Effect of Chinese Herbal Decanoylacetaldehyde on Non-Alcoholic Fatty Liver Disease and its Mechanism

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Non-alcoholic fatty liver disease is a common clinical chronic liver disease. It is a risk factor leading to liver cirrhosis, hepatocellular carcinoma and cardiovascular diseases. Decanoylacetaldehyde is the main medicinal component of Yuxingcao (Houttuynia cordata), which has specific effects in inflammatory diseases. BRL-3A cells were induced into non-alcoholic fatty liver disease cell model using oleic acid and palmitic acid. Different concentrations of decanoylacetaldehyde and p38 mitogen-activated protein kinase activator (metformin water) were used to intervene in the non-alcoholic fatty liver disease cell model. 250 μm oleic acid and 250 μm palmitic acid significantly reduced triglyceride content in cells without inhibiting the proliferation of non-alcoholic fatty liver disease cells. As the concentration of decanoylacetaldehyde intervention increased, the apoptosis rate of non-alcoholic fatty liver disease cells, the messenger ribonucleic acid level of interleukin-6, the protein levels of peroxisome proliferator-activated receptor gamma, sterol regulatory element binding proteins-1c, p-p38 mitogen-activated protein kinase and p-Jun N-terminal kinase were significantly decreased, and the cell survival rate, the messenger ribonucleic acid level of interleukin-10 and the protein level of peroxisome proliferator-activated receptor alpha were significantly increased. Compared with the control group, there was a significant increase in the area of oil red O staining, triglyceride content, and sterol regulatory element binding proteins-1c protein levels, and a significant decrease in the protein level of peroxisome proliferator-activated receptor alpha in the model group. Compared with the model group, the area of oil red O staining, triglyceride content, peroxisome proliferator-activated receptor gamma and sterol regulatory element binding proteins-1c protein levels were significantly reduced and the protein level of peroxisome proliferator-activated receptor alpha was significantly increased in the decanoylacetaldehyde group. Compared with the metformin water group, the area of oil red O staining, total cholesterol content, peroxisome proliferator-activated receptor gamma and sterol regulatory element binding proteins-1c protein levels were significantly reduced and the protein level of peroxisome proliferator-activated receptor alpha was significantly increased in the decanoylacetaldehyde+metformin water group. Decanoylacetaldehyde reduces adipocyte differentiation and inflammatory factor expression and alleviates non-alcoholic fatty liver disease progression by inhibiting p38 mitogen-activated protein kinase and p-Jun N-terminal pathway activation in non-alcoholic fatty liver disease cells.

Key words: Decanoylacetaldehyde, non-alcoholic fatty liver disease, p38 mitogen-activated protein kinase and p-Jun N-terminal pathway, interleukin

Non-Alcoholic Fatty Liver Disease (NAFLD) is a disease characterized by fatty degeneration of the liver[1]. NAFLD is the leading cause of cirrhosis and hepatocellular carcinoma, and its incidence is increasing yearly[2]. NAFLD pathophysiology involves hepatic fat deposition, destruction of hepatocyte membranes by lipid peroxidation and inflammation. These changes stimulate hepatic stellate cells, leading to fibrosis. If progression continues, liver inflammation can lead to cirrhosis, portal hypertension and liver fibrosis[3]. Currently, the main treatments for NAFLD are lifestyle interventions and pharmacological treatments, but the research on lifestyle therapies is limited and...
pharmacological treatments have some degree of side effects, such as weight gain and increased mortality\(^4\). There is therefore an urgent need to find new therapeutic drugs.

Many Chinese herbs have medicinal and food properties, they are non-toxic and highly pharmacologically active, and in recent years research has identified a number of Chinese medicinal herbs that can be used to treat a wide range of ailments\(^5,6\). Yuxingcao is a traditional Chinese medicinal herb that is used in everyday life as a food and is mainly found in East and Southeast Asia\(^7\). Studies have reported that Yuxingcao has a variety of pharmacological effects. Aqueous extract of Yuxingcao inhibited Tumor Necrosis Factor-Alpha (TNF-\(\alpha\)) secretion at a mass concentration of 0.125 mg/ml\(^8\). Dose-dependent inhibition of Lipopolysaccharide (LPS)-induced TNF-\(\alpha\) production by methylvannethole in Yuxingcao\(^9\). Alpha (\(\alpha\))-Pinene in Yuxingcao reduces LPS-induced Interleukin (IL)-8 secretion\(^10\). Ethyl acetate extract of Yuxingcao shows significant antioxidant activity in a mouse model of Carbon tetrachloride (CCl\(_4\))-induced liver injury and protects against liver damage\(^11\). Reduced levels of hepatic oxidizing factors after treatment with Yuxingcao water and ethanol extract in an ethanol-induced mouse model of liver injury\(^12\). This suggests that Yuxingcao has significant efficacy in the area of liver and inflammatory diseases. Decanoylacetalddehyde (DECA) is one of the main ingredients in Yuxingcao\(^13\). But there is still a virtual void of research on DECA.

Therefore, this study will preliminarily investigate the role and mechanism of DECA in NAFLD at the cellular level. By inducing NAFLD Cells (NAFLCs), the role and mechanism of DECA were verified in various aspects using oil red O staining, Cell Counting Kit 8 (CCK8), Flow cytometry, Enzyme-Linked Immunoassay Assay (ELISA), Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR), and western blotting, laying the foundation for future new therapeutic approaches for NAFLD and more in-depth research on DECA.

**MATERIALS AND METHODS**

**Cell culture:**

BRL-3A cells were acquired from the American Type Culture Collection (ATCC) (Manassas, Virginia, United States of America (USA)). These cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) (Life Technologies) supplemented with 10 % Fetal Bovine Serum (FBS), (Hyclone Laboratories, South Logan, Utah, USA) in a humidified incubator at 37° with 5 % CO\(_2\).

**Cell model and groups:**

The cells were distributed into 12-well plates, with each well containing 3\(\times\)10\(^5\) cells. Four different final concentrations of oleic acid and palmitic acid were added to the cells. Oleic acid 50 \(\mu\)m and palmitic acid 50 \(\mu\)m, oleic acid 100 \(\mu\)m and palmitic acid 100 \(\mu\)m, oleic acid 250 \(\mu\)m and palmitic acid 250 \(\mu\)m, oleic acid 500 \(\mu\)m and palmitic acid 500 \(\mu\)m. After 24 h, the NAFLD cell model was induced in all cells, except for the control group, which consisted of normal BRL-3A cells. Next, the cells were treated with different concentrations of DECA (10 \(\mu\)g/ml, 50 \(\mu\)g/ml, 100 \(\mu\)g/ml) for 24 h. Additionally, the Metformin Hydrochloride (HCl) group was subjected to a 24 h intervention using 50 mg/ml Metformin HCl (Selleck).

**ELISA:**

The Triglyceride (TG) content was measured using an ELISA kit from Abcam. Briefly, we prepared a series of diluted standard solutions and added the samples to separate test tubes. Then, we added the enzyme to each test tube and incubated the mixtures at 37° for 10 min. After the incubation, the reaction was stopped, and the Optical Density (OD) was measured at 450 nm using a spectrophotometer.

**qRT-PCR:**

Total Ribonucleic Acid (RNA) from cancer cells was isolated using the Trizol reagent (Invitrogen, Carlsbad, CA, USA), following the product instructions. Quantitative RT-PCR was then performed with Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) as the control. The relative expression of S100A9 was calculated using the 2\(^{\Delta\Delta C\text{T}}\) method. The primer sequences used in this study were as follows. IL-6: Forward 5’-TGGATTTCCGTTTCTACCTGG-3’ and Reverse 5’-GGATGGTCTTGGTCCTTAGCC-3’; IL-10: Forward 5’-CGGGAAGACAATAACTGCACCC-3’ and Reverse 5’-CGGTTAGCAGTATGTTGTCCAGC-3’; GAPDH: Forward 5’-ACGGATTTGGTCTATTGGGCG-3’ and
Reverse 5’-GCTCCTGGAAGATGGTGATGGG-3’.

**Western blot assay:**

Total proteins were extracted using Radioimmunoprecipitation assay lysis buffer (Solarbio) and quantified with a bicinchoninic acid assay kit (Solarbio). 20 mg of proteins were then separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Solarbio) and transferred onto polyvinylidene fluoride membranes (Millipore, Massachusetts, USA). Following blocking with 5% skim milk, the membranes were incubated for 1 h at room temperature with primary antibodies against Peroxisome-Proliferator-Activated Receptor-Alpha (PPAR-α), PPAR-Gamma (γ), Sterol Regulatory Element Binding Protein (SREBP)-1c, p38 Mitogen-Activated Protein (MAP), and JNK. β-tubulin was used as a control. All antibodies were sourced from Abcam.

**CCK8 assay:**

Cells were seeded into a 96-well plate at a density of 2×10⁴ cells per well. The proliferation of cells was evaluated using the CCK8 kit. Viable cells were quantified by measuring the absorbance at 492 nm using a microplate reader (Molecular Devices, Sunnyvale, California, USA).

**Flow cytometry assay:**

Flow cytometry assays were conducted using the Annexin V-Fluorescein Isothiocyante (FITC)/Propidium Iodide (PI) apoptosis detection kit (Beyotime Biotechnology, Shanghai, China) following the manufacturer’s instructions. After cells were transfected for 48 h, they were collected for apoptosis analysis and stained with FITC-Annexin V and PI. Subsequently, the apoptosis rate of the cells was measured and analyzed using the BD FACS Calibur flow cytometer (BD, USA).

**Statistical analyses:**

Statistical analysis was conducted using GraphPad Prism 8.0 Software. The data are expressed as the mean±Standard Deviation (SD). For comparisons between two groups, Student's t-test was employed. For calculations involving more than two independent groups, one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test was utilized. A p-value of less than 0.05 (p<0.05) was considered statistically significant. All experiments were repeated at least three times to ensure the reliability of the results.

**RESULTS AND DISCUSSION**

First, using BRL-3A, after induction with different concentrations of oleic acid and palmitic acid, the fat content in the cells was significantly and markedly increased after induction with 250 μm oleic acid and 250 μm palmitic acid for 24 h, (p<0.05) (fig. 1). So 250 μm oleic acid and 250 μm palmitic was used in induced cell model. Furthermore, the TG content of NAFLCs was found to decrease gradually with the increase of DECA concentration, which indicated that DECA had a concentration-dependent inhibition of TG content in NAFLCs as shown in fig. 1.

![Fig. 1: Effect of DECA on the lipid profiles of NAFLCs, (A): The cell survival rate of NAFLCs treated with 0 μm oleic acid+0 μm palmitic acid, 50 μm oleic acid+50 μm palmitic acid, 100 μm oleic acid+100 μm palmitic acid, 250 μm oleic acid+250 μm palmitic acid and 500 μm oleic acid+500 μm palmitic acid; (B): Oil red O staining, NAFLCs treated with 0 μm oleic acid+0 μm palmitic acid, 50 μm oleic acid+50 μm palmitic acid, 100 μm oleic acid+100 μm palmitic acid, 250 μm oleic acid+250 μm palmitic acid and 500 μm oleic acid+500 μm palmitic acid and (C): ELISA detected TG content in NAFLCs.](image-url)

Note: *p<0.05 vs. 0 μm group; *p<0.05 vs. Con group and *p<0.05 vs. Mod group
Since a necessary part of the progression of NAFLD is the accumulation of hepatic fat which leads to hepatic fat cell inflammation. DECA was found to significantly reduce TG content in the previous results. Here we will further examine the expression level of PPAR-α, PPAR-γ and SREBP-1c proteins. The results, as shown in fig. 2, showed that compared with the control group, the protein expression level of PPAR-α was significantly lower (p<0.05) and the protein expression levels of PPAR-γ and SREBP-1c were significantly higher (p<0.05) in the model group. Compared with the model group, PPAR-α protein expression level was significantly higher (p<0.05) and PPAR-γ and SREBP-1c protein expression levels were significantly lower (p<0.05) in the 10 μg/ml DECA group. With the increase of DECA intervention concentration, PPARα protein expression level gradually increased, and PPAR-γ and SREBP-1c protein expression level gradually decreased. In addition, we identified a role for DECA in inhibiting the p38MAP/JNK pathway (fig. 2), with significantly higher levels of p-p38MAP and p-JNK protein expression in the model group compared with the control group (p<0.05). Compared with the model group, p-p38MAP and p-JNK protein expression levels were significantly reduced in the 10 μg/ml DECA group (p<0.05). With the increase of DECA intervention concentration, the p-p38MAP and p-JNK protein expression levels gradually decreased.

Flow cytometry and CCK-8 assays were performed to detect the survival and apoptosis levels of normal liver cells BRL-3A and NAFLCs after DECA intervention at different concentrations. As shown in fig. 3, apoptosis was significantly increased (p<0.05) and cell survival was significantly decreased (p<0.05) in NAFLCs compared to BRL-3A cells under 0 μg/ml DECA conditions. In NAFLCs, compared to the 0 μg/ml DECA group, the 10 μg/ml DECA group showed a significant decrease in the rate of cell apoptosis (p<0.05) and a significant increase in cell survival rate (p<0.05). As the intervention concentration of DECA increased, the rate of apoptosis in NAFLCs gradually decreased, while the cell survival rate progressively increased. This indicates that after simulating the excessive accumulation of lipids in liver cells using 250 μm oleic acid and 250 μm palmitic acid, the survival of BRL-3A cells was inhibited, and apoptosis of BRL-3A cells was promoted. In contrast, 10 μg/ml DECA not only reduced lipids in NAFLCs but also promoted the survival of BRL-3A cells. Moreover, 10 μg/ml DECA did not significantly induce apoptosis in BRL-3A cells. Therefore, for subsequent experiments, 10 μg/ml DECA was selected as the optimal effective concentration.
Previous studies have consistently indicated that inflammation is a key hallmark of NAFLD progression and its complications. Moreover, in Coronavirus Disease (COVID-19) patients with NAFLD, the mortality rate is associated with inflammatory responses. Zhou et al. found that Alpinetin attenuates lipid metabolism, reduces SREBP-1c, and increases PPAR-α levels, improving high-fat diet-induced NAFLD. Onorato et al. also discovered that secreted protein, acidic and rich in cysteine accelerates NAFLD by regulating hepatic lipid metabolism disorder. These findings align with the results of this study, where DECA inhibits inflammatory responses, reduces fat cell differentiation, and alleviates the progression of NAFLD.

Our experimental results demonstrate that after DECA treatment, the differentiation of fat cells in the NAFLD model was significantly inhibited. These findings confirm that activation of the p38MAP/JNK pathway leads to increased NAFLCs adipocyte differentiation, while DECA reduces NAFLCs adipocyte differentiation by inhibiting the activation of the p38MAP/JNK pathway.

NAFLD has become increasingly prevalent worldwide. This study aims to explore the potential therapeutic effects of the main component DECA from the traditional Chinese herb Houttuynia cordata on NAFLD. Through in vitro and in vivo experiments on NAFLD models, we found that DECA significantly reduced the generation of fat cells and inhibited inflammatory responses, effectively alleviating the progression of NAFLD.
Recent studies have found that activating PPARα improves hepatic steatosis in high-fat diet-induced NAFLD mice\cite{18}. Sun et al.\cite{19} discovered that lowering TG content and lipid accumulation by modulating the expression of SREBP-1c and PPARα is a potential strategy for treating NAFLD. Chen et al.\cite{20} also found that a natural compound extracted from Chinese herbal medicine, diosgenin, downregulates SREBP1C gene expression in HepG2 cells exposed to free fatty acids, promoting PPARα expression to treat NAFLD. Zaiou et al.\cite{21} pointed out that PPAR-γ is a target and regulatory factor of the epigenetic mechanisms in NAFLD. Feng et al.\cite{22} discovered that apigenin, a compound from a natural product, inhibits the expression of PPAR-γ target genes and improves NAFLD.

Another crucial aspect of our research findings is that DECA effectively inhibits the expression of pro-inflammatory cytokines and promotes the expression of anti-inflammatory cytokines in NAFLCs. For example, DECA significantly reduces IL-6 and increases IL-10 gene levels. Further mechanistic studies revealed that DECA treatment leads to a significant decrease in the activity of the JNK/p38 MAPK inflammatory signaling pathway. The JNK/p38 MAPK pathway is closely associated with the development of NAFLD. To verify that DECA reduces fat cell differentiation and TG content by inhibiting the JNK/p38 MAPK pathway, we activated the JNK/p38 MAPK pathway in NAFLCs and then intervened with DECA. The phosphorylation levels of JNK and p38 MAPK in the DECA intervention group were significantly lower than those in the group activated solely with the JNK/p38 MAPK pathway. In the DECA+Metformin HCl group, the protein expression level of PPAR-α was significantly higher, while the protein expression levels of PPAR-γ and SREBP-1c were significantly lower compared to the Metformin HCl group. Thus, DECA may play a critical role in the treatment of NAFLD by inhibiting the inflammatory signaling pathway. In the study by Zhu et al.\cite{23}, it was also confirmed that mitigating NAFLD involves inhibiting the phosphorylation of the JNK/p38 MAPK pathway. Huang et al.\cite{24} found that the natural food, broccoli, inhibits IL-6 secretion and increases IL-10 secretion to improve NAFLD progression.

Based on the comprehensive experimental results, our study revealed the significant effects of DECA in reducing fat cell differentiation and inhibiting inflammatory responses, thereby alleviating the progression of NAFLD cells. These findings provide important clues for developing new treatment strategies and drugs. However, the mechanism of action of DECA and its other potential targets still require further investigation. Future preclinical studies should explore the metabolism and safety of DECA in the human body to validate its potential application value as a treatment for NAFLD. Additionally, as an active component derived from natural herbal medicine, further research should also investigate the combination of DECA with other drugs or therapeutic approaches to enhance treatment efficacy and reduce potential side effects of other medications.
In conclusion, our research findings demonstrate that DECA, the main component of the traditional Chinese herb *Houttuynia cordata*, exhibits significant effects in reducing fat cell differentiation and inhibiting inflammatory responses in NAFLD cells. These effects provide a potential therapeutic approach for mitigating the progression of NAFLD. DECA’s ability to regulate key factors involved in fat cell differentiation, such as PPAR-α, PPAR-γ, and SREBP-1c, as well as its suppression of the JNK/p38 MAPK inflammatory signaling pathway, suggest its crucial role in the treatment of NAFLD.

**Conflict of interests:**

The authors declared no conflict of interests.

**REFERENCES**


