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Tephrosia purpurea Fraction Attenuates Lipid Accumulation and Adipogenesis in 3T3-L1 Adipocytes and Reduces Body Weight in High Fat Diet Induced Obese Rats

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Abstract

The anti-adipogenic and anti-obesity activity of chloroform fraction of *Tephrosia purpurea* (CFTp) on 3T3-L1 adipocytes and high fat diet (HFD)-fed obese rats was evaluated in this study. A substantial and dose dependent inhibition of *α*-glucosidase (81%) and lipase (75%) activities by CFTp was noticed. Treatment with CFTp (250 μg/mL) significantly inhibited 3T3-L1 adipocytes differentiation and lipid accumulation. A semi-quantitative RT-PCR analysis of 3T3-L1 cells revealed down regulation of mRNA expression of peroxisome proliferator-activated receptor-*γ* (PPAR-*γ*), fatty acid synthase (FAS) and acetyl CoA carboxylase-2 (ACC-2), while glucose transporter type-4 (GLUT-4) expression was up-regulated in a dose dependent manner with CFTp. Further, oral administration of CFTp (200 mg/kg.b.wt.) significantly reduced body weight gain, fat mass, blood glucose and leptin levels in high fat diet (HFD)-induced obese rats. Taken together, these findings demonstrate that CFTp possesses potent anti-obesity activities.

Keywords

Body Weight, Cell Viability, Enzyme Inhibition, Lipolysis, 3T3-L1 Cells

1. Introduction

The prevalence of obesity and associated ailments reached epidemic proportions across the world in recent decades. Over weight-obesity plays a central role in metabolic syndrome (MetS) which includes in its cluster other disorders like diabetes mellitus, hypertension, dyslipidemia and cardiovascular diseases (CVDs). The World Health Organization report-2016 mentions that 1.9 billion adults are

overweight of which 650 million are obese in the world [1] [2]. A sea change in food habits, work culture, increased snaking frequency, reduced physical activity and sedentary life styles have been the prime causes for enhanced MetS cases world over, especially in developing countries. The situation of childhood obesity is more alarming than adults [3].

Although a few FDA approved drugs are available to treat obesity or diabetes, drugs that can target both diabetes and obesity are lacking. In fact, some of the anti-obesity drugs have been withdrawn from the market due to their side effects [4] [5] [6]. In view of the high demand for safe, effective anti-obesity and anti-diabetic drugs and considering the side effects associated with existing synthetic drugs, there is a growing necessity to explore natural product based therapeutic alternatives. Targeting key carbohydrate and lipid metabolizing enzymes or molecules that reduce adipogenesis or/and insulin resistance have been considered as potential means to develop effective drugs to attenuate obesity or/and diabetes [7].

Compounds that interfere in the transcriptional regulation of key genes associated with lipid metabolism, insulin resistance and adipogenesis like peroxisome proliferator-activated receptor-γ, fatty acid synthase, acetyl CoA carboxylase-2, glucose transporter type-4 etc., have been found to be useful in developing effective therapeutics [8] [9] [10] [11]. Adipokines like leptin and adiponectin have been reported to play decisive roles in the regulation of obesity and insulin resistance and hence could be targeted as therapeutic molecules [12]. Leptin is produced from white adipose tissue and plays a negative feedback role in the regulation of energy expenditure through controlling specific neuronal groups of hypothalamus. In overweight/obese subjects a condition called leptin resistance occurs that leads to addicted food intake and eventually to more obesity. The expression of adiponectin is inversely related to obesity and exerts its action through modulating PPAR-γ and AMPK pathways [13].

Tephrosia purpurea (L.) Pers. is a perennial herb belonging to the family Fabaceae, distributed in Asian countries. It is commonly known as "Sarapunkha" in classical Ayurvedic texts and is traditionally used to treat cough, cold, cirrhosis, splenomegaly, abdominal swelling and also as an antidote in folklore medicine. Previous studies have identified several active components including flavonoids and other phytochemicals such as pongamol, semiglabrin, lanceolatins A and B, lupeol, rutin and β-sitosterol in *T. purpurea* extract [14]. Pharmacological studies on *T. purpurea* extracts showed hepatoprotective, anti-inflammatory, anti-allergic, antioxidant and antimicrobial activity [15]. In the present work, we evaluated anti-adipogenic and anti-obesity activity of *T. purpurea* extract, CFTp in 3T3-L1 adipocytes and HFD-fed obese rat model.

2. Materials and Methods

2.1. Chemicals and Reagents

Acarbose, orlistat, α-glucosidase (catalog no. G5003), pancreatic lipase (catalog

no. L3126), paranitrophenyl-glucopyranoside (p-NPG), p-nitro phenyl butyrate (p-NPB), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and Oil Red O (ORO) staining solution were procured from Sigma Aldrich. Nonidet P-40, morpholine propane sulphonic acid (MOPS), isopropyl alcohol, Insulin, Dulbecco's modified Eagle's medium (DMEM), fetal Bovine serum (FBS), 3-isobutyl-1-methyl-xanthine (IBMX), penicillin and streptomycin were procured from Thermo Scientifics. Other chemicals, solvents and reagents used were of Analytical Grade.

2.2. Plant Material Collection and Fraction Preparation

Fresh whole plants of *T. purpurea* were obtained from Chinthalapatteda area near Nagari and Tirupati, Andhra Pradesh, India. Their identity was authenticated by a taxonomist (Voucher Specimen Accession Number-1259) and deposited in the herbarium of Department of Botany, S.V. University, Tirupati. *T. purpurea* plants were shade dried, crushed to crude powder and extracted with chloroform following cold extraction method. The chloroform extract was then fractionated using hexane, ethyl acetate, chloroform and methanol based on their polarity in a column chromatography using silica gel as the column material. All the filtrates were concentrated under reduced pressure in Heidolph rotary-evaporator. Based on phytochemical analysis, chloroform fraction of *T. purpurea* (CFTp) was used for further studies.

2.3. DPPH Antioxidant Assay

Briefly, a 0.3 mM solution of DPPH was prepared in methanol and 500 µL of this solution was added to 1 mL of CFTp at different concentrations (100 - 500 µg/mL) [16]. These solutions were mixed and incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm against a blank lacking scavenger. Vitamin C was used as a standard. The antioxidant or free radical inhibitory activity was calculated according to the following formula

$$\%\text{inhibition} = ((Ac - As)/Ac) \times 100$$

where, Ac—Absorbance of control, As—Absorbance of sample.

2.4. Ferric-Reducing Antioxidant Power (FRAP) Assay

A 2.5 mL aliquot of CFTp was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [17]. The mixture was incubated at 50˚C for 20 min, followed by addition of 2.5 mL of 10% trichloro acetic acid and centrifuged at 3000 rpm for 10 min. Then, 2.5 mL of the upper layer of the solution was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride, after 10 min, absorbance was measured at 700 nm. An increase in the absorbance of the reaction mixture indicated increased reducing power of CFTp. The experiment was carried out in triplicate, using vitamin C as a positive control.

2.5. Assay of α-Glucosidase Activity

Briefly, 500 μL of CFTp and/or standard inhibitor (acarbose) at a concentrations of 100 - 500 μg/mL were incubated with 54 μL (1.0 U/mL) of α-glucosidase solution (in 100 mM phosphate buffer pH 6.8) and 446 μL of phosphate buffer for 15 min at 37˚C [18]. To this, 250 μL of p-nitrophenyl D-glucoside solution (5 mM) in 100 mM phosphate buffer (pH 6.8) was added and incubated for 20 min at 37˚C. Absorbance of liberated yellow colour p-nitrophenol was read at 405 nm using UV-visible spectrophotometer. All the readings were measured in triplicate and the average was considered. The percentage of enzyme inhibition was calculated using the formula specified below and the inhibitory activity was expressed as percentage of the control without inhibitor

$$\%\text{inhibition} = ((\text{Ac} - \text{As})/\text{Ac}) \times 100$$

where, Ac—Absorbance of control, As—Absorbance of sample.

2.6. Assay of Pancreatic Lipase Activity

Briefly, an enzyme-buffer was prepared by the addition of 30 μL of lipase solution (2.5 mg/mL) in 10 mM morpholine propane sulphonic acid (MOPS) and 1 mM EDTA, (pH 6.8) to 850 μL of Tris buffer (100 mM Tris-HCl and 5 mM $CaCl_2$, pH 7.0) [19]. Then 100 μL of CFTp (100 - 500 μg/mL) was mixed with 880 μL of enzyme buffer and incubated for 15 min at 37˚C. To this, 20 μL substrate solution (10 mM p-nitro phenyl butyrate in dimethyl formamide) was added and incubated for 15 min at 37˚C. The lipase activity was determined spectrophotometrically by measuring the hydrolysis of p-nitrophenyl butyrate to p-nitrophenol at 400 nm. All assays were carried out in triplicate and the calculation was done according to the following formula. Orlistat was used as standard drug.

$$\%\text{inhibition} = ((\text{Ac} - \text{As})/\text{Ac}) \times 100$$

where, Ac—Absorbance of control, As—Absorbance of sample.

2.7. Cell Viability by MTT Assay

Pre-confluent pre-adipocytes (3T3-L1 cells, from NCCS Pune), 2500 cells/well or mature adipocytes 5000 cells/well were seeded in 96 well culture plates using DMEM medium supplemented with 10% FBS and 1% antibiotic and incubated at 37˚C with 5% CO_2 for 48 or 72 h [20]. Then, cells were treated with CFTp. After overnight incubation, cytotoxicity/cell viability were determined by adding 10 μL of MTT [3-(4,5-dimethylthizol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tet razolium salt] (0.5 mg/mL in PBS), incubated at 37˚C for 4 h. At the end of incubation, culture media was discarded and the wells were washed with PBS. Later, 150 μL of dimethyl sulfoxide (DMSO) was added to all the wells, and incubated for 30 min at room temperature with constant shaking. Absorbance was read at 540 nm using Microplate Reader and subsequently percentage (%) of cell viability will be calculated using following equation to determine the formazan

concentration, which is proportional to the number of live cells.

$$\%\text{Inhibition of proliferation} = \%\text{untreated cell viability } (100) - \%\text{drug treated cell viability.}$$

2.8. Adipocyte Differentiation—Measurement of Cellular Lipid Contents by Oil Red O Staining

3T3-L1 cells were cultured ingrowth media (GM) consisting of DMEM supplemented with 10% fetal Bovine serum (FBS) and 2mM glutamine. The cells were grown according to a well-established protocol described previously. Briefly, for differentiation, 3T3-1 cells were cultured in GM to full confluence. Two days after confluence (referred to as day 0), the cells were switched to differentiation media (DM) consisting of DMEM supplemented with 10% FBS, 10 mg/mL insulin, 1 M dexamethasone and 0.5 mM IBMX (isobutylmethylxanthine) and cultured for three days. Next, the cells were maintained in DM but containing only insulin (10 mg/mL) and the medium was changed every 2 - 3 days. The cells normally differentiate into mature adipocytes in a week. The 3T3-L1 preadipocytes were differentiated as described above in the presence of CFTp or vehicle (PBS).

For Oil Red O staining, at the end of incubation period, cell monolayers were washed twice with PBS (pH 7.4) and, fixed in 10% buffered formalin solution in PBS for 1 h, washed twice with DW and then stained with 0.5% Oil Red O stain for 30 min at room temperature. Excess Oil Red O dye was washed with DW and photographs were taken in inverted microscope using digital camera system. In another set of experiment, the stained adipocytes were treated with 60% isopropanol (to extract intracellular Oil Red O stain) and the absorbance (Optical density, OD) was read at 520 nm [21].

%Adipogenesis was calculated as OD of treated cells/OD of untreated cells × 100.

2.9. Lipolysis: Measurement of Glycerol Content

Glycerol release was measured to assess the lipolytic effect from adipocytes and examined according to the Millipore kit procedure. Briefly, differentiated adipocytes were incubated at 37˚C in 5% CO_2 atmosphere with the CFTp in sterile Hank's balanced salt solution containing 2% Bovine serum albumin (BSA). At the interval of 12 h and 24 h, the 10 μL supernatant from the 96 well plates were withdrawn and mixed with the 80 μL of glycerol assay reagent in a separate 96 well plate. After incubation of 1 h, the absorbance of the solution was measured at 540 nm using a microplate reader [22]. The amount of glycerol released was calculated by the equation of glycerol standard curve. Nor epinephrine, quercetin and forskolin were used as positive standards.

2.10. RT-PCR—mRNA Expression

Total RNA was isolated from 3T3-L1 cells/adipose tissue by using tri-reagent (Sigma Aldrich, USA) according to manufacturer's protocol and reverse transcribed to obtain cDNA using DNA synthesis kit (Applied Bio Systems, Foster City, USA) [23]. Two nanograms of cDNA were used for semi-quantitative

RT-PCR. The PCR amplification was performed for 38 cycles using the following cycling conditions: 30 sec of denaturation at 94˚C, 30 sec of annealing at 59˚C and 1 min of extension at 72˚C, with the specific the primers. The primer sequences used for PCR analysis were as follows:

PPAR-γ F: 5'-GACCGAGTGTGACGACAAG-3';
R: 5'-CGTGATTTCTCAGCCGCGT-3'
Glut4 F: 5'-AAAAGTGCCTGAAACCAGAG-3';
R: 5'-TCACCTCCTGCTCTAAAAGG-3'
FAS F: 5'-ATGTGGTACGGAAGGTGGAG-3';
R: 5'-TGGCTACCTTCGTCTGTGTG-3'
ACC2 F: 5'-ACCTTGTTGGGGAGAAGTGC-3';
R: 5'-AGGGCCAAGGTGTCATAAGC-3'
β-Actin F: 5'-ACCTTCCAGCAGATGTGGAT-3';
R: 5'-AGAAGCACTTGCGGTGCACGA-3'.

2.11. Animals and Diets

Male WNIN rats and diets were obtained from National Institute of Nutrition (NIN), Hyderabad, India. After one-week quarantine period for safe health of experimental rats, they were fed with either normal diet or freshly prepared HFD (15 g/rat/day) for 16 weeks as mentioned in experimental design and water *ad libitum* and standard laboratory conditions (temperature: 22˚C ± 2˚C; humidity: 40% - 60%) were maintained. Normal diet contained all the recommended macro and micronutrients (carbohydrate-56%, protein-18.5%, fat-8%, fiber-12% and adequate levels of minerals and vitamins). High fat diet contained starch-42%, casein-23%, lard oil-23%, cholesterol-2%. Cellulose-5%, mineral mixture (AIN-93G)-3.5%, vitamin mixture (AIN-93VX)-1%, L-cystine-0.3%, choline bitartrate-0.2%. Rats initially weighing 180 - 200 g were randomly divided into six groups of six each (n = 6). To test the activity of CFTp, 100 or 200 mg/kg b.wt. of CFTp was suspended in 0.5% carboxy methyl cellulose (CMC), and orally administered for 42 days from 10th week onwards using an intra-gastric tube. All experimental protocols were followed as per institutional animal ethical committee guidelines (No: 55/2012/(i)/a/CPCSEA/IAEC/SVU/MBJ, Dt: 08.07.2012).

2.12. Experimental Design

Group 1: Normal control (normal diet control)
Group 2: High fat diet (HFD) control
Group 3: HFD + orlistat 5 mg/kg b.wt.
Group 4: HFD + CFTp 100 mg/kg b.wt.
Group 5: HFD + CFTp 200 mg/kg b.wt.

2.13. Measurement of Body Weight and Body Composition Parameters

The body composition, body weight, fat percent of each rat was measured by

Total Body Electrical Conductivity (TOBEC) using small animal body composition analysis system (EM-SCAN, Model SA-3000 Multi detector, Springfield, USA). At the end of the experiment, blood was collected from overnight fasted animals under inhalation of anesthesia by heart puncture method; plasma was separated by centrifugation at 2500 rpm for 15 min.

2.14. Estimation of Leptin and Adiponectin Levels

Plasma leptin and adiponectin are important adipokines and their levels were measured in experimental rats by using enzyme-linked immunosorbent assay kits (Crystal Chem, Downers Grove, IL, USA), performed in duplicate, as per the manufacturer's guidelines and were expressed in ng/mL.

2.15. Oral Glucose Tolerance Test (OGTT)

At the end of the experiment OGTT was performed [24]. After overnight fasting of experimental rats, glucose was administered orogastrically at a dose of 2.0 g/kg b.wt. and blood samples were collected from supraorbital sinus at 0, 30, 60, 90 and 120 min. Glucose levels were estimated at all intervals.

2.16. Statistical Analysis

Statistical analysis was performed using Turkey's-HSD multiple range post hoc test $p < 0.05$ IBM SPSS version 23. Data were expressed as the mean ± standard deviation (SD).

3. Results

3.1. Antioxidant Activity of CFTp by DPPH and FRAP Assays

The antioxidant capacity of the chloroform fraction of *T. purpurea* (CFTp) was measured by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays. *In-vitro* antioxidant activity of CFTp on DPPH free radicals had shown significant scavenging activity in a dose dependent manner and its activity was close to vitamin C at 500 μg/mL of CFTp (**Figure 1(A)**). Similarly, the dose-dependent ferric reducing power of CFTp which was found to be about 75 % of Ascorbic acid, indicating its potential free radical scavenging activity (**Figure 1(B)**).

3.2. Inhibitory Effect of CFTp on α-Glucosidase and Pancreatic Lipase Activities

In the present study as shown in **Figure 2(A)** and **Figure 2(B)**, a significant and dose-dependent inhibition of both α-glucosidase and pancreatic lipase was noticed. At 500 μg/mL of CFTp, the maximum inhibition of 81% and 75% was observed for α-glucosidase and pancreatic lipase respectively.

3.3. Effect of CFTp on Cell Viability of 3T3-L1 Cells

The cytotoxic effects of CFTp on the viability of 3T3-L1 cells was analysed at 48

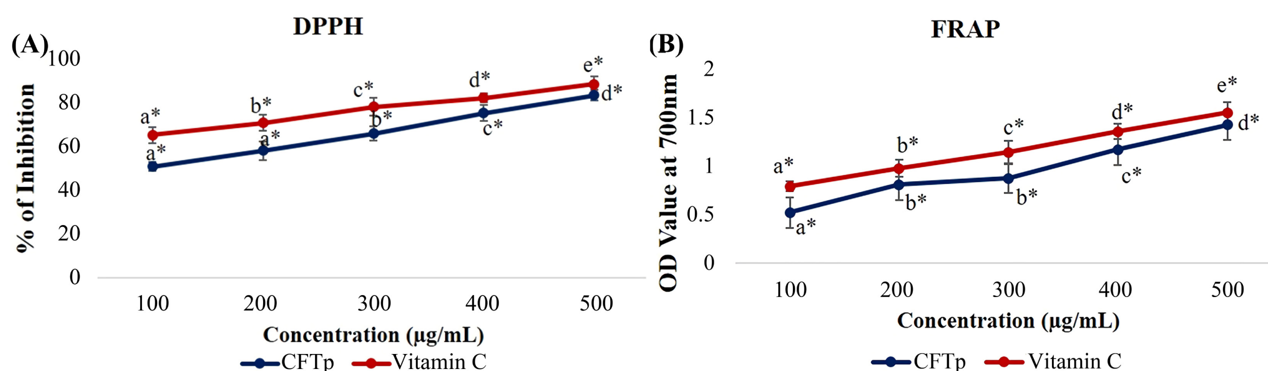


Figure 1. Effect of CETp on free radicals scavenging activities. (A) *In-vitro* antioxidant activity of CFTp on DPPH free radicals. (B) Ferric reducing antioxidant power of CFTp. Data are presented as mean ± SD of triplicate. Bars with different superscript letters are significantly different from one another ($p < 0.05$).

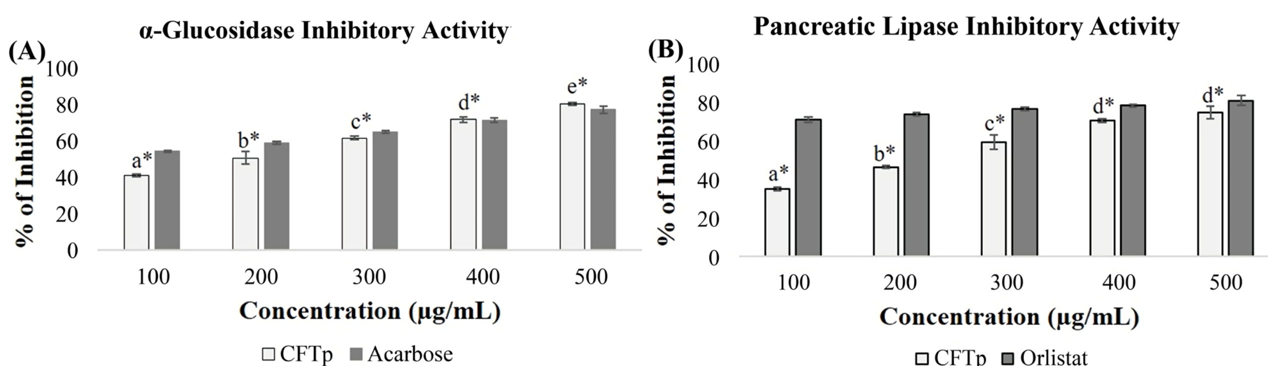


Figure 2. Effect of CFTp on carbohydrate and lipid metabolizing enzymes by *in-vitro* studies. (A) *α*-glucosidase activity. (B) Pancreatic lipase activity. Data are presented as mean ± SD of triplicate. Bars with different superscript letters are significantly different from one another ($p < 0.05$).

h using MTT assay. No cytotoxic effect was observed up to a dose of 250 µg/mL of CFTp, but thereafter, about 10% - 15% of cell death was noticed (Figure 3(A)).

3.4. Effect of CFTp on Adipocyte Differentiation, Lipid Content and Glycerol Release in 3T3-L1 Cells

The microscopic observation of Oil Red O stained 3T3-L1 cells indicates that, groups treated with CFTp shows decreasing number of adipocytes and reduced lipid accumulation in adipocytes (in a dose dependent manner) when compared to untreated cells (Figure 3(D)). In addition, measurement of absorbance of Oil Red O stain, extracted (using isopropanol) from lipid droplets of 3T3-L1 cells, indicates the extent of adipocytes differentiation. Our results showed that, CFTp (250 µg/mL) could considerably inhibit adipocyte differentiation when compared to the untreated cells (Figure 3(B)). To understand the effect of CFTp on lipolysis of 3T3-L1 cells, glycerol release into the surrounding medium was estimated spectrophotometrically. A significant increase in glycerol content was observed in groups treated with CFTp when compared to untreated cells and the maximum lipolytic activity was noticed at a concentration of 250 µg/mL (Figure 3(C)).

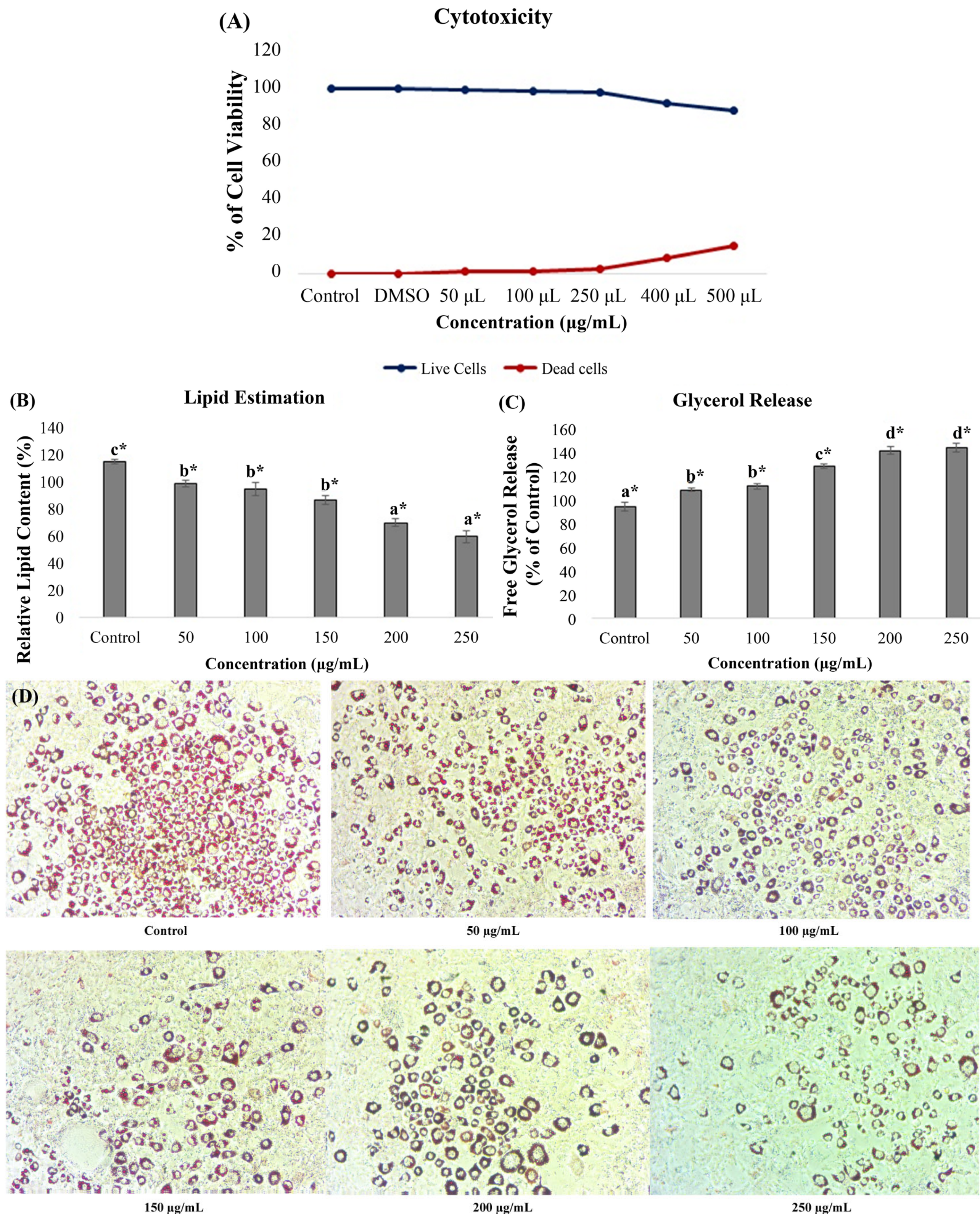


Figure 3. Effect of CFTp on 3T3-L1 adipocytes. (A) Percentage of cell viability shown at 48 h. (B) Relative lipid content in adipocytes. (C) Glycerol content released into media. (D) Oil Red O stained pictures showing decreased adipocytes and lipid accumulation (Magnification-20×). Data are presented as mean ± SD of triplicate. Bars with different superscript letters are significantly different from one another ($p < 0.05$).

3.5. Effect of CFTp on mRNA Expression of FAS, GLUT4, ACC-2 and PPAR-γ

The mRNA expression of FAS, GLUT4, ACC-2 and PPAR-γ in 3T3-L1 cells in the presence and absence of CFTp (Figure 4). The expression of FAS, PPAR-γ and ACC-2 were down-regulated, while GLUT4 was up-regulated with increasing concentration of CFTp. Their expression was compared to that of house-keeping gene β-actin.

3.6. Effect of CFTp on Body Composition and Body Weight of WNIN Rats

Table 1 depicts change in body weight and body composition of experimental rats. Consumption of HFD for 16 weeks resulted in significant increase in body weights (458 ± 3.45 g) and total fat levels (53.245 ± 2.75 g) in HFD control group, compared to normal control group of rats whose body weight and total fat were 302 ± 7.11 g and 29.22 ± 3.04 g, respectively. Oral administration of CFTp (100 and 200 mg/kg b.wt.) for 42 days (from 12 to 16 weeks) considerably reduced body weight and body composition in a dose dependent manner. At a dose of 200 mg/kg b.wt., CFTp could substantially limit the body weight gain and total fat to 312 ± 8.16 g and 96.265 ± 4.78 g respectively.

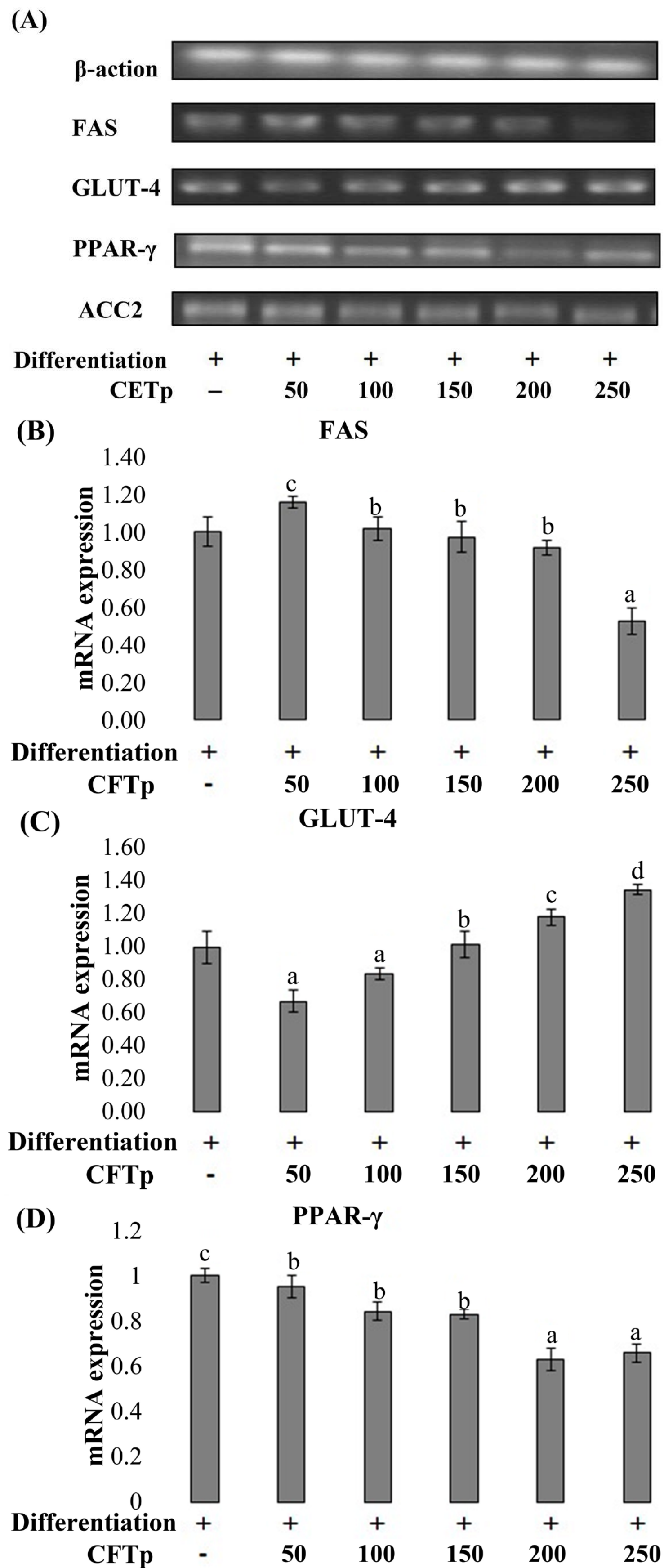
3.7. Effects of CFTp on Leptin and Adiponectin Levels

Figure 5 depicts the levels of leptin and adiponectin in control and experimental obese rats. There was a marked increase in leptin levels, while decrease in adiponectin levels found in HFD-fed obese rats compared to the normal rats. Interestingly, treatment with CFTp has significantly ($p < 0.05$) decreased the levels of leptin, while the levels of adiponectin increased in HFD-fed obese rats.

Table 1. Effect of CFTp on body weight and body composition parameters of treated and untreated rats.

Parameters	ND	HFD	HFD + Orlistat	HFD + CFTp1	HFD + CFTp2
Initial body weight (g)	185 ± 5.72	183 ± 7.12*	187 ± 4.93#	180 ± 8.14#	182 ± 6.58#
Final body weight (g)	302 ± 7.11	478 ± 23.45*	423 ± 14.56#	452 ± 17.25#	446 ± 12.16#
Body weight gain (g)	117 ± 9.58	295 ± 27.41*	236 ± 12.85#	272 ± 16.94#	264 ± 14.65#
Lean body mass (g)	286.78 ± 12.68	350.755 ± 11.79*	353.28 ± 26.49#	363.675 ± 9.11#	381.16 ± 10.52#
Total body fat (g)	15.22 ± 3.04	127.245 ± 2.75*	69.72 ± 2.11#	88.325 ± 3.49#	64.835 ± 4.78#
body fat (%)	5.03 ± 1.8	26.62 ± 0.7*	16.48 ± 1.9#	19.54 ± 1.6#	14.53 ± 2.1#
Fat free mass (g)	173.224 ± 3.76	182.164 ± 1.89*	197.384 ± 4.48#	198.74 ± 3.69#	214.172 ± 5.43#
Total body H_2O (mg)	737.748 ± 23.58	777.978 ± 30.64*	846.468 ± 37.8#	852.57 ± 29.72#	922.01 ± 32.45#
Total body Na (mg)	1022.8 ± 45.2	1078.675 ± 67.54*	1173.8 ± 89.18#	1182.27 ± 36.7#	1278.7 ± 27.56#
Total body K (mg)	2106.582 ± 76.48	2217.21 ± 112.31*	2405.56 ± 59.7#	2422.34 ± 44.5#	2613.3 ± 72.71#

All data in the table are shown as mean ±SD (n = 6). * indicates significant difference between normal control and HFD control groups. # indicates significant difference between HFD control and other groups. CFTp1 = 100 μg/mL; CFTp2 = 200 μg/mL.


(A)
β-action
FAS
GLUT-4
PPAR-γ
ACC2
Differentiation + + + + + +
CETp – 50 100 150 200 250
(B)
FAS
mRNA expression
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
c
b
b
b
a
Differentiation + + + + + +
CFTp - 50 100 150 200 250
(C)
GLUT-4
mRNA expression
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
a
a
b
c
d
Differentiation + + + + + +
CFTp - 50 100 150 200 250
(D)
PPAR-γ
mRNA expression
1.2
1
0.8
0.6
0.4
0.2
0
c
b
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a
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Differentiation + + + + + +
CFTp - 50 100 150 200 250

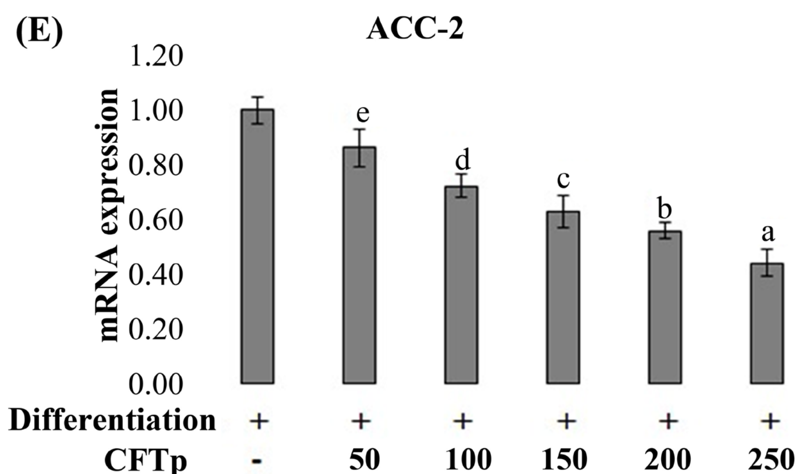

Figure 4. (A) Effect of CFTp on mRNA expression of FAS, GLUT-4, PPAR-γ and ACC-2 in 3T3-L1 adipocytes. (B), (C), (D) and (E) are graphical representation of expression levels of these genes. Bars with different superscripts indicate significant ($p < 0.05$) differences from one another.

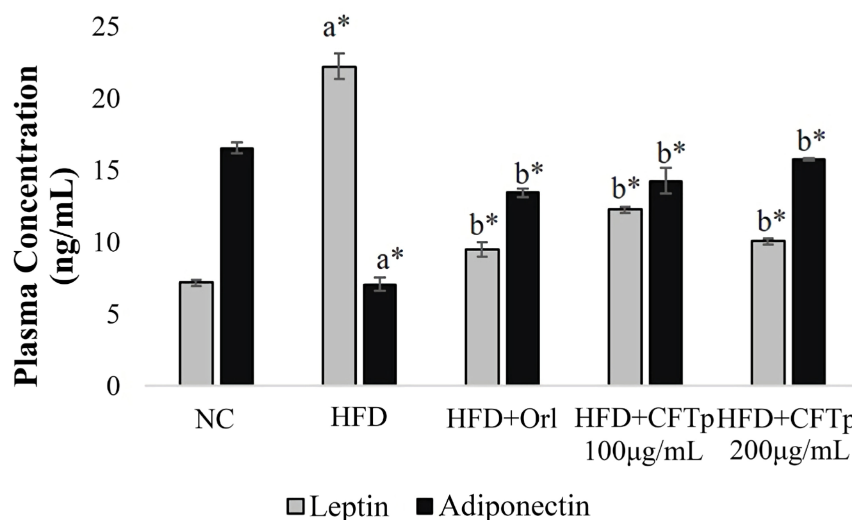

Figure 5. Effect of CFTp on leptin and adiponectin levels in treated and untreated rats. Values are mean ± SD, n = 6. Values are statistically significant at $^*p < 0.05$. a* significantly different from normal control and b* significantly different from HFD control.

3.8. Administration of CFTp Decreases Blood Glucose Levels in HFD-Fed Obese Rats

Figure 6 depicts the results of oral glucose tolerance test performed on control and experimental obese rats. In the normal control group of rats, blood glucose level reached its maximum value at 60 min after glucose load and declined to near basal level at 120 min, whereas, in HFD-induced obese rats, the peak increase in blood glucose level was noticed even after 60 min and remained high over the next 60 min. Administration of CFTp (100 and 200 mg/kg b.wt) or orlistat to obese rats elicited a significant decrease in blood glucose level at 60 min and beyond when compared with HFD control rats.

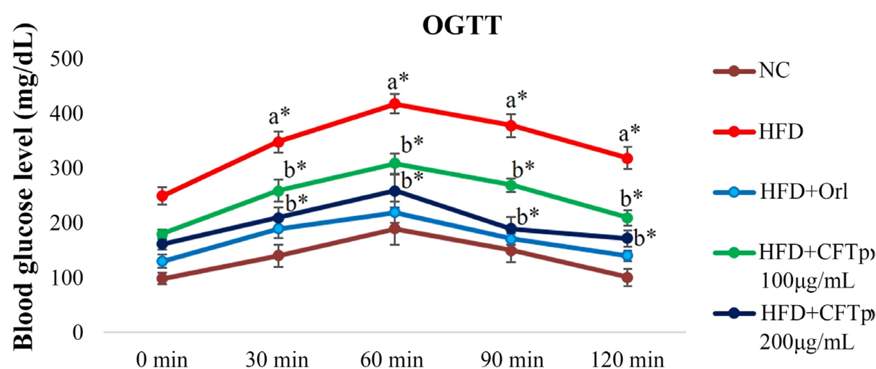

Figure 6. Effect of CFTp on blood glucose level during OGTT in treated and untreated rats. Values are mean ± SD, n = 6. Values are statistically significant at $*p < 0.05$. a* significantly different from normal control and b* significantly different from HFD control.

4. Discussion

The cases of obesity, diabetes, hypertension and CVDs have tremendously increased in recent times making metabolic syndrome (MetS) a common global public health issue. There remains a growing public interest towards natural product based therapeutics in place of synthetic drugs due to their side effects to treat such diseases. Adipogenesis is the key process that involves growth and division of adipocytes. Agents that modulate adipocyte differentiation, lipid metabolism, adipokines and insulin resistance have been reported to reduce obesity and diabetes. Here, we have demonstrated anti-obesity and anti-insulin resistance effects of CFTp in HFD-fed obese rats. Further, *in vitro* treatment of CFTp in 3T3-L1 cells exhibited inhibition of adipogenesis and increased lipolysis. Overall, these findings support pharmacological effects of CFTp, which can be treated as natural product therapy in patients with obesity-related compilations.

Inhibiting the activity of key enzymes of carbohydrate and lipid metabolism has been considered as potential therapeutic target to contain obesity and diabetes [25]. In the present study CFTp substantially inhibited the activity of α-glucosidase and pancreatic lipase (**Figure 2**). Previous studies on phytochemicals of *Oncoba spinosa* and *Ficus carica* have shown inhibition of α-amylase, α-glucosidase and pancreatic lipase activity leading to anti-obesity and anti-diabetic activity [26] [27]. Piper and Capsicum extracts have brought about weight reduction in diet induced obese rat models through inhibition of key lipid metabolizing enzymes [28] [29].

Our studies on mRNA expression level showed down-regulation of PPAR-γ, FAS and ACC-2 but up-regulation of Glut-4 in the presence of CFTp. This indicates that, CFTp exerts anti-adipogenic activity through modulation of master transcriptional regulator PPAR-γ as well as ACC-2 and FAS [10] [11]. Down-regulation of ACC-2 by CFTp might lead to increased oxidation of fatty acids and favours glucose uptake (as evident from increased expression of Glut-4) leading to its oxidation and might contribute to decrease insulin resistance [9].

Enhanced levels of reactive oxygen species (ROS) are observed during obesity development. Phytochemicals with potent antioxidant activity could support in attenuation of obesity ailments. In the present study CFTp showed potent antioxidant and free radical scavenging activity which might augment the therapeutic efficiency of *T. purpurea* to treat obesity and insulin resistance (**Figure 1(A)**, **Figure 1(B)**). Particularly, the presence of 4-(4-methoxyphenyl)-1H-1,2,3-triazole in CFTp could act on NADPH oxidase (NOX) or Toll-like receptor (TLR-4) leading to reduced inflammation, insulin resistance and obesity [30] [31]. Our results suggest that, CFTp has beneficial effects in the management of adipogenesis, hyperlipidemia and insulin resistance.

5. Conclusion

These findings suggest that, CFTp attenuates insulin resistance and obesity through inhibition of key enzymes of carbohydrate and lipid metabolism, modulation of leptin and adiponectin levels in HFD-induced obese rats and through transcriptional regulation of mRNA expression of PPAR-γ, FAS, ACC-2 and Glut-4 levels in 3T3-L1 adipocytes. Thus, CFTp could be considered as an effective therapeutic agent to treat obesity and insulin resistance.

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Conflicts of Interest

The authors have no conflict of interest to declare.

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List of Abbreviations

CFTp, chloroform fraction of T. purpurea; HFD, high fat diet; HMGR, HMG-CoA reductase; PPAR-γ, peroxisome proliferator-activated receptor-γ; FAS, fatty acid synthase; ACC-2, acetyl CoA carboxylase-2; GLUT-4, glucose transporter type-4; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power.