Gao Z. Y et al. (2023) EXAMINING THE SYSTEMIC IMPACTS OF GLYCYRRHETINIC ACID: OXIDATIVE STRESS AND INFLAMMATORY RESPONSES IN LO2 CELLS AND THEIR POTENTIAL INFLUENCE ON PHYSICAL FITNESS AND MENTAL HEALTH. Revista Internacional de Medicina y Ciencias de la Actividad Física y el Deporte vol. 23 (93) pp. 446-462. **DOI:** https://doi.org/10.15366/rimcafd2023.93.029

ORIGINAL

EXAMINING THE SYSTEMIC IMPACTS OF GLYCYRRHETINIC ACID: OXIDATIVE STRESS AND INFLAMMATORY RESPONSES IN LO2 CELLS AND THEIR POTENTIAL INFLUENCE ON PHYSICAL FITNESS AND MENTAL HEALTH

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Recibido 24 de agosto de 2022 **Received** August 24, 2022
Aceptado 29 de octubre de 2023 **Accepted** October 29, 2023

ABSTRACT

Glycyrrhetinic Acid (GA) is renowned for its anti-inflammatory and antioxidative properties, particularly in treating various liver diseases. However, its effects on normal hepatocytes, like human LO2 cells, are not well-documented. This study aimed to explore these effects and extrapolate potential systemic impacts on physical fitness and mental health. In this research, LO2 cells were exposed to varying concentrations of GA. We assessed cell viability, levels of reactive oxygen species (ROS), superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA). Additionally, whole-transcriptome sequencing (RNA-Seq) was employed to identify changes in gene expression post-GA treatment. The results showed that GA significantly inhibited LO2 cell viability and led to increased intracellular ROS and MDA levels, alongside decreased SOD and GSH contents. Post-treatment, pro-inflammatory cytokines (TNF-α, IL-1, and IL-6) were notably increased. A total of 2856 differentially expressed genes were identified in the GA-treated group compared to controls, predominantly enriched in inflammation-related gene ontology (GO) terms and pathways, with the NF-κB pathway being notably activated at both mRNA and protein levels. These findings indicate that GA induces oxidative stress and inflammatory responses in LO2 cells through the activation of the NF-κB pathway. Given the critical role of oxidative stress and inflammation in systemic health, these cellular-level changes might have broader implications. Specifically, they could

influence physical fitness and mental health, considering the interconnectedness of liver function with overall physiological and psychological wellbeing. This study lays the groundwork for further exploration into how GA's effects on hepatocytes could translate into systemic health outcomes, emphasizing the need for a holistic understanding of its impacts beyond liver-specific applications.

KEYWORDS: glycyrrhetinic acid; oxidative stress; inflammatory responses; NF-κB pathway; LO2 cells

1. INTRODUCTION

Glycyrrhetinic Acid (GA), a key constituent of licorice root, has long been recognized for its anti-inflammatory and antioxidative properties, primarily in the context of liver diseases(Heidari, Mehri, & Hosseinzadeh, 2021). Its therapeutic applications, derived from traditional medicine, have been substantiated through various modern pharmacological studies(F. Wu et al., 2018; Zhao, Ding, Cao, & Cao, 2012). However, the exploration of GA's effects has predominantly been confined to pathological states, leaving a gap in our understanding of its impact on normal cellular function, particularly in hepatocytes(F. Wu et al., 2018; Zhao, Ding, Cao, & Cao, 2012). This study aims to bridge this knowledge gap by investigating the effects of GA on normal human hepatocytes, using LO2 cells as a model. LO2 cells, a line of normal human hepatocytes, provide an ideal platform for examining the cellular-level impacts of compounds like GA in a controlled environment(Markov et al., 2020; Markov, Sen'kova, Zenkova, & Logashenko, 2018; Quan et al., 2021). The liver's central role in metabolic processes, detoxification, and immune regulation makes it a critical organ for overall health and well-being. (Doan, Truong, & Nguyen, 2021; Y. L. Li, Zhu, Liang, Orvig, & Chen, 2021). Therefore, understanding how GA interacts with hepatocytes is crucial, as these interactions can have far-reaching implications beyond the liver itself(Doan, Truong, & Nguyen, 2021; Y. L. Li, Zhu, Liang, Orvig, & Chen, 2021).

Recent scientific inquiry has broadened to consider how liver health and function can influence systemic conditions, including physical fitness and mental health. Physical fitness, a key determinant of overall health, is closely linked to liver function due to the liver's role in energy metabolism, muscle function, and inflammation regulation(Hasan et al., 2015; X. Li, Sun, & Liu, 2019; F. Wu et al., 2018). Similarly, mental health is increasingly being understood in the context of systemic inflammation and oxidative stress, areas where liver function plays a significant role(Asrani, Devarbhavi, Eaton, & Kamath, 2019). This research, therefore, extends beyond the cellular effects of GA, hypothesizing that the oxidative stress and inflammatory responses induced by GA in hepatocytes could have systemic implications(Robinson, Harmon, & O'Farrelly, 2016; R. Wang et al., 2021). (Y. He et al., 2021). These implications

might manifest as changes in physical fitness and mental health, given the liver's interconnectedness with these aspects of health(Gatmaitan, Werner-Gibbings, Sallam, Bell, & Gkoutzios, 2020; C. Y. Wang, Kao, Lo, & Yen, 2011). By employing whole-transcriptome sequencing (RNA-Seq) technology and various biochemical assays(B. Gao, Ahmad, Nagy, & Tsukamoto, 2019; Koyama & Brenner, 2017; Wree, Holtmann, Inzaugarat, & Feldstein, 2019), the study aims to provide a comprehensive analysis of the cellular changes induced by GA in LO2 cells and to discuss the potential systemic impacts of these changes(B. Gao, Ahmad, Nagy, & Tsukamoto, 2019; Koyama & Brenner, 2017; Wree, Holtmann, Inzaugarat, & Feldstein, 2019). Although some studies reveal many protective effects of GA in various cell lines or mouse models, the detailed mechanism of GA's impact on everyday human hepatocytes still needs to be clarified. Most previous studies pay more attention to the beneficial aspects of GA(F. Wu et al., 2018). Side effects-related reports are limited. Herein, the purpose of our research is to explore the impact of GA on inflammatory reactions in normal human hepatocytes via transcriptome sequencing (RNA-Seq). Finally, our results suggest that GA induces oxidative stress and inflammatory responses in LO2 cells via activating NF-κB pathway. Our findings provide more insights into understanding the effects of GA on human liver cells(Z. Y. He et al., 2010; F. Wu et al., 2018). (BILECENOGLU & ÇELIK, 2021).

2. Materials and methods

2.1. Preparation for Glycyrrhizic acid, Glycyrrhetinic acid, and Liquiritin

Glycyrrhizic acid, GA, and liquiritin were purchased from sigma, respectively.

2.2. Culture of LO2 cell line

Our study was conducted in human hepatocyte cell line L02, purchased from the Chinese Academy of Sciences (Shanghai, China). LO2 cell line was cultured in DMEM medium, supplemented with Penicillin-Streptomycin Solution, in a 37 ℃, 5%CO_2 incubator.

2.3. Cell viability detecting

To evaluate the impacts of glycyrrhizic acid, GA, and liquiritin on LO2 cell line, cell viability was tested using Cell Counting Kit-8 (CCK-8,).

2.4. Detection of intracellular reactive oxygen species (ROS) and superoxide dismutase (SOD)

The intracellular ROS level of LO2 cells was determined using 2,7-dichlorofluorescein diacetate (DCFH-DA; Solarbio, D6470). The LO2 cells were cultured in 6-well plates and supplemented with 10 μM DCFH-DA, which were

incubated for 20 mins at 37 ℃. Then the cells were washed with serum-free medium for three times. The cells were treated with 0, 25, 50, and 100 μM GA. After treatments, the cells were collected, which were then detected with a fluorescence microplate reader. Additionally, the total contents of SOD in LO2 cells were also evaluated using the Total Superoxide Dismutase Assay Kit with WST-8 (Beyotime, S0101). LO2 cells treated with 0, 25, 50, and 100 μM GA were washed with PBS, and the sample preparation liquid was added (100-200 μL/ 1×10^6 cells). The WST-8 reaction system (160 μL) was prepared by mixing 151 μL SOD detection buffer, 8 μL WST-8, and 1 μL enzyme solution. Then SOD detection buffer, WST-8 reaction system, and reaction-priming liquid were added in the samples, which were together incubated for 30 mins at 37 ℃. The SOD contents in LO2 cells were then determined at 450 nm by using a microplate reader.

2.5. Determination of glutathione (GSH) and malondialdehyde (MDA)

The intracellular GSH contents in LO2 cells were determined utilizing GSH and GSSG Assay Kit (S0053, Beyotime, Shanghai, China). After treatments with 0, 25, 50, and 100 μM GA, LO2 cells were firstly washed with PBS. Then cells were harvested after centrifuging, and a triple amount of M solution was added. The sample solution undergone fully vortex, freezing-thawing, and centrifuging, the GSH contents of which were then detected according to the manufacturer's instructions. Moreover, the level of MDA in LO2 cells was detected using Lipid Peroxidation MDA Assay Kit (S0131S, Beyotime, Shanghai, China). LO2 cells with 0, 25, 50, and 100 μM GA treatments were then prepared as cell homogenate samples. MDA contents were determined according to the manufacturer's instructions.

2.6. RNA-Seq and bioinformatics analysis

LO2 cells were cultured in 6-well plates (1×10^6 cells per well), then cells were treated with 100 μM GA. Next, the cells were washed with PBS and collected for subsequent total RNA extraction. Total RNA extraction was conducted according to the instructions. After quality control, RNA was used to build cDNA libraries. The libraries were then sequenced on Agilent SurePrint G3 Human Gene Expression v3 8x60K Microarray. The differentially expressed genes (DEGs) were identified between control cells and GA-treated cells using Feature Extraction. Those DEGs with FDR (false discovery rate) of $p < 0.05$ and |FC (fold-change) | ≥2 were screened. The selected DEGs were then subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. GO terms and KEGG pathways with $p < 0.05$ were taken as significantly enriched ones.

2.7. Analysis of GA's impact on NF-κB pathway in LO2 cells

The protein expression levels of NF-κB p105/p50, NF-κB p100/p52,

TRAF1 (TNF receptor-associated factor 1), TRAF3 (TNF receptor-associated factor 3), CyclinD1, Survivin, MMP9 (matrix metallopeptidase 9), CXCL8 (C-X-C motif chemokine ligand 8), and TRIM25 (tripartite motif containing 25) were detected using western blot. After treatments with 0, 25, 50, and 100 μM GA, the above proteins in LO2 cells were determined using previous standard methods. Detailed reagents information is listed in Table 1.

Table 1: Detailed reagents used in western blot.

PROTEINS	PRIMARY ANTIBODY	SECONDARY ANTIBODY
NF-KB P105/P50	NF-κB p105/p50 (CST, #3035, 1:1200)	Goat anti-rabbit IgG-HRP (Bioss, bs-0295G-HRP, 1:4000)
NF-KB P100/P52	NF-κB p100/p52 (CST, #4882, 1:1500)	Goat anti-rabbit IgG-HRP (Bioss, bs-0295G-HRP, 1:4000)
TRAF1	TRAF1 (Abcam, ab155268, 1:1200)	Goat anti-rabbit IgG-HRP (Bioss, bs-0295G-HRP, 1:4000)
TRAF3	TRAF3 (Abcam, ab155298, 1:800)	Goat anti-rabbit IgG-HRP (Bioss, bs-0295G-HRP, 1:4000)
CYCLIND1	CyclinD1 (Abcam, ab226977, 1:1000)	Goat anti-rabbit IgG-HRP (Bioss, bs-0295G-HRP, 1:4000)
SURVIVIN	Survivin (Abcam, ab76424, 1:1500)	Goat anti-rabbit IgG-HRP (Bioss, bs-0295G-HRP, 1:4000)
MMP9	MMP9 (Abcam, ab58803, 1:1500)	Goat anti-mouse IgG-HRP (Bioss, bs-0296G-HRP, 1:4000)
CXCL8	CXCL8 (Abcam, ab18672, 1:2000)	Goat anti-rabbit IgG-HRP (Bioss, bs-0295G-HRP, 1:4000)
TRIM25	TRIM25 (Abcam, ab16754, 1:2000)	Goat anti-rabbit IgG-HRP (Bioss, bs-0295G-HRP, 1:4000)
GAPDH	GAPDH (Abcam, ab9485, 1:2000)	Goat anti-rabbit IgG-HRP (Bioss, bs-0295G-HRP, 1:4000)

2.8. Statistical analysis

Our experiments were conducted more than three times with similar results, and the final data obtained were expressed as the mean standard deviation (SD). GraphPad Prism Version 7 software (GraphPad, San Diego, CA, USA) was used for analysis. Statistical differences between various groups were detected by t-tests and one-way analysis of variance (ANOVA). Differences with p-values ≤0.05 were considered statistically significant.

3. Results

3.1. Glycyrrhizic acid, Glycyrrhetinic acid, and Liquiritin's toxic effects on cell viability

Firstly, the toxic effects of glycyrrhizic acid, GA, and liquiritin on LO2 cell

viability have been preliminarily evaluated. The chemical structure of glycyrrhizic acid, GA, and liquiritin were displayed in Fig 1A. Among these, low concentrations of glycyrrhizic acid briefly promoted the LO2 cell growth, and more than 250μM glycyrrhizic acid inhibited the viability (Fig 1B). GA had inhibited the LO2 cell viability, and the cell viability significantly reduced with the concentration increasing (IC50 = 65.3 μM) (Fig 1C). Moreover, with the liquiritin treatment, cell viability decreased with the concentration increasing (Fig 1D). Under the microscope, LO2 cells with different doses of GA treatments indicated that living cells decreased with the GA concentration increasing (Fig 1E). Therefore, our study then mainly explored the impact of GA treatment in LO2 cells.

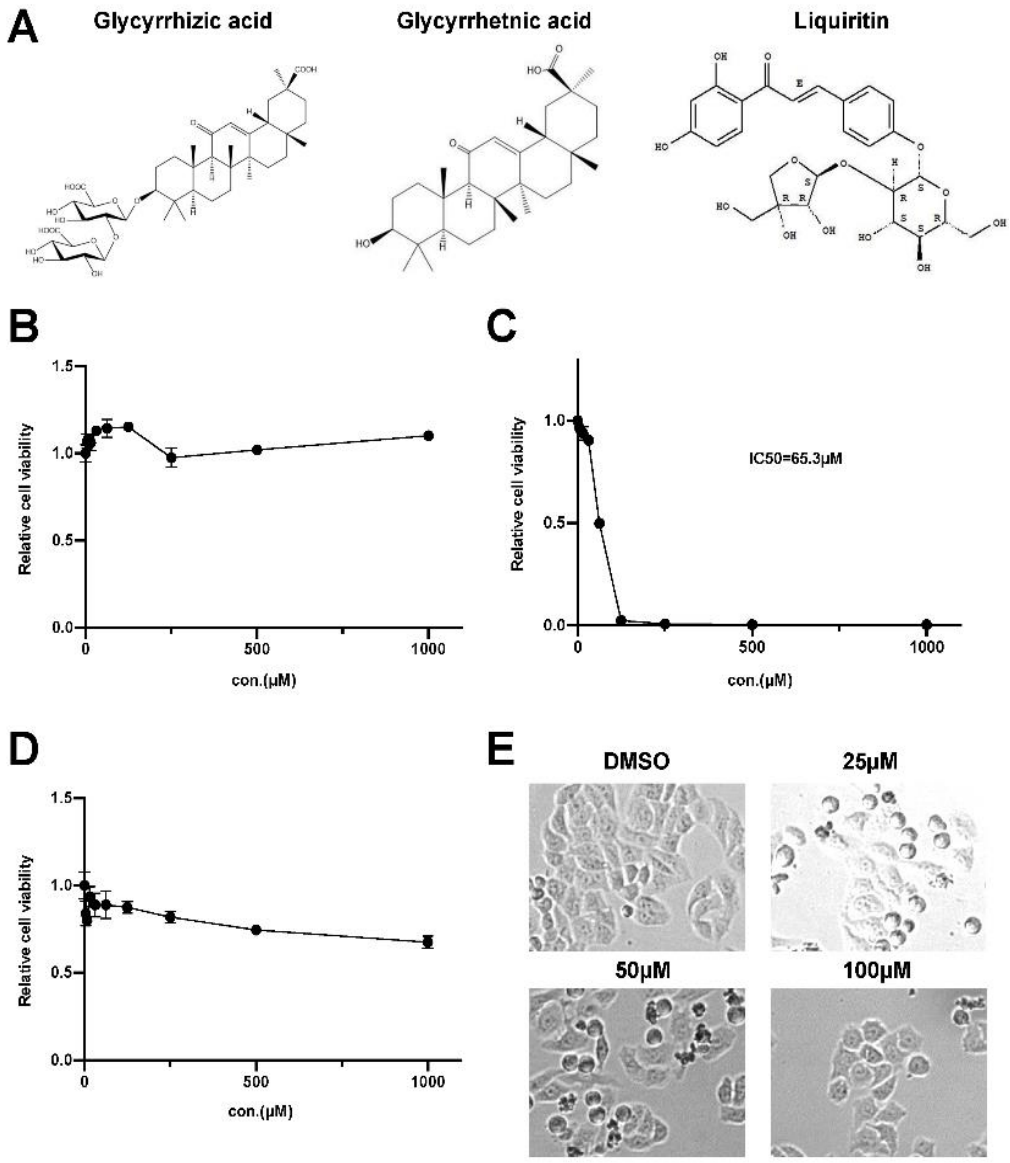

Figure 1: Glycyrrhizic acid, Glycyrrhetinic acid, and Liquiritin' s toxic effects on cell viability. (A) The chemical structure of glycyrrhizic acid, GA, and liquiritin. (B-D) The toxic effects of glycyrrhizic acid, GA, and liquiritin on LO2 cells, separately. (E) The impacts of 25 μM, 50 μM, 100 μM GA treatments on LO2 cells.

3.2. Impact of Glycyrrhetinic acid on ROS and SOD in LO2 cells

The ROS and SOD levels in LO2 cells with 25, 50, and 100 μM GA treatments were then detected separately. Our results indicated that all three doses of GA stimulated the increase in intracellular ROS levels (Fig 2A). Additionally, SOD changes were also detected in LO2 cells with 25, 50, and 100 μM GA treatments, suggesting that three doses of GA showed different impacts on intracellular SOD activity (Fig 2B). We found that 25 μM GA stimulated the increase of SOD activity, while 50 μM and 100 μM GA inhibited the SOD activity in LO2 cells.

3.3. Impact of Glycyrrhetinic acid on GSH and MDA contents in LO2 cells

Next, the GSH and MDA contents in LO2 cells with 25, 50, and 100 μM GA treatments were also detected. Compared with the DMSO group, the GSH levels were all decreased in three doses of GA treatment groups. Besides, the decreasing tendency of GSH content was observed with the increasing doses of GA (Fig 2C). On the contrary, three doses of GA significantly stimulated the increasing of MDA levels in LO2 cells (Fig 2D). And a dose-dependent increase of MDA content was observed in LO2 cells with 25, 50, and 100 μM GA treatments.

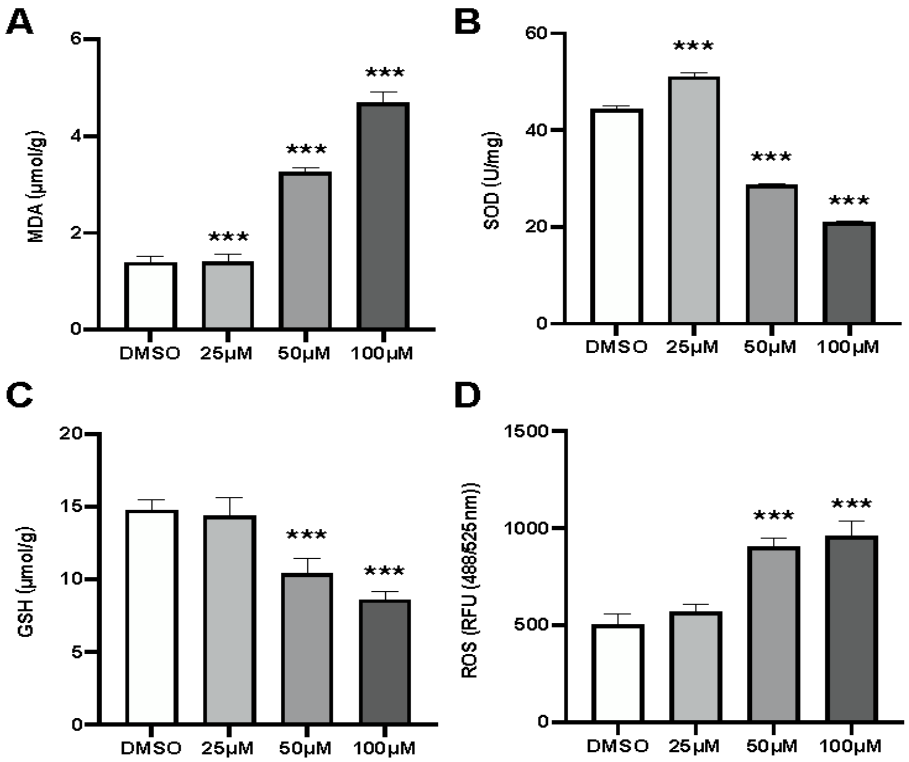

Figure 2: Impacts of different doses of GA on MDA, SOD, GSH, and ROS in LO2 cells. (A) The 25, 50, 100 μM GA treatments' impacts on MDA. (B) The 25, 50, 100 μM GA treatments' impacts on SOD. (C) The 25, 50, 100 μM GA treatments' impacts on GSH. (D) The 25, 50, 100 μM GA treatments' impacts on ROS. *** P <0.001.

3.4. Transcriptomic analysis of the effects of Glycyrrhetinic acid on LO2 cells

Furthermore, we have also explored the effects of GA treatment on LO2 cells, utilizing the transcriptomic analysis. Compared with the control group, we identified 2856 differentially expressed genes (DEGs) in the GA treatment group, including 1351 upregulated genes and 1505 downregulated genes (Fig 3A). All DEGs' expression levels were significantly different between the control group and treatment group (Fig 3B). Our findings implied that these DEGs were probably associated with the influence of GA on LO2 cells. Subsequently, we performed the functional enrichment analysis to get more practical information on these DEGs and GA's impacts on LO2 cells. These 2856 DEGs were significantly enriched in 236 GO terms, comprising 27 CC terms, 162 BP terms, and 47 MF terms. Among them, the top 30 significant GO terms were shown in Fig 3C. Besides, 2856 DEGs were significantly enriched in 54 KEGG pathways, the top 30 of which were shown in Fig 3D.

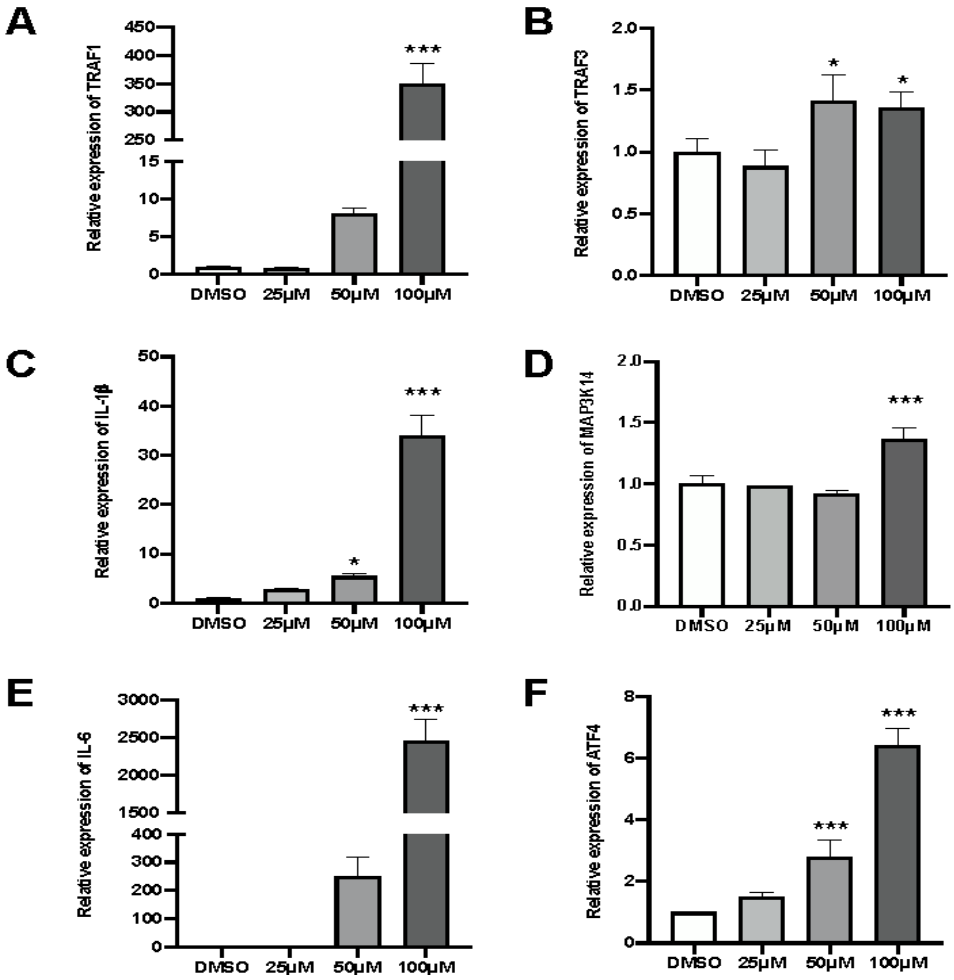

Figure 3: GA treatments' effects on crucial targets of NF-κB pathway. (A) Relative expression of TRAF1. (B) Relative expression of TRAF3. (C) Relative expression of IL-1β. (D) Relative expression of MAP3K14. (E) Relative expression of IL-6. (F) Relative expression of ATF4. * $p < 0.05$, *** $p < 0.001$.

3.5. Effects of Glycyrrhetinic acid treatment on NF-κB pathway in LO2 cells

The mRNA expression levels of four critical genes in NF-κB pathway were determined by qRT-PCR. Our results showed that GA treatments promoted the expression of TRAF1 and NF-κB2 in LO2 cells (Fig 4A-4B), and the expression levels of TRAF1 and NF-κB2 increased gradually along with the dose increase of GA. Besides, in LO2 cells treated with different doses of GA, the expressions of IL-1β and CXCL8 were significantly elevated compared with the control group (Fig 4C-4D). On the other hand, some crucial NF-κB pathway downstream proteins in LO2 cells with GA treatments were measured using western blot. Along with the doses of GA increasing, these proteins' expression levels were elevated, including NF-κB p105/p50, NF-κB p100/p52, TRAF1, TRAF3, CyclinD1, Survivin, MMP9, CXCL8, and TRIM25. Our data indicated that GA treatments probably activated NF-κB pathway in LO2 cells.

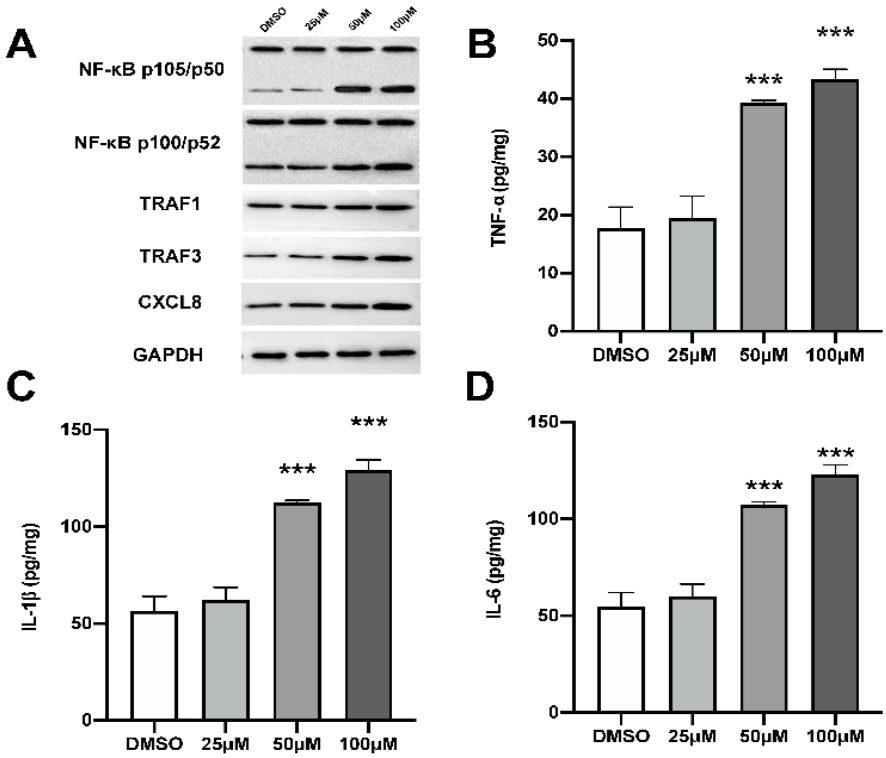

Figure 4: Effects of Glycyrrhetinic acid treatment on pro-inflammatory cytokines' expression in LO2 cells. (A) Effects of GA treatments on NF-κB pathway target proteins. (B-D) The 25, 50, 100 μM GA treatments' impacts on TNF-α, IL-1β, and IL-6 expression in LO2 cells, respectively.

3.6. Effects of Glycyrrhetinic acid treatment on pro-inflammatory cytokines' expression in LO2 cells

The protein expression levels of three critical pro-inflammatory cytokines

in LO2 cells with 25, 50, and 100 μM GA treatments were determined. Our results showed that three concentrations of GA all stimulated the increase of pro-inflammatory cytokines' protein expression levels, including TNF-α, IL-1, and IL-6 (Fig 5A-5C). Additionally, 50 μM and 100 μM GA treatments significantly promoted the expressions of TNF-α, IL-1, and IL-6 (Fig 5A-5C).

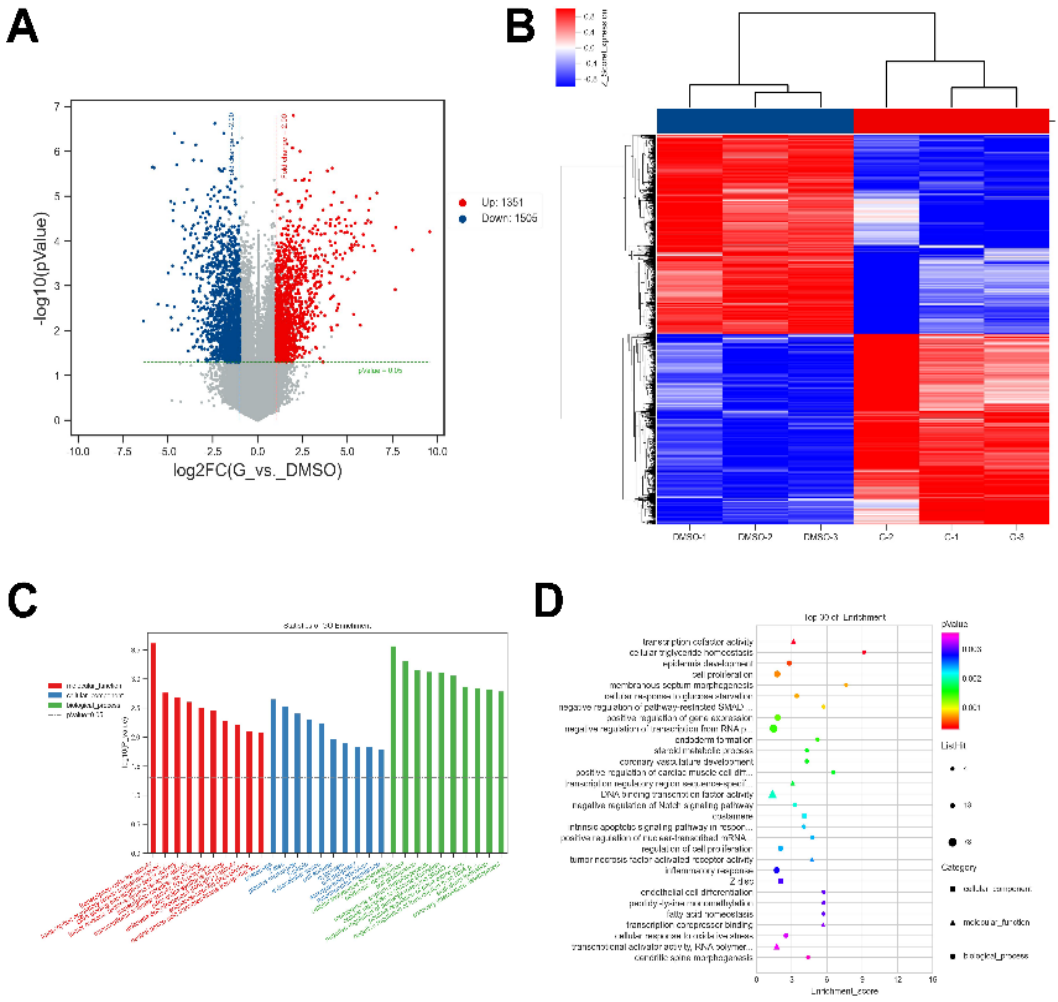

Figure 5: Transcriptomic analysis of the effects of Glycyrrhetinic acid on LO2 cells. (A-B) The differentially expressed genes between GA treatment group and control group. (C) Top 30 significant GO terms. (D) Top 30 significant KEGG pathways.

4. Discussion

In the development of liver diseases, the cell death of hepatocytes is a pivotal event as its inflammation might contribute to fibrosis (Shojaie, Iorga, & Dara, 2020). GA exhibits hepatoprotective properties in many liver injuries, such as non-alcoholic fatty liver disease (Shi et al., 2020), ischemia/reperfusion injury (Jiang et al., 2019), liver cancer (Stecanella et al., 2021), and so on. However, the effect of GA has been seldom studied in normal hepatocytes. In our study, LO2 cells were treated with 0, 25, 50, and 100 μM GA, respectively, which

suggested that cell viability was significantly inhibited after GA exposure. Moreover, GA treatments significantly elevated the intracellular ROS and MDA levels and decreased SOD and GSH contents, implying the harmful effects of GA on LO2 cells.

The redox-regulated processes in hepatocytes influence the function of the liver, including iron homeostasis, detoxification processes, and so on (Mello, Zanieri, Ceni, & Galli, 2016). Oxidative stress refers to an imbalance between free radicals and antioxidant capacity (Ma et al., 2021; Valko et al., 2007). The protective effects of GA in gastric cancer (GC) have been demonstrated that GA suppressed the formation of ROS and finally indirectly inhibited the invasion of GC cells (Cai, Chen, Zhang, & Wang, 2018). However, in another recent study, it has been evidenced that GA could elevate ROS levels, decrease GSH contents and induce oxidative/nitrative stress, subsequently triggering ferroptosis in triple-negative breast cancer cells (Wen et al., 2021). In hepatocytes, mitochondrial damage might lead to excessive ROS, which then activates mitochondrial permeability transition causing pyroptosis or ferroptosis (Shojaie et al., 2020), whereas the more detailed role of GA in hepatocytes still needs to be clarified. GA could alleviate skin lesions in a mouse model by inducing cell apoptosis via increasing ROS levels (J. Gao et al., 2020). The effects of GA in various cells or models seem different. In our work, additional doses of GA increased the ROS formation in LO2 cells, which is consistent with some previous reports. Our data indicated that oxidative stress was an essential part of the toxic effects of GA.

The elevated ROS would then lead to lipid peroxidation and MDA accumulation (Jelic, Mandic, Maricic, & Srdjenovic, 2021), which could also be observed in our study. Additionally, GSH plays a crucial role in detoxifying intracellular ROS via collaborating with other antioxidant enzymes (G. Wu, Fang, Yang, Lupton, & Turner, 2004). Meanwhile, the potential toxicity of ROS is also controlled by SOD (Y. Wang, Branicky, Noe, & Hekimi, 2018). Accordingly, reduced levels of GSH and SOD further confirmed that GA induced oxidative stress in LO2 cells. Furthermore, the impacts of GA treatment on three key pro-inflammatory cytokines were also investigated in LO2 cells, including TNFα, IL-1, and IL-6, which indicated that GA significantly promoted the expressions of these cytokines. Although the effects of GA have been widely investigated in varying from cells to models, the results might come to conflicting conclusions. Yan et al. have reported that in mice and LO2 cells, GL rather than GA could attenuate acetaminophen-induced liver injury through suppressing TNFα-induced apoptosis (Yan et al., 2016). In rat primary hepatocytes, GA might attenuate NF-κB activation to protect liver against inflammation (H. J. Chen, Kang, Lee, & Lin, 2014). The results were not that compatible with ours, which might result from the time and dose of GA administration. Additionally, the effects of GA treatment were also evaluated using RNA-seq technology. We found that 2856 DEGs between the control and

GA treatment groups were significantly enriched in several oxidative stress and inflammation-related GO terms and KEGG pathways, such as NF-κB signaling pathway, TNF signaling pathway, MAPK signaling pathway, and so on. Among these, the NF-κB signaling pathway has been reported as a crucial pathway in various functions of GA (Chang, Chen, Kuo, Chen, & You, 2010; X. Chen et al., 2018; Su et al., 2018).

Our data also evidenced that GA treatment activated the NF-κB signaling pathway from mRNA and protein levels. NF-κB is an essential mediator of inflammatory processes, regulating the survival and activation of inflammatory cells and multiple immune cells (Barnabei, Laplantine, Mbongo, Rieux-Laucat, & Weil, 2021; Liu, Zhang, Joo, & Sun, 2017). Intracellular ROS could also interact with NF-κB signaling pathway (Morgan & Liu, 2011). The complex interactions between them might also help to illustrate the activation of NF-κB signaling pathway in LO2 cells with GA treatment. GA treatment induced oxidative stress and inflammatory responses in LO2 cells by activating the NF-κB pathway. Moreover, the functional enrichment results gave us more inspirations about the role of GA in LO2 cells. MAPK signaling pathway were usually activated or inhibited together with NF-κB signaling pathway during the GA administration (B. Li et al., 2017), and this synergistic effect could also be found in our study. In short, the impacts of GA on oxidative stress and inflammatory responses were preliminarily explored in this study, while more details still deserve more exploration.

5. Conclusions

The findings from this study provide a comprehensive insight into the cellular impacts of Glycyrrhetinic Acid (GA) on normal human hepatocytes, specifically LO2 cells. Our results demonstrate that GA induces significant oxidative stress and inflammatory responses, evidenced by increased intracellular reactive oxygen species (ROS), malondialdehyde (MDA) levels, and pro-inflammatory cytokines, along with decreased superoxide dismutase (SOD) and glutathione (GSH) levels. The activation of the NF-κB pathway further substantiates the role of GA in these cellular processes.

While the study primarily focuses on the cellular level, the implications of these findings are far-reaching. The liver plays a crucial role in overall physiological homeostasis, and disturbances in liver cell function can have systemic effects. The oxidative stress and inflammatory responses triggered by GA in LO2 cells may potentially influence systemic health, particularly in the realms of physical fitness and mental health. This is due to the intricate interplay between liver health, systemic inflammation, oxidative stress, and overall well-being. Physical fitness and mental health are closely interconnected with the body's inflammatory and oxidative status. For instance, increased systemic inflammation and oxidative stress can adversely affect muscle function and

endurance, impacting physical fitness. Similarly, chronic inflammation is often linked to various mental health issues, including mood disorders and cognitive impairments. Thus, while our study is grounded in cellular-level observations, it opens up avenues for further research into how substances like GA, known for their liver-targeted effects, might influence broader aspects of health. It underscores the need for a holistic view when considering the application of such compounds, especially in therapeutic contexts.

In conclusion, this research highlights the complex nature of GA's impact on liver cells and suggests potential systemic implications that warrant further exploration. Understanding the broader effects of GA, particularly on physical fitness and mental health, could be crucial for developing more comprehensive and effective treatment strategies for conditions involving oxidative stress and inflammation.

Acknowledgments

Financial supports of the National Key Research and Development Program, the National Natural Science Foundation.

Funding

This work is supported by grants from the National Key Research and Development Program of PR China (No.2019YFC1604900) and the National Natural Science Foundation of PR China (No.81901577).

Conflicts of Interest

The authors declare no conflict of interest.

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