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ORIGINAL ARTICLE

Hydroxycitric Acid-Induced Activation of Peroxisome Proliferator-Activated Receptors in 3T3-L1 Adipocyte Cells

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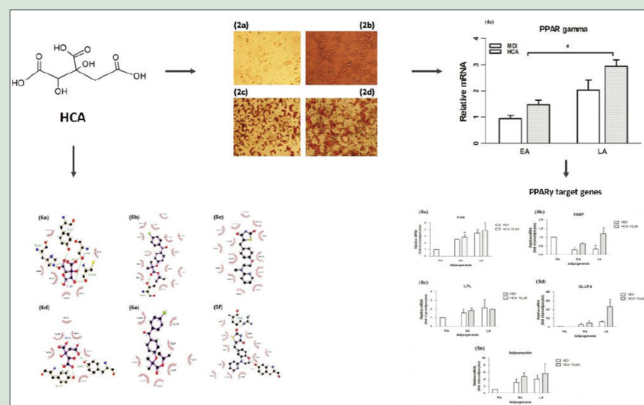
ABSTRACT

Background: Increase in obesity incidence has become serious threat to civilized human population. Traditional Indian System of Medicines may have many potential leads in managing obesity. (-)-Hydroxycitric acid (HCA), a unique compound which is present in *Garcinia* species, has been safely used for centuries in Southeastern Asia for various purposes. **Objective:** To determine the effect of HCA treatment on peroxisome proliferator-activated receptors (PPARs) and their target genes. **Materials and Methods:** Effect of the calcium salt of HCA on adipogenic transcription factors PPARs and their target genes (lipoprotein lipase, fatty acid synthase, fatty acid binding protein, glucose transporter 4, and adiponectin) during adipogenesis in 3T3-L1 was investigated. The extent of adipogenesis was checked by measuring the lipid accumulation and glucose uptake in the presence and absence of HCA. **Results:** HCA treatment modulated the differentiation of adipocytes in a dose-dependent manner, wherein lower concentrations showed increased accumulation of lipid depots. HCA treatment increased PPARγ and its target genes during adipocyte differentiation, which emphasize the role of HCA in adipocity and obesity. Moreover, the binding conformations of HCA and PPARα and PPARγ were predicted using flexible docking and confirmed with known agonists, which also confirms the bioactivity. **Conclusion:** HCA decreases circulating lipids through raised levels of adipogenic-specific genes activated by the PPARs, thereby augmenting adiposity and related complications.

Key words: 3T3-L1, adipocyte cells, *Garcinia*, hydroxycitric acid, peroxisome proliferator-activated receptor γ

SUMMARY

- 1. HCA improved adipogenesis by increasing PPARγ expression and its target genes.
- 2. Docking studies showed that HCA in part acts as a partial agonist of PPARγ.



Abbreviations Used: HCA: Hydroxycitric Acid, PPARs: Peroxisome proliferator activated receptors, GLUT: Glucose transporter, ORO: Oil red O, MDI: Methylxanthine dexamethasone insulin, LPL: Lipoprotein lipase, FAS: Fatty acid synthase, FABP: Fatty acid binding protein, LBD: Ligand binding domain.

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INTRODUCTION

Alarming incidence of obesity has become a serious threat to human population since obesity is implicated with the development of other consequential conditions, including insulin resistance, hyperglycemia, abnormal lipid profile, and hypertension, which contributes to cardiovascular diseases. Obesity is contributed to increased mass of adipose tissue as the preadipocytes proliferate and differentiate to adipocytes,[1] a cellular process called adipogenesis.[2] The successful differentiation of preadipocyte fibroblasts to mature adipocytes relies on the expression of specific adipogenic genes. Expression of various adipogenic factors is dependent on transcription factor, peroxisome proliferator-activated receptor (PPAR)-γ. PPAR-γ, the master regulator for most of the adipogenic-specific genes,[3] is a ligand-activated nuclear transcription factor which plays an important role not only in adipogenesis but also in immunity, inflammation, atherosclerosis, cell proliferation, cell growth, and macrophage function.[4] Many studies have shown that the role of PPAR-γ in metabolic disorders makes it as a molecular target for pharmacological ligands which induce adipocyte differentiation and improve insulin sensitivity by expressing glucose transporter 4 (GLUT4) and lipogenic factors.[5]

Plant-derived compounds that can modulate PPAR activity have been reported by many research groups.[6-8] These compounds through PPAR may have a positive impact on metabolism and have become promising natural drugs for the treatment of obesity and its associated complications. Hydroxycitric acid (HCA), also known as 1,2-dihydroxypropane-1,2,3-tricarboxylic acid, with a monoisotopic mass of 208.021917 Da, is the principle organic acid found in the fruit rinds of *Garcinia cambogia*, *Garcinia indica*, and *Garcinia atroviridis* with its contents ascending as reported.[9-11] Conventionally, the fruit of *Garcinia* is widely employed as a food ingredient and as a folklore medicine in curing many ailments; it also shows antioxidant and antidiabetic activities as mentioned by Deore *et al.*[12] and Kirana and Srinivasan,[13] respectively. Physiological importance of HCA has been extensively studied and attributed for its role in body weight reduction,

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lipogenesis, appetite control, and increased fat oxidation. HCA exists as a free acid or in the lactone form, and the former is biologically active but unstable. To prevent the cyclization of HCA into its less potent lactone, the organic acid is combined with various counterions to form stable salts. This enhances the solubility and confers higher bioavailability.[14] Herein, the present study aimed to evaluate the role played in adipogenesis by the calcium salt of HCA which confers higher solubility. The modulatory effect of this organic acid commonly available in *Garcinia* species to the tune of 16%–30% on adipocyte differentiation and carbohydrate and lipid metabolism-associated genes was assessed in murine 3T3-L1 cells and was also confirmed by *in silico* analysis in the present study.

MATERIALS AND METHODS

Materials

HCA calcium salt (HCAca) <95% purity by high-performance liquid chromatography was obtained from Natural Remedies Private Limited, Bangalore, India. All cell culture chemicals were bought from HiMedia, India, and the induction chemicals/fine chemicals were purchased from Sigma-Aldrich, USA. Biochemical kits were procured from Spinreact, Spain, whereas the ELISA kits for PPAR-α and PPAR-γ were from Cusabio Biotech Co., Limited. 3T3-L1 murine preadipocyte cell line was purchased from National Centre for Cell Science, Pune, India.

Effect of hydroxycitric acid on 3T3-L1 adipocyte cell growth

3T3-L1 preadipocytes were seeded in a 96-well plate at a density of 1×10^4 and treated with HCA at different concentrations (1–100 µM) for 24 h. After the medium was removed, the cells were treated with 50 µl/well 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution at 5 mg/ml for 2 h. Produced formazan crystals were dissolved in dimethyl sulfoxide after the removing the MTT solution. The absorbance was measured at 570 nm using a Bio-Rad iMARK microplate reader.

Table 1: Oligonucleotide primer sequence used for RT-PCR

Gene	Sequence
β-actin	F 5′- GGCCAACCGTGAAAAGATG-3′ R5′-GGATCTTCATGAGGTAGTCTGTC-3‘
Glut-4	F 5‘-CTCATGGGCCTAGCCAATG-3‘ R 5‘-GGGCGATTTCTCCCACATAC-3‘
LPL	F 5‘-TTCCATTACCAAGTCAAGATTCAC-3‘ R 5‘-TCAGCCCGACTTCTTCAGAGACTT-3‘
FAS	F 5‘-GGCTCAGCATGGTCGCTT-3‘ R 5‘-CTCCCGCCAGCTGTCATT-3‘
PPARγ	F 5‘-GGATTCATGACCAGGGAGTTCCTC-3‘ R 5‘-GCGGTCTCCACTGAGAATAATGAC-3
PPARα	F 5‘-GCCATCTTCACGATGCTGTCCTCC-3‘ R 5‘-GTAGATCTCTTGCAACAGTGGGTGC-3‘
FABP4	F 5‘-TCAGCTGGGAATAGAGTTCGAC-3‘ R 5‘-TAGTTAGTGTTGTCTCCTGCCC-3‘

3T3-L1 differentiation

Differentiation of 3T3-L1 cells was done based on the method used by Kong *et al*.[15] with little modifications. Cells were maintained in Dulbecco's modified Eagle medium (DMEM) with 10% (v/v) fetal calf serum, 100 U/ml penicillin, and 10 µg/ml streptomycin at 37°C in a humidified 5% CO_2 atmosphere. After 2 days of postconfluence (considered as day 0), adipocyte differentiation was induced by differentiation medium (MDI) cocktail with or without various concentrations of HCA. MDI consists of 250 mM 3-isobutylmethylxanthine, 1 mM dexamethasone, and 670 nM insulin in DMEM with 10% fetal bovine serum (FBS). On the 4th day, MDIs were replaced with the insulin medium (10% FBS + DMEM with 10 µg/ml insulin) with or without HCA, and thereafter, the medium was changed with insulin media for every 2 days. Maximum differentiation is achieved between 8 and 10 days. Well-differentiated adipocytes were used for further analysis.

Oil red O staining

The extent of preadipocyte differentiation in the presence of HCA was quantified using oil red O (ORO) staining. Cells were treated with HCA at various concentrations during the entire period of differentiation. When the maximum differentiation was obtained, the cells were fixed with 10% formalin in phosphate-buffered saline for 1 h. It was followed by 60% isopropanol washing and staining with ORO solution for 10 min and subsequently washing with water. Pictures were taken at this stage and the quantity of lipid accumulation was colorimetrically estimated by eluting the lipid-bound dye using 100% isopropanol at 500 nm.

Glucose consumption of preadipocytes and differentiated adipocytes

Glucose consumption assay was performed simultaneously in preadipocytes and differentiated adipocytes.[14] Preadipocyte cells and differentiated adipocytes were incubated in serum-starved conditions with a known concentration of glucose (20 mM) medium with and without HCA in incremented concentrations. Subsequently, the glucose content in the medium was estimated biochemically at 1 h and 24 h using glucose oxidase kit. The readings were measured using a Semi-automated Biochemical Autoanalyzer at 492 nm.

Glucose uptake of 3T3-L1 cells

Glucose uptake assay was performed similar to glucose consumption assay with some slight modifications. This assay employs the use of a fluorescent

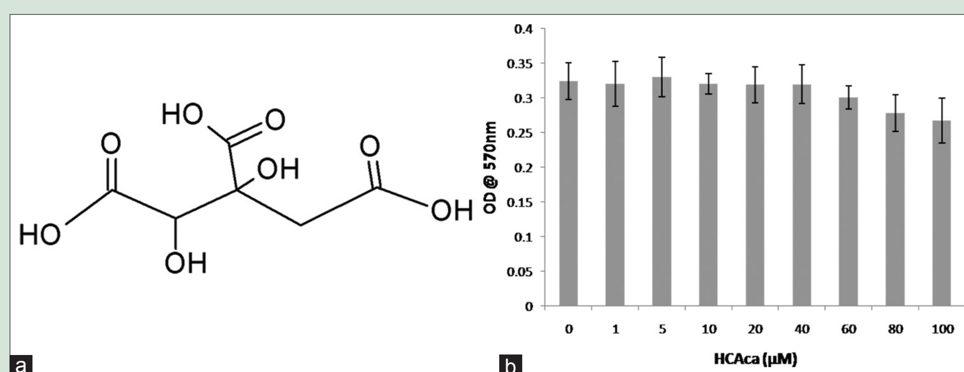

Figure 1: Effects of hydroxycitric acid calcium salt on of 3T3-L1 cells. (a) Chemical structure of hydroxycitric acid. (b) Cytotoxic effects and effects on cell viability in 3T3-L1 cells. Data are represented as mean ± standard deviation ($n = 3$)

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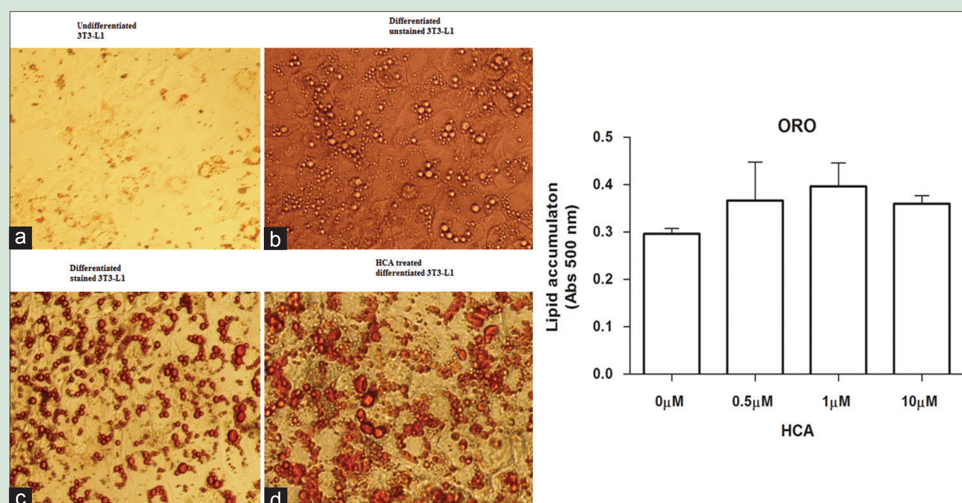

Figure 2: Effects of hydroxycitric acid calcium salt on differentiation and lipid accumulation in 3T3-L1 cells. Postconfluent preadipocytes were incubated in differentiation medium cocktail to induce differentiation with or without the various concentrations of hydroxycitric acid calcium salt. At day 8, cells were fixed and stained with oil red O and quantified by the absorbance at 500 nm. Data are represented as mean ± standard deviation; $n = 3$. (a) stained preadipocytes, (b) unstained, (c)differentiated adipocytes, (c) stained differentiated adipocytes, (d) HCA treated stained adipocytes

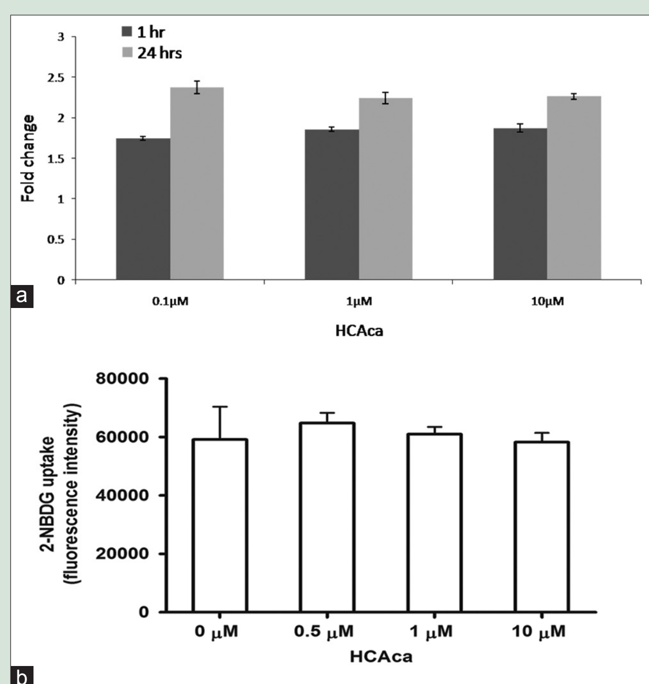

Figure 3: Effect of hydroxycitric acid calcium salt on glucose consumption and uptake in 3T3-L1 adipocytes. (a) Postdifferentiation, 3T3-L1 cells were starved in serum-free medium, then treated with glucose and different concentrations of hydroxycitric acid calcium salt. After 1 h and 24 h, concentration of glucose was examined. (b) Differentiated adipocytes were serum starved and treated with or without hydroxycitric acid calcium salt in various concentrations, and then glucose uptake was assayed. Data are represented as mean ± standard deviation; $n = 3$

analog 2-NBDG (40 nM) which was incubated with differentiated adipocytes with and without HCA at different concentrations. The intensity of the fluorescence was spectrofluorimetrically measured with excitation at 465 nm and emission at 540 nm wavelength which is proportional to the glucose uptake of the cells.

Peroxisome proliferator-activated receptor-γ and peroxisome proliferator-activated receptor-α protein quantification

The PPAR-α and PPAR-γ proteins expressed in 3T3-L1 cells were quantified using ELISA kits. Briefly, cells were allowed to differentiate in MDI cocktail media in the presence of HCA for 8 days. The cells were lysed using the lysis buffer and centrifuged. The supernatant was used for the PPAR-γ and PPAR-α quantification according to the manufacturer's instruction individually.

Reverse transcription polymerase chain reaction

The cells were incubated with the HCA during the period of differentiation. Total cellular RNA was prepared using the Trizol reagent (Gibco-BRL, Grand Island, NY, USA). Two micrograms total RNA was reverse-transcribed using Moloney murine leukemia virus reverse transcriptase and an antisense primer to generate cDNA under standard conditions. cDNA samples were amplified by polymerase chain reaction (PCR) in a AbiPrism 7500 Research Thermocycler, and the mRNA levels of PPAR-α, PPAR-γ, lipoprotein lipase (LPL), fatty acid synthase (FAS), fatty acid-binding protein (FABP), GLUT4, and adiponectin were quantified. The PCR primers used for amplification are shown in Table 1. The reaction consisted of 30 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 58°C, and elongation for 1 min at 72°C.

Molecular docking studies

To understand the binding interaction mechanism of HCA with PPARs Autodock v4.2, a molecular docking tool that predicts the interaction of ligands with biomacromolecular targets was used. Three-dimensional structure information on the target proteins was taken from the protein data bank (PDB ID: 1I7G-PPAR-α, 1KNU-PPAR-γ). The protocol followed for docking studies of virtual hits included processing of the protein and ligand preparation. To run Autodock, we used a searching grid extended over the selected amino acids in receptor protein; polar hydrogens and atomic solvation parameters were added. Small molecules HCA and the known agonists rosiglitazone and fenofibrate were docked to the receptor proteins

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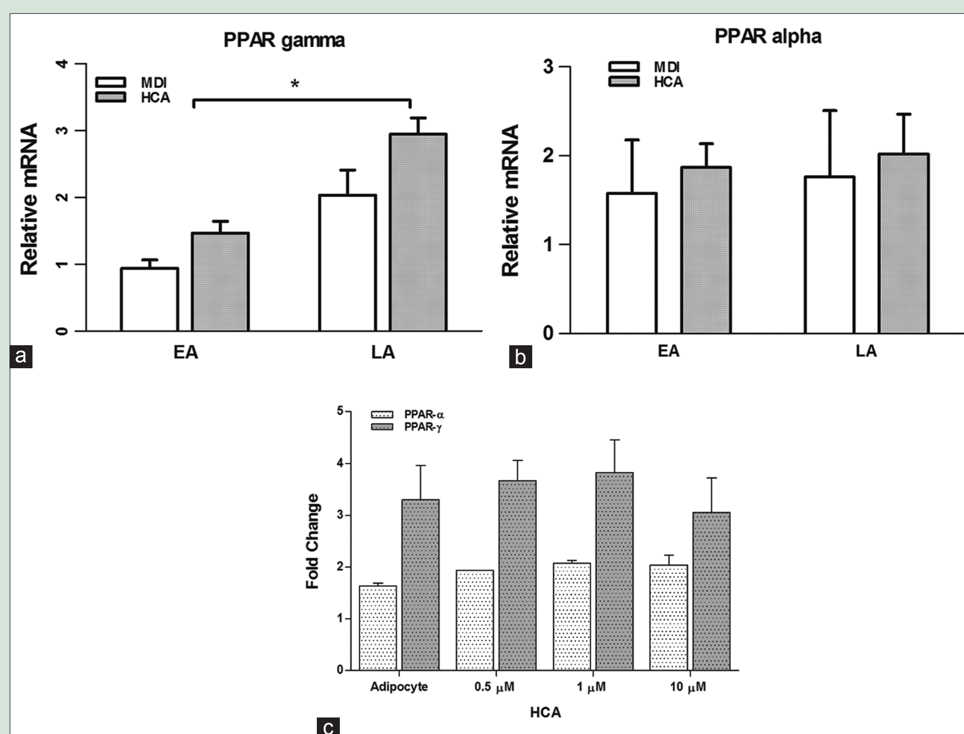

Figure 4: Effect of hydroxycitric acid calcium salt on expression of peroxisome proliferator-activated receptor-γ and peroxisome proliferator-activated receptor-α. At day 4 and day 8, total RNA was extracted and mRNA levels of peroxisome proliferator-activated receptors were assayed using real-time polymerase chain reaction. (a) Relative mRNA expression of peroxisome proliferator-activated receptor-γ, (b) relative mRNA expression of peroxisome proliferator-activated receptor-α in early and late stages of adipogenesis. (c) Protein expression of peroxisome proliferator-activated receptors was measured using ELISA kit. Postdifferentiated adipocytes were harvested and total cell lysate was subjected to quantify the protein expression. Data are represented as mean ± standard deviation; $n = 3$. EA: Early adipocyte, LA: Late adipocyte. *$P < 0.05$ compared to EA

PPAR-α and PPAR-γ, with the macromolecule considered as a rigid body and the ligands being flexible. The search was carried out with the Lamarckian Genetic Algorithm. Evaluation of the results was done by sorting the different complexes with respect to the predicted binding energy.

RESULTS

3T3-L1 cells are viable upon hydroxycitric acid treatment

To evaluate the effects of HCA [Figure 1a], on cell viability of 3T3-L1 cells, MTT assay was performed and the result in the form of histogram is shown in Figure 1b. Preadipocytes seeded in a 96-well plate and treated with HCA at different concentrations (0–100 μM) for 24 h revealed no cytotoxicity effect till 40 μM and showed less effect beyond 40 μM. Hence, the biological assays were carried out at a concentrations at or lesser than 40 μM.

Effect of hydroxycitric acid on adipocyte differentiation

To determine the effects of HCA on cell differentiation, preadipocytes were treated with MDI in the presence of various concentrations of HCA, and accumulation of lipid depots was visualized by ORO staining [Figure 2]. Treatment of preadipocytes with HCA increased the accumulation of intracellular lipid droplets. A lower concentration (0.5 μM and 1 μM) of HCA showed enhanced lipid depots and was dose responsive [Figure 2e] but was reduced at a concentration beyond 10 μM which may be a saturation concentration.

Effect of hydroxycitric acid on glucose consumption and uptake

Glucose consumption stimulatory effect of HCA examined in 3T3-L1 preadipocyte cells as well as in differentiated adipocyte by glucose oxidase method is represented in Figure 3a. In the preadipocytes, glucose consumption was maintained near-to-normal levels when compared to untreated preadipocytes at both the time periods (1 and 24 h), whereas in the differentiated adipocytes, glucose consumption of the medium was increased more than 2-fold as compared to the untreated adipocytes. Subsequently, the cellular glucose uptake determined by 2-NBDG method indicated that glucose uptake was moderately improved in the presence of HCA compared to the control [Figure 3b].

Effect of hydroxycitric acid on adipocyte-specific genes

Expression of adipocyte-specific genes is expressed in a sequential manner to bring adipogenesis. Hence, impact of HCA was investigated at two stages: early adipogenesis at day 3 and late adipogenesis at day 7 of induction. Our results show that during adipogenesis, the expression of PPAR-γ gene [Figure 4a] was increased to 1.4-fold and 2.9-fold in the early and late stage, respectively. However, PPAR-α gene expression [Figure 4b] exhibited minimum changes during the adipogenesis. To validate the changes in the PPAR gene expression, the protein levels of PPAR-γ and PPAR-α were analyzed by an ELISA kit wherein the levels of PPAR-γ protein were higher than the control groups. This indicates that HCA may improve adipogenesis by enhancing the expression of transcription factors essential for the cell differentiation [Figure 4c].

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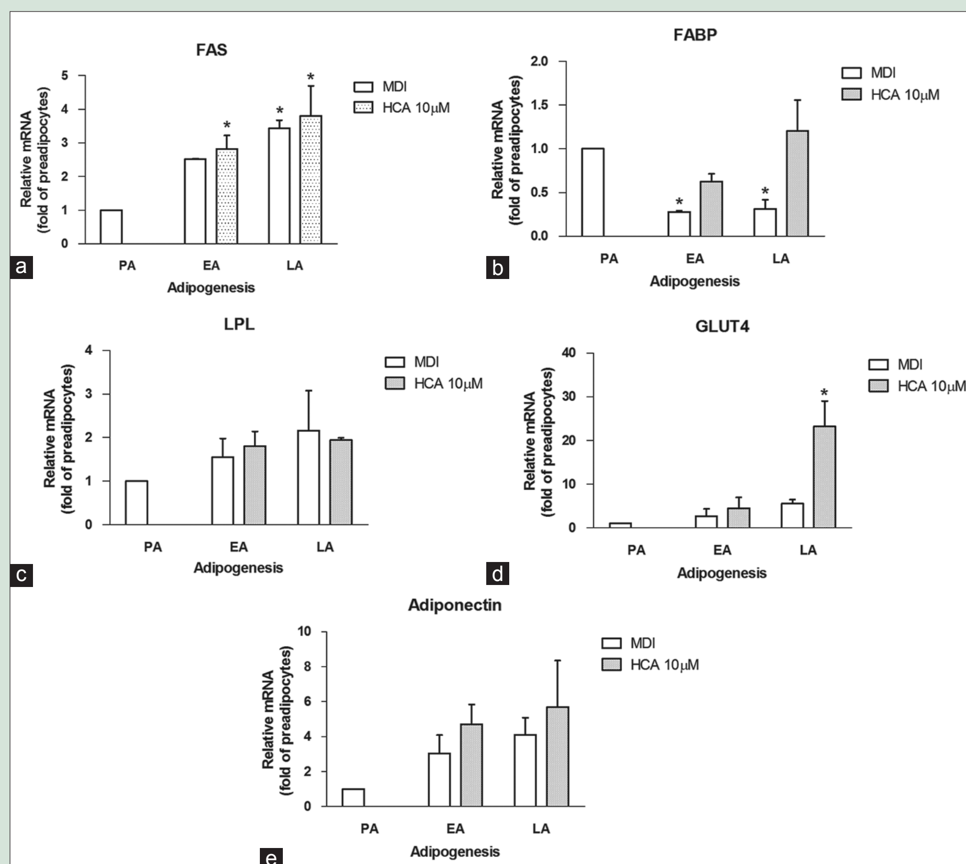

Figure 5: Effect of hydroxycitric acid calcium salt on mRNA expression levels of peroxisome proliferator-activated receptor-γ target genes in 3T3-L1 cells. The relative mRNA expression of peroxisome proliferator-activated receptor-γ target genes was measured in differentiated 3T3-L1 cells after hydroxycitric acid calcium salt treatment. RNA was extracted from differentiating adipocytes at day 4 and day 8 mRNA levels of peroxisome proliferator-activated receptor-γ target genes were measured using β-actin as a reference. Data are represented as mean ± standard deviation; $n = 3$. EA: Early adipocyte, LA: Late adipocyte. $^*P < 0.05$ compared to EA for FAS, $^*P < 0.05$ compared to HCA for FABP, $^*P < 0.05$ compared to EA for GLUT4

Likewise, expression of lipid and glucose metabolism genes, namely FAS, FABP, LPL, GLUT4, and adiponectin which are targets for PPAR-γ transactivation and markers of adipocyte differentiation, was studied to further assess the PPAR-γ-enhanced activity. Lipid metabolism markers analyzed such as FAS [Figure 5a], FABP [Figure 5b], and LPL [Figure 5c] showed noticeable changes in expression at the transcriptional level. Increase in mRNA levels of lipid-metabolizing genes when compared to normal control adipocytes indicates that adipogenesis and PPAR-γ activity were positively altered on HCA treatment. Furthermore, GLUT4 mRNA expression [Figure 5d] was also increased substantially during early (4.5-fold) and late (23.2-fold) adipogenesis stages, which substantiates it as a marker for adipocyte sensitization to insulin. Adiponectin, an adipocytokine produced by adipocytes, mRNA also substantially increased in early (4.7-fold) and late stages (5.6-fold) of differentiation of 3T3-L1 preadipocytes [Figure 5e].

Hydroxycitric acid is a partial agonist for peroxisome proliferator-activated receptors

With such an arrangement, it appears evident that the interaction between a plant-derived small molecule HCA and PPARs (α and γ) is tighter than a synthetic known PPAR agonist fenofibrate shown in Ligplot representation [Figure 6]. The binding sites of PPARs consist of a large T-shaped cavity flanked by helices 3, 5, 7, 11, and 12. The residue Ser280, Tyr314, and His440 belonging to helices 3, 5, and 11, respectively, form the coactivator binding site. The most active small molecule HCA has scored a good docking score value of – 4.99 [Figure 6a] and – 5.14 [Figure 6d] with PPAR-α and PPAR-γ, respectively. Specific agonists finofibrate [Figure 6b and 6e] and rosiglitazone [Figure 6c and 6f] for PPARα and PPARγ respectively were used for comparative study. Compared to fenofibrate and rosiglitazone, HCA showed better interaction in both PPAR-α and PPAR-γ ligand-binding domain (LBD). It has formed a hydrogen bond network with Cys276, Ser280, Tyr314, and His440 in PPAR-α; furthermore, it has hydrophobic interaction with Phe318, Leu321, Ile317, Val332 Val444, Leu247, and Met355, whereas in PPAR-γ, only two amino acids Tyr327 and Tyr473 were involved in hydrogen bonding. Known PPARs agonist fenofibrate did not score significant docking score compared to HCA. The binding interaction patterns observed during docking studies were complementary with that of *in vitro* results, which confirm that the developed molecular docking have good predictability.

DISCUSSION

The global prevalence of obesity and related metabolic disorders such as coronary heart disease, diabetes, hyperlipidemia, hypertension, and cancer is rising rampantly, increasing the burden on our healthcare.[16] The plethora of natural products and foods with the potential benefits for obesity continue to expand. Studies on various food sources exhibiting antiobesity effects have focused on the development of plant extracts or functional food which can suppress the accumulation of body fat.[14] As mentioned earlier, increased mass of adipose tissue due to a cellular

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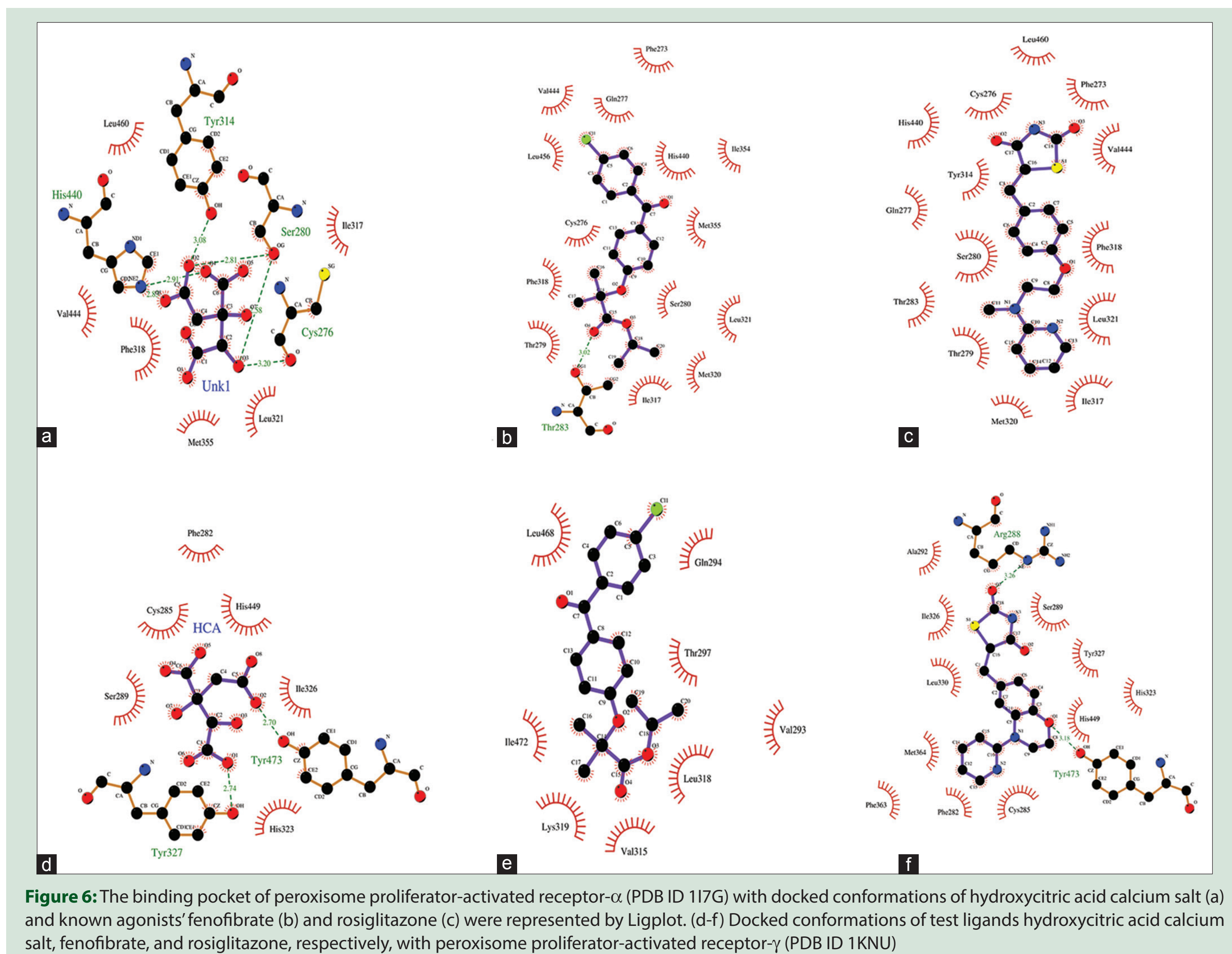

Figure 6: The binding pocket of peroxisome proliferator-activated receptor-α (PDB ID 1I7G) with docked conformations of hydroxycitric acid calcium salt (a) and known agonists' fenofibrate (b) and rosiglitazone (c) were represented by Ligplot. (d-f) Docked conformations of test ligands hydroxycitric acid calcium salt, fenofibrate, and rosiglitazone, respectively, with peroxisome proliferator-activated receptor-γ (PDB ID 1KNU)

process called adipogenesis leads to obesity. Targeting the adipose tissue, which exhibits both autocrine and endocrine functions, aids in maneuvering obesity-related complications. Moreover, one of the most active areas of research into potential strategies for the management of metabolic diseases is in the role of nuclear receptors as therapeutic targets for both glucose and lipid metabolism. The PPARs are ligand-activated transcription factors belonging to the nuclear receptor superfamily; as transcription factors, they are activators of key metabolic pathways that control fatty acid oxidation, adipocyte differentiation, and insulin sensitivity.[17] Herein, in the present study, the role of a specific plant-derived ligand HCA from *Garcinia* species was studied for its role on adipocyte differentiation. In the mammalian system, metabolically less active preadipocytes differentiate to metabolically active adipocytes which ultimately become a depot of lipid storage. It is interesting to note that treatment of preadipocytes with HCA increased the accumulation of intracellular lipid droplets as evidenced by ORO staining [Figure 2]. A lower concentration (0.5 μM and 1 μM) of HCA showed enhanced lipid depots and was dose responsive. The adipogenic potential of HCA may aid in minimizing the circulating lipids in the plasma, thereby decreasing the risk of coronary artery disease.

In the preadipocytes, glucose consumption was maintained to near-normal levels when compared to untreated preadipocytes at both the time periods (1 and 24 h), whereas in the differentiated adipocytes, glucose consumption of the medium was increased more than 2-fold as compared to the untreated adipocytes. However, the cellular glucose uptake as measured by the fluorescence of 2-NBDG was only moderately elevated on HCA administration.

PPAR-γ is indispensable for the proliferation, growth, and metabolism of the adipose tissue. However, the downstream targets that trigger adipogenesis *per se* are not known clearly. In differentiated cells and tissues, in addition to TNFα and leptin, two genes of fatty-acid metabolism, LPL and the FABP, appear to be direct targets of PPAR activation.[18] Likewise, the expression of GLUT4 is increased in cultured adipocytes and fat tissue through PPAR-γ activation by activators such as thializodones, which has a direct effect on insulin sensitivity.[19] In the present study, the mRNA expression of the genes of the fatty acid metabolism, namely FAS, LPL, and FABP,[4] was determined. When compared to normal control, adipocytes indicate that adipogenesis and PPAR-γ activity were positively altered on HCAca treatment which is more conspicuous in the late stage of adipogenesis. This is in conformation with an earlier report that PPAR-γ along with transcription factor CAAT/enhancer-binding protein (C/EBPα) furthers terminal differentiation of adipocytes by upregulating adipocyte-specific genes which are required for glucose and lipid metabolism.[1] Similarly, the GLUT4 mRNA expression was also increased substantially during early (4.5-fold) and late (23.2-fold) adipogenesis stages, substantiating

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its role in insulin sensitivity. Apart from adipocyte-specific gene expression, functional genes such as adiponectin were also investigated. Adiponectin is one of the many adipocytokines produced by the adipose tissue which is an important modulator of metabolic homeostasis. In 3T3-L1 adipocytes, adiponectin expression and secretion are increased during differentiation and also promote the differentiation of preadipocytes, lipid accumulation, and increased GLUT4 gene expression. Li *et al.* have shown that activation of PPAR-γ signaling in adipose tissue increased adiponectin production.[7] In the present study, treatment of 3T3-L1 cells with HCA upregulated the mRNA levels of adiponectin in both stages of differentiation. Likewise, PPAR-α target of fibrates exhibited only marginal variations in the gene expression in the adipocytes but not as PPAR-γ.

Many natural compounds have been reported to show PPAR-binding ability and act as an agonist or antagonist.[20] As HCA increased PPARγ mRNA and its target genes, in the present study, we tried to predict binding affinities between HCA and PPAR transcription factors (α and γ) through *in silico* molecular protein-ligand docking analysis. The ability of the nuclear receptors such as PPAR-α and -γ to promote or suppress the transcription of responsive genes depends on interaction of its LBD with ligands that stabilize receptor conformation.[21] HCA possesses eight hydrogen bond acceptors, five hydrogen bond donors, and seven freely rotating bonds,[22] which aid in the binding and probably aid in its biological action. Based on the interaction of HCA in the LBD, it can be considered that HCA elicits its action on the target genes (LPL, FAS, and FABP) in 3T3-L1 cells and activates GLUT4 similar to rosiglitazone and luteolin mediated by the PPARs.[23] In the future, natural compounds should be developed to replace these pharmaceutically efficient drug but toxic on long-term ingestion. HCA could be one such natural compound which needs further scientific scrutinization, especially on the aspect of toxicity and also its impact on other pathways and markers

CONCLUSION

Our study showed that HCA stimulated PPAR-γ mRNA level in both preadipocytes and differentiated adipocytes and enhanced mRNA levels of GLUT4 and lipid metabolism genes during early and late adipogenesis. In addition, our results indicate that HCA might have played a role in differentiation of 3T3-L1 preadipocytes which is evidenced by increase in lipid content in the adipocytes. Although HCA increased GLUT4, mRNA expression glucose uptake of adipocytes was not concomitantly improved and this issue warrants further studies. Moreover, due to the side effects of synthetic PPAR's agonists, identification of dual agonists of both PPAR-γ and PPAR-α receptors may be of great importance in the future. Further *in vivo* studies on HCA may throw light on the multiple actions of the molecule on the entire class of nuclear receptors.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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