unk.

From the table, it has been found that out of 10 components in FK, the component with Rf value 0.74 was more predominant as the percentage area is 47.72% (Table 3).

Table 3: HPTLC profile of the HELEFK scanned at 366nm

Track	Peak	Rf	Height(mm)	Area(mm²)	Assigned substance
Sample A	1	0.03	83.8	1309.2	Unk.
A	2	0.11	84.5	2545.5	Unk.

A	3	0.25	26.7	916.2	Unk.
A	4	0.31	39.8	984.2	Unk.
A	5	0.36	51.3	1755	Unk.
A	6	0.49	65.6	2343.9	Unk.
A	7	0.54	44.7	469.7	Unk.
A	8	0.56	47.9	578.9	Unk.
A	9	0.60	65.1	1021	Unk.
A	10	0.74	160.2	10883.6	Rutin

NMR spectra of compounds

NMR was taken on Avance 300,1HNMR (CDCl3) 300 MHz, TMS as standards shown in the figure below, and the spectrum was attached.

Description of NMR spectra of Quercetin

2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one (***Quercetin***)*:* ^{1}H NMR (500 MHz, DMSO)δ (ppm) 12.50 (s, 1H), 10.79 (s, 1H), 9.59 (s, 1H), 9.35 (s, 1H), 9.31 (s,1H), 7.70 (d, *J* = 2Hz, 1H), 7.56 (dd, *J* = 2, 8.5 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 6.42 (s, 1H), 6.20 (s, 1H). ^{13}C NMR (125 MHz, DMSO)δ (ppm) 175.77, 160.65, 156.07, 147.63, 146.73, 144.98, 135.66, 121.90, 119.91, 115.53, 115.00, 102.95, 98.11, 93.28.

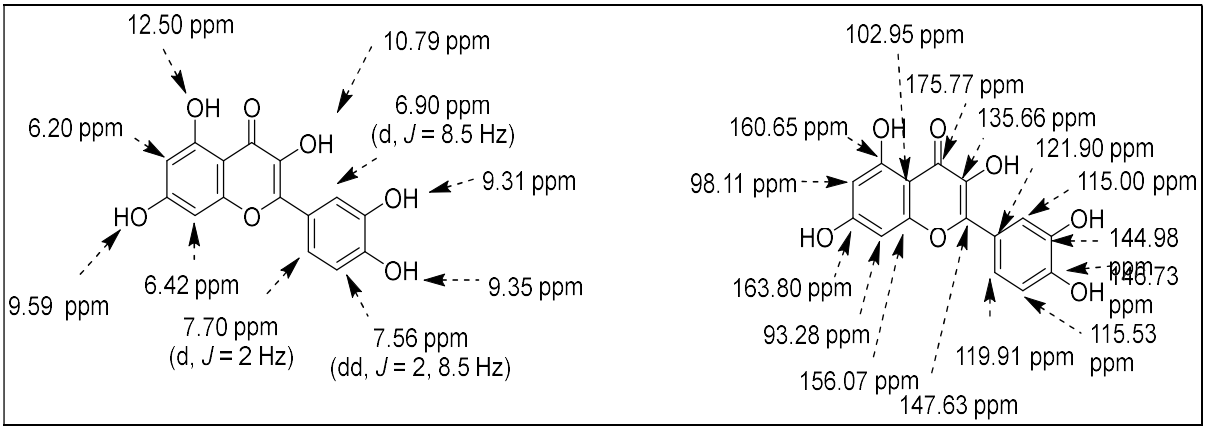


Figure 1.: Proton and carbon resonances of Quercetin

Description of NMR spectra of Rutin

NMR was taken on Avance 300,1HNMR (CDCl3) 300 MHz, TMS as standards shown in the figure below, and the spectrum was attached.

NMR Analysis

2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one(**Rutin**):

^{1}H NMR (500 MHz, DMSO)δ (ppm) 12.61 (s, 1H), 10.86 (s, 1H), 9.68 (s, 1H), 9.19 (s, 1H), 7.57 (m, 2H), 6.87 (s, 1H), 6.40 (s, 1H), 6.21 (s, 1H), 5.35 (d, *J* = 4 Hz, 1H), 5.30 (m, 1H), 5.12-5.08 (m, 2H), 4.54 (s, 1H), 4.40 (m, 2H), 4.36 (s, 1H), 3.72 (d, *J* = 10.5 Hz, 1H), 3.64-3.41 (m, 1H), 3.33-3.24 (m, 6H), 3.11-3.07 (m, 2H), 1.01 (s, 3H).

^{13}C NMR (125 MHz, DMSO) δ (ppm) 177.36, 164.04, 161.20, 156.61, 156.41, 148.38, 144.72, 133.28, 121.58, 121.17, 116.26, 115.21, 103.96, 101.16, 100.72, 98.67, 93.58, 76.42, 75.88, 74.05, 71.83, 70.55, 70.36, 69.99, 68.22, 66.98, 17.70.

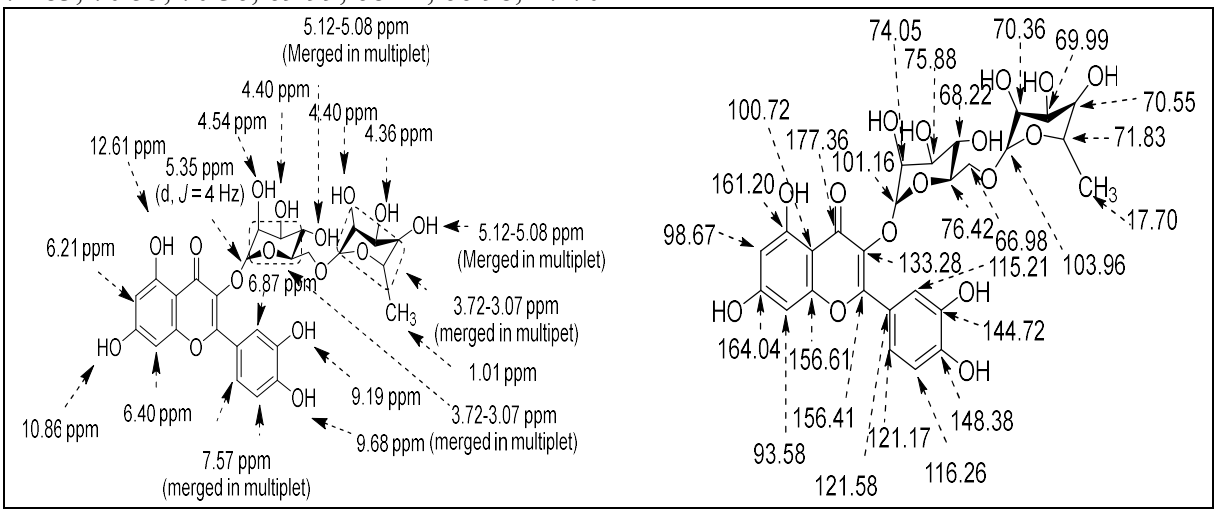


Figure 2.: Proton and carbon resonances of Rutin

***In-vitro* antioxidant assay:**

An *in-vitro* antioxidant test was used to evaluate the FK plant extract's antioxidant capability.

Effect of FK plant ethanolic and hydroalcoholic extracts on the ability to scavenge DPPH radicals:

The antioxidant efficacy of FK extracts was assessed by their capacity to scavenge DPPH radicals.Significant antioxidant effects ($P<0.05$) were observed with both HELEFK and ELEFK extracts at different concentrations, as shown in Table 5. Both extracts' ability to scavenge DPPH radicals depended on their concentration.

Table 5: DPPH radical-scavenging activity of ELEFK and HELEFK

Samples	Treatment	Dose Concentration	IC_{50} Values
1	Ascorbic Acid	(1-10µg/ml)	38.50 ± 0.94 µg/ml
2	ELEFK	(1-600µg/ml)	86.68 ± 9.21 µg/ml
3	HELEFK	(1-600µg/ml)	78.56 ± 7.67 µg/ml

The mean ± SD of three replicates is used to represent the data.

Effect of ethanolic and hydroalcoholic extracts of FK plants on hydrogen peroxide scavenging activity:

H_2O_2 scavenging activity was measured to assess the antioxidant efficacy of FK extracts. Both extracts' ability to scavenge hydrogen peroxide depended on their concentration.

Table 6: H_2O_2 scavenging activity of ELEFK and HELEFK

Samples	Treatment	Dose Concentration	IC_{50} Values
1	Ascorbic Acid	(100 µg/ml)	90.12 ± 3.94%
2	ELEFK	(1-160 µg/ml)	52.22 ± 1.67%
3	HELEFK	(1-160 µg/ml)	66.22 ± 3.99%

The mean ± SD of three replicates is used to represent the data.

Effect of Ethanolic and Hydroalcoholic Extracts of FK Plants on Reducing Power Activity:
Compared to ethanolic extracts, hydroalcoholic extracts have the greatest capacity to diminish power(table 6). The results were in line with BHA and quercetin guidelines. This might be because the extract contains biologically active substances that have strong donating properties.

Table 6: Assessing the reducing power activity of various doses of HELEFK and ascorbic acid.

S. No	Conc. (µg/mL)	ELEFK	HELEFK	Ascorbic Acid
04	160	47.44±1.82	59.55±2.10	79.22±2.45

The mean ± SD of three replicates is used to represent the data.

***In vitro* anti-obesity**
Effect of FK extracts on the test for pancreatic lipase inhibition:
The enzymatic activity in terms of % inhibition of HELEFK was found 12.10-50.21 % at doses of 5-25 micro/ml, respectively, whereas in the case of orlistat, the % inhibition was found in a range of 17.16-70.32% (table 7). The standard orlistat also showed significant enzymatic inhibitory activity.
Table 7: Pancreatic lipase activity

Conc (micromole)	5	10	15	20	25
Orlistat (% inhibition)	17.16	31.22	43.04	56.26	70.32
HELEFK (% inhibition)	12.10	23.35	40.08	44.87	50.21

The mean ± SD of three replicates is used to represent the data.

***In-vivo* Anti-Obesity Activities:**
Effect of Orlistat and FK plant leaves extracts on body weight, BMI, Lee Index, Feed Intake of HFD-fed rats:
Table 8 displays body weight, BMI, Lee Index, and feed intake. A significant rise in body weight, BMI, Lee Index, and feed intake was seen following eight weeks of a high-fat diet (HFD). While the lower dose (100 mg/kg) had no impact, daily administration of 30 mg/kg orlistat resulted in a

significant ($p<0.05$) decrease in body weight, BMI, Lee Index, and feed intake when compared to HFD control rats. Higher doses of HELEFK (200 and 400 mg/kg) also significantly decreased these parameters in a dose-dependent manner.

Table 8: Effect of Orlistat and FK plant leaf extracts on body weight, BMI, Lee Index, Feed Intake of HFD-fed rats

Time point	Normal Control	HFD	Orlistat (30mg/kg	HELEFK (100mg/kg)	HELEFK (200mg/kg)	HELEFK (400mg/kg)
Body Weight (g)						
Basal	227.5 ± 3.1	228.3 ± 4.3	229.1 ±3.8	228.3 ±4.2	231.6 ± 3.4	233.3 ± 5.2
4th week	258.3 ± 4.3	353.7±13.7[a]	278.7±9.6[b]	352 ± 12.3	326.4±11.3[b]	304.7 ± 8.2[b]
8th Week	281.8±2.74	405.7 ± 9.3[a]	289.5±7.6[b]	393.5±15.9	340.8±9.1[b]	314.5±8.95[b]
BMI (g/cm^2)						
Basal	1.15 ± 0.04	1.19 ± 0.03	1.12 ±0.06	1.14 ± 0.06	1.13 ± 0.04	1.14 ± 0.05
4th Week	1.30 ± 0.03	1.72 ± 0.06[a]	1.34±0.05[b]	1.67 ± 0.08	1.48 ±0.06[b]	1.34 ± 0.07[b]
8th Week	1.35 ± 0.06	1.91 ± 0.08[a]	1.34±0.06[b]	1.82 ± 0.09	1.67 ±0.08[b]	1.50 ± 0.09[b]
Lee Index						
Basal	275.30±5.1	296.21 ±4.3	298.40±6.5	295.21 ± 4.2	297.50 ±5.0	294.70 ± 4.3
4th week	277.45 ± 3.3	339.40±10.8[a]	287.21±8.7[b]	335.30 ± 9.6	317.22±7.8[b]	298.70 ± 9.5[b]
8th Week	281.82 ± 4.7	360.50±12.3[a]	285.20±8.5[b]	352.40±12.2	329.20±8.79[b]	301.30 ±7.19[b]
Feed Intake (g/day)						
Basal	88.50 ± 4.1	90.30 ± 3.9	90.70 ±2.7	90.34 ± 3.9	89.67 ± 4.2	88.88 ± 3.2
4th Week	93.65 ± 3.8	120.67±6.2[a]	98.50±4.2[b]	117.20±6.6	110.34±5.9[b]	101.40±5.3[b]
8th Week	98.76 ± 2.7	134.33±9.5[a]	100.43±5.1[b]	131.85±9.2	117.43±6.7[b]	108.98±6.1[b]

In this case, "[b]P<0.05 in comparison to the HFD control group; [a]P<0.01 in comparison to the Control Group.

"Effect of orlistat and FK plants leaves extracts on fat depots of HFD fed rats

Significant increases in epididymal, mesenteric, retroperitoneal, and total fat pads were observed in rats fed a HFD for eight weeks. Higher doses of HELEFK (200 and 400 mg/kg) significantly reduced all fat pads in a dose-dependent manner. In addition, daily management by 30 mg/kg orlistat in a single dose for 8 weeks showed a significant ($p<0.05$) reduction in all fat pads when compared to rats fed a high-fat diet.

Table 9: Effect of orlistat and FK plant leaves extracts on fat depots of HFD-fed rats

Fat depot(g)	Control (without HFD)	HFD	Orlistat (30mg/kg	HELEFK (100 mg/kg)	HELEFK (200 mg/kg)	HELEFK (400 mg/kg)
Epididymal Fat	2.64±0.6	16.21 ± 1.1	2.98 ± 0.5	15.27±2.35	11.13±1.04	8.56 ± 1.12
Mesenteric Fat	2.91±0.5	9.30±0.96[a]	3.70 ±0.7[b]	9.0 ± 0.9	7.10 ± 0.8[b]	5.10 ± 0.4[b]
Retroperito	2.96±0.08	11.45±1.26	4.20 ±0.6[b]	10.90±	8.86 ± 0.5[b]	6.20 ± 0.6[b]

neal Fat		[a]		1.21		
Total Fat	8.51 ±1.3	36.96±3.8[a]	10.88±1.2[b]	35.17±5.3	27.08±4.3[b]	19.86±3.2[b]

In this case, "[b]P<0.05 in comparison to the HFD control group; [a]P<0.01 in comparison to the Control Group."

Biochemical estimation

Effect of orlistat and FK plant leaves on HFD-fed rats' blood glucose, TC, TG, HDL, LDL, and VLDL

In HFD-fed rats, glucose, TC, TG, LDL, and VLDL levels significantly decreased ($p<0.05$), while HDL increased at the 4th and 8th weeks. Orlistat (30 mg/kg) also decreased high levels, and HELEFK at 200 and 400 mg/kg markedly improved these parameters in a dose-dependent manner.

Table 10: Effect of orlistat and FK plant leaves extracts on blood glucose, TC, TG, HDL, LDL, and VLDL of HFD-fed rats

Time point	Normal Control	HFD	Orlistat (30mg/kg	HELEFK (100 mg/kg)	HELEFK (200 mg/kg)	HELEFK (400 mg/kg)
Effect on Blood Glucose level (mg/dl)						
Basal	70.34 ± 3.2	72.78 ± 3.5	69.54 ± 2.4	70.67 ± 4.1	71.71 ± 3.9	69.50 ± 2.5
4th week	78.14 ± 3.7	170.87 ± 8.4[a]	79.140 ± 4.8[b]	165.3 ± 10.1	135.12 ± 9.2[b]	119.89 ± 8.3[b]
8th week	77.67 ± 3.08	184.38 ± 11.4[a]	90.89 ± 6.6[b]	180.21 ± 10.3	137.61 ± 7.2[b]	125.62 ± 6.6[b]
Effect on Total Cholesterol (TC) level (mg/dl)						
Basal	89.33 ± 3.43	88.83 ± 5.32	89.00 ± 4.50	87.67 ± 3.35	89.17 ± 4.24	86.67 ± 3.12
4th week	89.67 ± 3.89	151.20 ± 9.68[a]	88.30 ± 4.21[b]	144.30 ± 6.09	125.80 ± 3.23[b]	109.80 ± 3.98[b]
8th week	89.17 ± 4.03	170.30 ± 13.26[a]	98.50 ± 5.03[b]	166.20 ± 8.21	137.8±5.25[b]	105.20 ± 6.10[b]
Effect on Triglyceride (TG) level (mg/dl)						
Basal	67.83±2.98	69.00±3.05	67.50±4.60[b]	67.50±3.54	66.17±5.43[b]	66.67±4.20[b]
4th Week	69.00± 3.05	139.00 ± 12.1[a]	100.00±5.65[b]	136.50±8.76	120.60±4.50[b]	106.30 ± 2.92[b]
8th Week	69.71± 2.90	165.33±15.21[a]	78.50 ± 4.89[b]	126.83±9.23	112.30±5.50[b]	99.30 ± 5.12[b]
Effect on High-Density Lipoprotein (HDL) level (mg/dl)						
Basal	46.17± 2.12	47.88 ± 3.82	46.33 ± 2.15	49.33 ± 3.67	47.67 ± 2.29	45.83 ± 2.14
4th week	47.60± 3.59	29.40 ± 1.35[a]	42.50 ± 3.49[b]	29.90 ± 2.18	33.20 ± 3.02[b]	38.20 ± 2.80[b]
8th	47.33±	15.20 ± 1.24[a]	43.40 ± 3.29[b]	20.50 ± 2.12	28.89 ± 1.25[b]	36.83 ±

week	3.76					2.76[b]
Effect on Low-Density Lipoprotein (LDL) level (mg/dl)						
Basal	29.59± 2.07	27.20 ± 2.32	29.17 ± 1.95	24.84 ± 2.29	28.27 ± 3.14	27.51 ± 2.90
4th Week	28.27± 2.32	94.00 ± 6.20[a]	25.80 ± 2.65[b]	87.10 ± 6.17	68.48 ± 4.69[b]	50.34 ± 2.67[b]
8th Week	28.01± 2.14	122.03 ± 8.08[a]	39.40 ± 3.12[b]	120.83±5.22	86.45 ± 5.76[b]	48.51 ± 3.05[b]
Effect on Very Low-Density Lipoprotein (VLDL) (mg/dl)						
Basal	13.57± 1.20	13.80 ± 1.10	13.50 ± 1.09	13.50 ± 0.92	13.23 ± 0.99	13.22 ± 1.01
4th Week	13.80± 0.90	27.80 ± 1.64[a]	20.00 ± 1.80[b]	27.30 ± 1.09	24.12 ± 1.05[b]	21.26 ± 1.87[b]
8th Week	13.83± 1.01	33.07 ± 1.9[a]	15.70 ± 0.90[b]	25.37 ± 1.10	22.46 ± 1.03[b]	19.86 ± 0.87[b]

In this case, "[b]P<0.05 in comparison to the HFD control group; [a]P<0.01 in comparison to the Control Group."

Effect of Orlistat and FK plants leaves extracts on plasma liver function test of HFD Fed Rats

Following eight weeks of a high-fat diet (HFD), elevated AST, ALT, and ALP values indicated liver damage. In a dosage-dependent way, HELEFK at 200 and 400 mg/kg dramatically decreased these enzymes, whereas the 100 mg/kg dose had no impact. Comparing orlistat (30 mg/kg) to HFD control rats, AST, ALT, and ALP levels were also considerably ($p<0.05$) reduced.

Table 11: Effect of Orlistat and FK plants leaves extracts on plasma liver function test of HFD Fed Rats

Liver Markers	**Control (without HFD)**	**HFD**	**Orlistat (30mg/kg**	**HELEFK (100mg/kg)**	**HELEFK (200mg/kg)**	**HELEFK (400mg/kg)**
AST (IU/ml)	28.98±1.65	70.56±5.08	32.56±2.2	68.90± 3.5	52.70±3.87	39.50±3.12
ALT (IU/ml)	21.67±1.67	67.50±3.91[a]	27.80±1.9[b]	63.20± 5.4	49.67± 3.3[b]	32.65±2.4[b]
ALP (IU/ml)	70.23 ± 4.38	142.76±9.29[a]	77.67±3.8[b]	136.54±9.32	108.40±5.3[b]	91.43 ± 6.8[b]

In this case, "[b]P<0.05 in comparison to the HFD control group; [a]P<0.01 in comparison to the Control Group."

Table 12. Effect of Orlistat and FK plants leaves extracts on plasma and Liver oxidant-anti-oxidant levels of HFD Rats

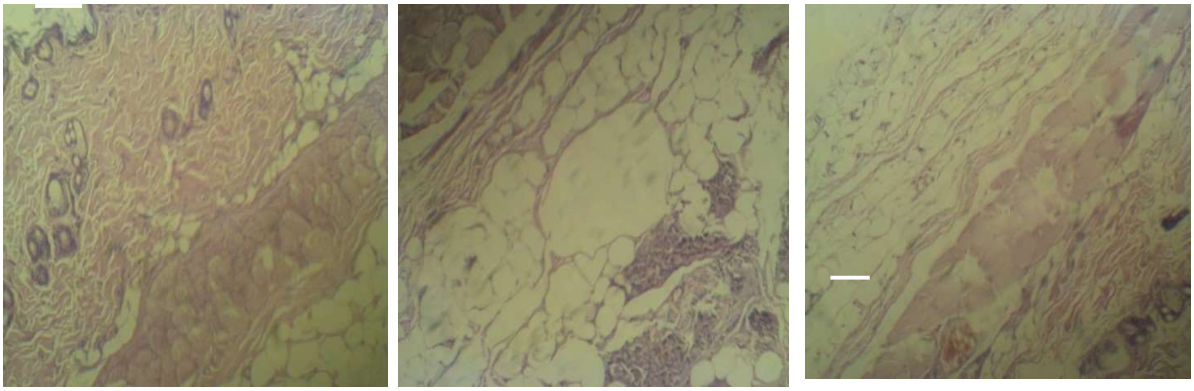
Parameters	**Normal Control**	**HFD**	**Orlistat (30mg/kg**	**HELEFK (100mg/kg)**	**HELEFK (200mg/kg)**	**HELEFK (400mg/kg)**
A) Effect on Plasma oxidant-anti-oxidant levels of HFD Fed Rats						
TBARS (nmol	10.67± 1.06	16.21±1.41	11.64±0.92	15.82±1.66	14.21±0.9	13.55±1.02

MDA/ml						
GSH(µmol/ml)	8.35 ± 0.93	3.20± 0.09[a]	8.10± 0.81[b]	3.55 ± 0.09	5.01±0.20[b]	5.99± 0.32[b]
SOD (u/ml)	6.15 ± 0.78	3.57± 0.26[a]	5.89± 0.46[b]	3.59 ± 0.21	4.26±0.65[b]	5.02± 0.71[b]
CAT (u/ml)	5.11 ± 0.53	2.96± 0.07[a]	4.98± 0.31[b]	3.05 ±0.07	3.78±0.08[b]	4.70± 0.10[b]
B) Effect on Liver oxidant-anti-oxidant levels of HFD Fed Rats						
TBARS (nmol MDA/gtissue)	3.22 ± 0.08	7.23 ± 0.90	3.89 ± 0.08	7.16 ± 1.01	5.86 ± 0.69	4.25 ± 0.20
GSH(µmol/g tissue)	4.99 ± 0.09	1.91± 0.02[a]	4.02± 0.08[b]	2.01 ± 0.09	2.97± 0.08[b]	3.67± 0.07[b]
SOD(U/mg protein)	3.88 ± 0.07	1.53± 0.02[a]	3.65± 0.06[b]	1.60 ± 0.04	2.16± 0.05[b]	3.06± 0.09[b]
CAT(U/mg protein).	2.56 ± 0.05	1.12± 0.01[a]	2.29± 0.03[b]	1.29 ± 0.06	1.62± 0.03[b]	1.92± 0.04[b]

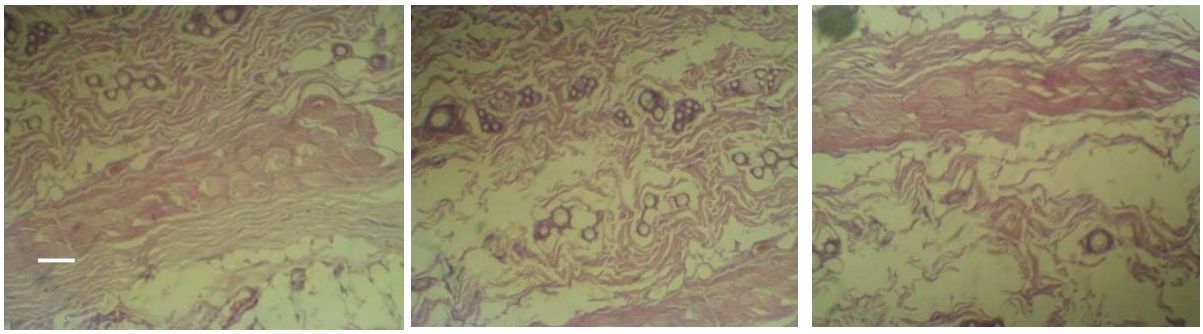
In this case, "[b]P<0.05 in comparison to the HFD control group; [a]P<0.01 in comparison to the Control Group."

GSH, SOD, and CAT levels dropped after eight weeks of a high-fat diet (HFD), although TBARS levels rose. HELEFK dramatically decreased TBARS and raised antioxidant levels in a dose-dependent manner at 200 and 400 mg/kg. The 100 mg/kg dose had no effect. In contrast to HFD control rats, orlistat (30 mg/kg) significantly decreased TBARS while raising GSH, SOD, and CAT levels.

Effect of Orlistat and FK plants leaves extracts on histopathological changes in adipose tissue of obese rats.



Normal Control **HFD**



(*Ficus Krishnae*) MD (*Ficus Krishnae*) HD

LD = Lower Dose (100 mg/kg) **MD = Middle Dose (200mg/kg)** **HD = Higher Dose (400mg/kg)**

Figure 3.: Histopathological analysis of adipose tissue in experimental rats

In comparison to normal rats, eight weeks of a high-fat diet (HFD) resulted in aberrant adipose tissue distribution and increased adipocyte size and obesity, according to histological study of epididymal adipose tissue. After eight weeks of HELEFK treatment, adipocyte size and adiposity decreased, tissue distribution improved, and body weight dropped; the most significant effects were observed at higher dosages (200 and 400 mg/kg). There was no discernible effect from the lesser dosage (100 mg/kg).

Discussion

The increase in obesity in recent decades is primarily attributable to the predominance of modified food selections and a sedentary lifestyle. High-fat diets (HFD) promote excessive fat accumulation by disturbing the equilibrium between energy use and expenditure, altering lipid metabolism, and increasing lipid storage in adipose tissues. HFDs, particularly rich in saturated and trans fats, cause hypertrophy and hyperplasia of adipocytes, leading to weight gain and insulin resistance, a key feature of metabolic syndrome, by impairing glucose metabolism in the liver and muscles. [(xvi)]

Considering the restricted accessibility of FDA-sanctioned anti-obesity medications including orlistat[(xvii)], liraglutide, [(xviii)] semaglutide. [(xix)]and their associated side effects, the search for novel, effective, and safer natural product-based therapies remains ongoing. This persistent need underscores the growing interest in discovering alternative treatments derived from natural sources to effectively address the global obesity epidemic. [(xx)] Among various therapeutic targets for obesity, those that modulate lipid mobilization and deposition, particularly influencing triglyceride levels, are considered crucial for effective intervention.[(xxi)]High fat diet (HFD)-induced obesity in rodent models is regarded as an effective analog for human obesity, as it demonstrates significant parallels in pathophysiology. [(xxii)] The characteristics of human obesity are closely linked to visceral adiposity, insulin resistance, dyslipidemia, hyperglycemia, and hepatic steatosis in rats fed a high-fat diet. [(xxiii)]

In this study, a high-fat diet-induced obesity paradigm in Wistar rats is used to test the anti-obesity potential of a hydroethanolic extract of *Ficus krishnae*.Enriched with bioactive flavonoids, particularly quercetin, and rutin, the extract demonstrates significant antioxidant activity, targeting oxidative stress and inflammation critical in managing obesity-related metabolic disorders. [(xxiv)] The parameters evaluated include body weight, BMI, Lee index, feed intake, lipid profiles, oxidative stress markers, liver function, and histological analysis of adipose tissue.

The physicochemical analysis of *Ficus krishnae* (FK) leaves revealed high ash and hydroalcoholic extractive values, confirming the purity and the presence of polar bioactive compounds like

flavonoids and alkaloids. Further HPTLC analysis identified key constituents, rutin, and quercetin, with ethanolic and hydroethanolic extracts demonstrating the highest bioactive compound content, supporting their selection for in-depth study and therapeutic analysis. [xxv]

The in vitro antioxidant activity of Ficus krishnae (FK) highlights its exceptional potential to significantly reduce oxidative stress, a critical factor linked to obesity and related diseases. [xxvi] Our findings demonstrate that FK possesses strong antioxidant properties and effectively decreases obesity levels. Notably, the hydroethanolic extract of FK shows the best results, which supports its further use in our studies. By alleviating oxidative stress, which is closely associated with obesity, FK serves as a promising antioxidant solution.

Interestingly, oral doses of HELEFK have decreased feed intake, suggesting an appetite-suppressing effect, which is similar to studies showing certain compounds can affect feeding behavior and reduce caloric intake, contributing to weight loss. FK has shown the presence of flavonoids, tannins, phytosterols, and phenolic acids. Moreover, it is also well reported that flavonoids and polyphenols show potential antioxidant activity, which may be responsible for anti-obesity-like effect. [xxvii]

Lipid profile analysis revealed that HELEFK significantly improved serum lipid levels, including reductions in total cholesterol, triglycerides, and LDL; while increasing HDL levels. These changes indicate that HELEFK has a hypolipidemic effect, which is crucial in reducing the risk of cardiovascular diseases associated with obesity. [xxviii]

The liver is essential for detoxification and lipid metabolism, and HFD-induced obesity is often associated with hepatic steatosis and liver dysfunction. In this study, HELEFK treatment significantly diminished heightened levels of ALT, AST, and ALP, demonstrating its hepatoprotective effects. This finding aligns with previous studies suggesting that *Ficus* species possess the ability to prevent liver damage caused by high-fat diets.

The presence of terpenoids, phenolic acids, and flavonoids (quercetin, rutin), which are recognized for their anti-inflammatory and antioxidant qualities, was verified by phytochemical analysis. These substances might help lower oxidative stress, which is a major contributor to the development of obesity and associated metabolic diseases. In vitro tests showing HELEFK's antioxidant activity lend more credence to its possible function in reducing oxidative stress and enhancing metabolic health.

Additionally, to validate the anti-adipogenic effect, we performed a histological analysis of adipose tissue. Our results showed that HELEFK significantly reduced adipocyte size and improved adipose tissue morphology in HFD-fed rats. This finding suggests that HELEFK may directly influence fat cell differentiation and growth, further supporting its potential as an anti-obesity agent.

Conclusion

The research findings demonstrate that the hydroethanolic extract of HELEFK has substantial anti-obesity effects in rats subjected to obesity models induced by high-fat diets. Along with improvements in lipid profiles and liver function, The extract resulted in substantial reductions in body weight, body mass index, and adipogenesis. These results imply that, especially at larger dosages, It is possible that HELEFK will be created as a natural medication to treat obesity and associated metabolic disorders.

Conflict of interest:

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