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Synergistic anti-inflammatory effects of resveratrol and vitamin E in lipopolysaccharide-induced RAW264.7 cells

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Abstract

To determine the synergistic anti-inflammatory effects of resveratrol (RES) combined with vitamin E (VE) and its mechanism. Lipopolysaccharide (LPS)-induced RAW264.7 cells were used to determine the effect of resveratrol, vitamin E and their combination on the production of cellular inflammatory mediators, including NO, IL-6, TNF-α, IL-1β, TLR4 and p-NF-κBp65. The synergistic anti-inflammatory effect of the combination was evaluated by ELISA, and the effect of the combination on the TLR4, p-NF-κBp65 and p-IκBα pathway by Western blot. The results showed that resveratrol combined with vitamin E synergistically inhibited the production of NO, LDH, MPO and inflammatory factors IL-6, TNF-α, IL-1β and TLR4 by LPS-stimulated macrophages, and effectively inhibited the expression of TLR4, p-NF-κBp65 (P<0.01). Resveratrol combined with vitamin E have a synergistic anti-inflammatory effect. They can inhibit the expression of inflammatory mediators and further suppress the activation of TLR4, p-NF-κBp65 and p-IκBα signaling pathway in LPS-induced RAW264.7 cell, which might provide a new effective way for inflammation treatment.

Keywords: resveratrol; vitamin E; synergistic anti-inflammatory; RAW264.7 cells; NF-κ.

Practical Application: Resveratrol combined with vitamin E (3.13 μg/mL RES + 3.13 μg/mL VE) have a great synergistic anti-inflammatory effect in LPS-induced RAW264.7 cell, which provided a new effective way for the application of resveratrol and VE as natural anti-inflammatory ingredients in health food or adjuvant medicine in the future.

1 Introduction

Inflammation is an adaptive response triggered by noxious stimuli and conditions such as infection and tissue injury (Karboua et al., 2021). It has extensively been demonstrated that strong and complex interconnections occur between oxidative stress and the inflammatory response (Miguel et al., 2021). The plant active ingredients have been recently shown to effectively modulate the inflammatory response, which were considered "health promoting" effects on cellular biomolecules (Anusmitha et al., 2022). In addition, knowledge of the interaction of these molecules with enzymes, receptors, and transcription factors has recently emerged (Afonso et al., 2020; Reuter et al., 2010). Many natural bioactive substances have been proven to have excellent anti-inflammatory effects and are used as potential anti-inflammatory agents in treatment (Liu et al., 2021; Kim et al., 2022). Therefore, it is necessary to develop natural and effective anti-inflammation ingredients, and provide a new perspective for solving human anti-inflammation problems.

Resveratrol (trans-3, 5, 40 trihydroxystilbene, RES, Figure 1A) is a phenolic phytoalexin mainly found in grapes, plums, cranberries, and peanuts (Jen et al., 2021). It has been shown that RES had anti-inflammatory, antiseptic, anti-oxidative and anti-aging activities (Malaguarnera, 2019). Vitamin E (VE, Figure 1B) is the term for the four tocopherols (α-, β-, γ-, δ-tocopherol) and naturally produced only by plants, especially in fruits, vegetables and nuts (Abraham et al., 2019). It is a necessary fat-soluble vitamin, as an excellent antioxidant and nutritional agent, is widely used in food, health care products, cosmetics and medicine and other industries (Papada & Kaliora, 2019). VE has also been shown to have excellent anti-inflammatory properties. Studies have shown that VE or A-tocopherol can effectively cut down the content of reactive oxygen species in monocytes and reduce the production of pro-inflammatory cytokines such as TNF-α, IL-6, IL-1β and TLR4 (Lee & Han, 2018; Lan-Fang et al., 2022). Inflammation is a complex multifactorial defense response of the body to stimuli. In the course of treatment, a single drug may not have the best therapeutic effect on inflammation, and increasing the dosage of drugs alone may have the risk of toxic harm. More and more studies have found that synergistic effects can be achieved by combining drugs with different molecular targets (Upadhyay & Dixit, 2015). Synergistic intervention between different anti-inflammatory drugs can enhance the anti-inflammatory efficacy

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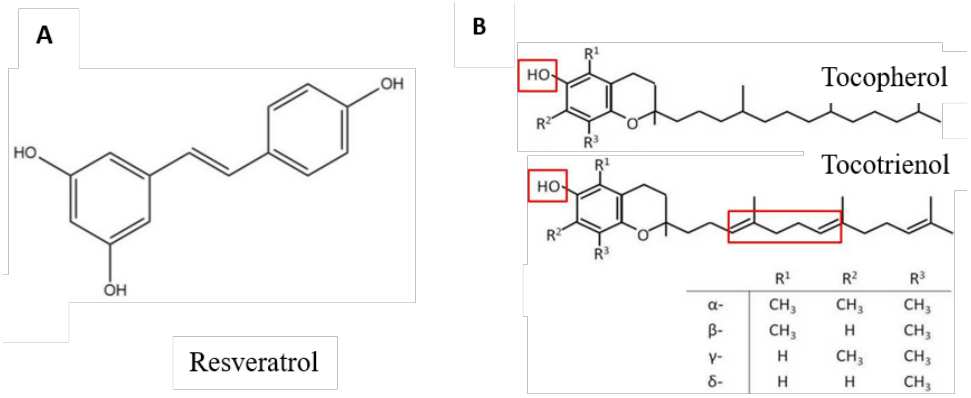

Figure 1. Chemical structure of RES (A) and VE (B).

of the body, helping to reduce the effective dose of each anti-inflammatory drug and minimize the potential toxicity and/or side effects of large doses (Nathan & Ding, 2010). RES and VE, as active substances in food sources, are safe and have no side effects, and both have certain anti-inflammatory effects, suggesting that they may have synergistic or overlapping effects in preventing inflammation. However, the synergistic anti-inflammatory effects of RES and VE remain unclear.

The purpose of this study was to determine the potential synergistic effect of RES and VE combination in inhibiting pro-inflammatory responses in LPS-induced RAW 264.7 cells. The cell proliferation, NO release, activity of inflammatory enzymes and expression of apoptosis-related proteins were evaluated under different doses of RES and VE. This may provide a theoretical basis for further elucidating the combination of various functional components in the diet in the treatment of inflammation.

2 Materials and methods

2.1 Chemicals and reagents

Resveratrol (RES, white powder, molecular weight 228.24, purity ≥ 98%) was purchased from Solarbio Co., Ltd. (Beijing, China). Vitamin E (VE, Light yellow to light brown liquid, molecular weight 430.71, purity degree (Type V, approx. 1000 IU/g) was purchased from Solarbio Co., Ltd. (Beijing, China). Polyclonal antibodies specific to TLR4, p-NF-κBp65, p-IκBα were bought from Santa Cruz Biotechnology (Dallas, Texas, United States).

2.2 Cell culture

RAW264.7 cells were cultured in Dulbecco's modified Eagle medium (DMEM; Solarbio, Beijing, China) supplemented with 10% fetal bovine serum (Gibco, USA), 100U/ml penicillin (Solarbio, Beijing, China), 100 μg/mL streptomycin (Solarbio, Beijing, China), and 2 mM glutamine, at 37 °C in a humidified incubator (5% CO_2, 95% air), respectively.

2.3 Experimental group

The RAW264.7 cells were randomly divided into seven groups: Normal control, CK; Inflammatory model group, LPS; Dexamethasone group, DEX; VE group, VE; RES group, RES; Low concentration synergistic group, L(R + V); High concentration synergistic group, H(R + V).

2.4 LPS-induced inflammatory cell model

RAW264.7 cells were treated with 0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20 and 40 μg/mL of LPS (Sigma, USA). The pretreated cells were then incubated in different concentrations of LPS for 24 h. After the experiment, cells were collected and analyzed for cell viability, cell morphology and lactate dehydrogenase (LDH kit; Jiancheng, Nanjing, China).

2.5 Cell viability assay

Briefly, the macrophages in logarithmic phase were seeds at a density of 9×10^4 cells/mL in 96-cell plates and cultured overnight. Then, the cells were respectively treated with 0, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100 and 200 μg/mL of RES and VE for a 24 h-incubation. Inject 10 μL CCK8 solution into each well. After 4 h, cells were collected using the CCK-8 kit (Beyotime, Shanghai, China) according to the manufacturer's agreement. Different stages of absorption were recorded at 450 nm. In addition, the effect of RES and VE on cell viability under LPS (1 μg/mL) stimulation were determined using the same method described above.

2.6 Measurements of NO release

As previously described, the NO production in cell culture supernatant was determined. The cells were seeded in 24-well plates at a density of 2×10^5 cells/mL. RAW264.7 cells were fused to 80% and treated with (1 μg/mL) LPS for 1 h, followed by RES and VE for 24 h. The NO production was determined using Griess reagent assay (Jiancheng, Nanjing, China) and absorbance was detected at 540 nm.

2.7 Determination of inflammatory related enzyme activity

After arriving at a density of 2×10^5 cells/mL, RAW264.7 cells were trypsinized and added into 24-well plates for overnight. After simulated by LPS (1 μg/mL), RES and VE were added and incubated for 24 h. Then, the myeloperoxidase (MPO; Jiancheng, Nanjing, China), LDH and Glutathione (GSH; Jiancheng,

Nanjing, China) activity in the LPS-stimulated RAW264.7 cells were measured by colorimetric method according to the manufacturer's specifications.

2.8 Measurement of the concentrations of cytokines

RAW264.7 cells were inoculated in 24-cell culture dishes and cultured overnight. Lps-stimulated cells (1 μg/mL) were treated with RES and VE, as previously described. According to the manufacturer's protocol, cell culture supernatants were collected and the concentrations of TNF-α, IL-6 and IL-1β were determined using an ELISA kit (Boster, Wuhan, China). The concentrations of cytokines and chemokines were measured at 450 nm using a microporous plate.

2.9 RNA isolation and qRT-PCR

The mRNA expressions of TNF-α, IL-6, IL-1β, TLR4 and NF-κB were detected by QRT-PCR (Singh et al., 2020). Total RNA was extracted with Trizol reagent (Invitrogen, USA) according to manufacturer's protocols. RNA (1 μg) was back-transcribed using the Reverse Transcription System Kit (Promega, USA). The reaction was placed in duplicates in 20 μL containing 10 μL of SYBR ® Premix Ex TaqII, 0.4 μL of ROX Reference Dye, 0.8 μL of each primer, 1 μL of cDNA template and 6 μL of H_2O. Each sample was tested three times. Primers are shown in Table 1.

2.10 Western blotting

Western blot analysis: After the experiment, total protein was extracted with protein extraction kit (Solarbio, Beijing, China), and protein concentration was determined with BCA quantitative kit (Solarbio, Beijing, China). The protein sample (100 μg) was transferred to a polyvinylidene fluoride (PVDF) membrane by 5%-12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis. After being blocked with 5% BSA for 1h, primary antibodies (TNF-α, IL-6, IL-1β, TLR4, NF-κB and GAPDH) were incubated overnight at 4 °C, and then secondary antibodies conjugated with horseradish peroxidase were incubated at temperature for 2 h. The proteins were detected by chemiluminescence detection kit and quantified by Gel-Pro-Analyzer 4.0 software (Eleawa et al., 2014).

2.11 Statistical analysis

All experiments were conducted in triplicates. All data were presented as means ± SD. Statistical analysis of comparisons between two groups was performed using Student's t-test, while one-way analysis of variance (ANOVA) with Duncan's multiple range test was used in the analysis of two or more groups (GraphPad Prism 9.0, USA). Statistical significance was assessed at a $P < 0.05$.

Table 1. qRT-PCR primer sequence used in this study.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
β-actin	AACTTTGGCATTGTGGAAGG	ACACATTGGGGGTAGGAACA
TNF-α	GCCACCACGCTCTTCTGTCT	TGAGGGTCTGGGCCATAGAAC
IL-6	ACA ACCACGGCCTTCCCTAC	CATTTCCACGATTTCCCAGA
IL-1β	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
TLR4	TTCAGAGCCGTTGGTGTATC	CTCCCATTCCAGGTAGGTGT
NF-κB	TCTCCTTCTGGCTCAGTGTCTTC	CACCACGGTGATCCACCTCT

3 Results

3.1 Establishment of inflammatory cell model

Effects of LPS on the proliferation of RAW264.7 cells

To construct an in vitro model of inflammation, RAW264.7 cells were stimulated with LPS at various concentrations to determine the most effective dose to induce an underlying inflammatory response, and the activity of LDH was tested in the RAW264.7 cells with different concentration and different time of LPS-stimulated. CCK-8 assay was used to measure cell viability, and cell morphology was observed by microscope as shown in Figure 2, The cells showed a good viability at the concentration of LPS (0.1-5 μg/mL). And the activity of LDH also significantly increased with the increase of LPS stimulation (concentration from 0.5-40 μg/mL and incubating time from 12-48 h). So we selected a dose of 1 μg/mL and a 24 h-incubation for further experiment. The morphology of LPS-stimulated RAW264.7 cells was observed furtherly. The cells in the control group had normal morphology structure, with round and good growth (Figure 3a). In contrast, after LPS (1 μg/mL) stimulation for 24 h, RAW264.7 cells exhibited activated macrophage characteristics, such as polygon and blurred contour (Figure 3b). All the above results indicated that the inflammatory model by LPS-stimulated (1 μg/mL, 24h) on RAW264.7 cells was successful.

3.2 Effect of RES and VE on cell viability

The potential cytotoxicity of RES and VE were evaluated in RAW264.7 cells after incubating 24 h with or without LPS (1 μg/mL) by CCK8. The results indicated that both the levels of RES (≤ 6.25 μg/mL) and VE (≤ 12.5 μg/mL) had no cytotoxicity in RAW264.7 cells without LPS (Figure 4). Then, we assessed the effect of RES and VE on cell viability in LPS-induced RAW264.7 cells. As the results shown in Figure 5, no toxicity were observed in the LPS-stimulated RAW264.7 cells after 24 h treatment with RES (≤ 6.25 μg/mL), VE (≤ 25 μg/mL) and the synergistic RES and VE (≤ 6.25 μg/mL), respectively. Based on these results, RES and VE of 3.13 μg/mL and the synergistic RES and VE of 0.78 μg/mL, 3.13 μg/mL were selected as the subsequent experiments to check the synergistic anti-inflammatory effects of resveratrol and vitamin E in LPS-induced RAW264.7 Cells.

3.3 RES and VE synergistically inhabit NO production in LPS-induced RAW 264.7 cells

Using the established non-toxic dose range, we tested the inhibitory effect of RES, VE and their combination on NO level in LPS-induced RAW 264.7 cells (Luo et al., 2022a). The Griess reagent method was used as we described previously.

The results were showed in Figure 6, the NO content of LPS group was significantly increased by contrast with CK group ($P < 0.01$), which indicated that LPS-induced RAW264.7 Cells were inflammatory. Compared with LPS group, VE caused a significant inhibition on LPS-induced NO production by 45% at

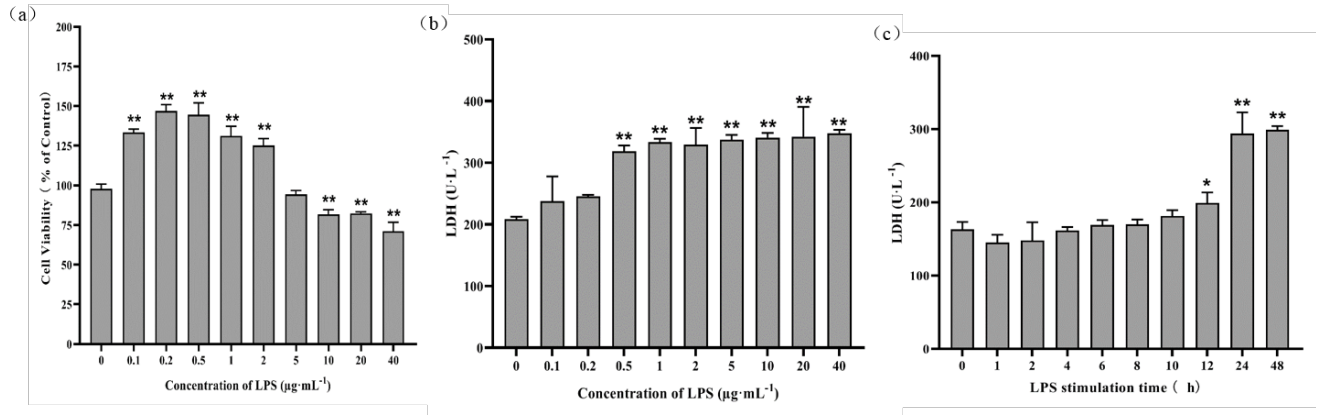

Figure 2. Effect of LPS-stimulated on RAW264.7 cells. (a) Effect of different concentrations of LPS on cells' viability; (b) Effect of different concentrations of LPS on the LDH activity; (c) Effect of LPS-induced at different times on the LDH activity. **P* < 0.05, relative to control group; ***P* < 0.01, relative to control group.

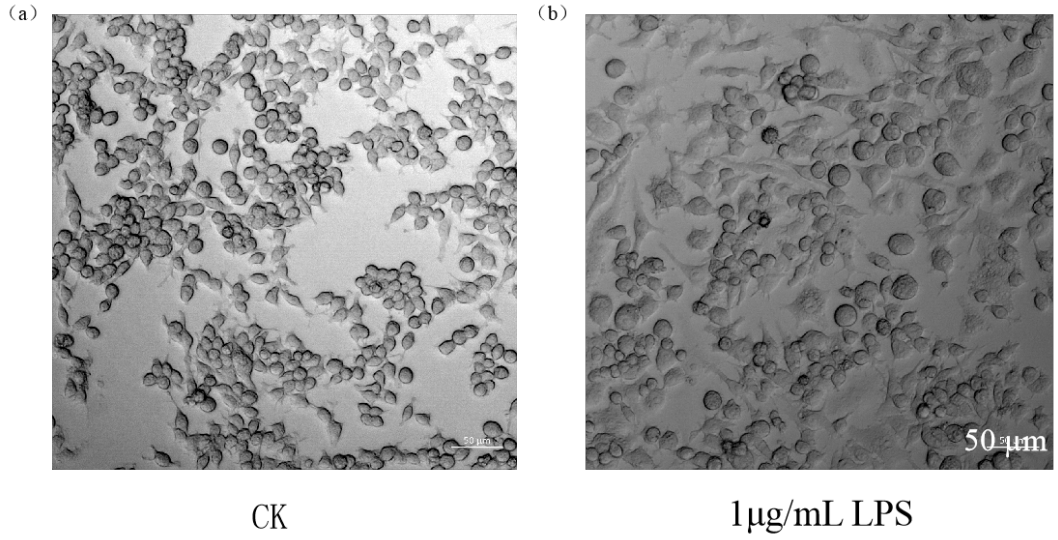

Figure 3. Comparison of cell morphology after LPS-stimulated for 24 h. (a) Control cells; (b) LPS 1 μg/mL treated cells.

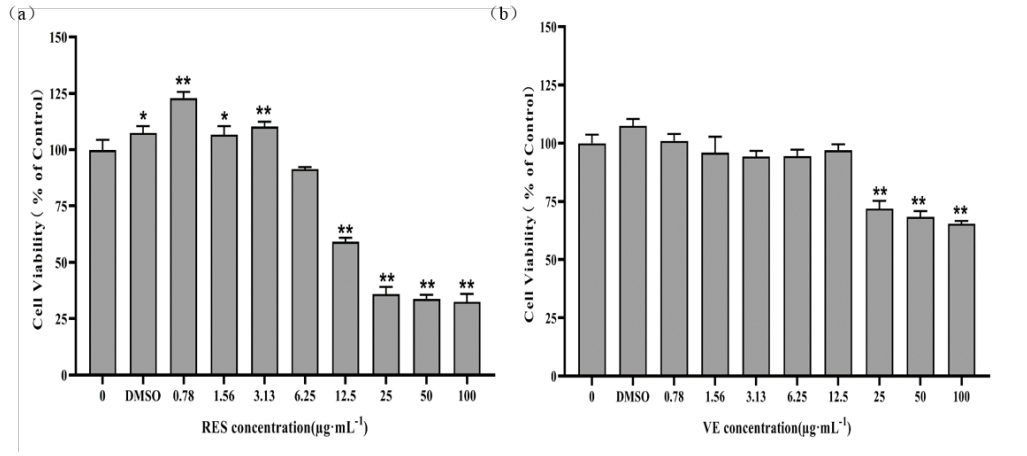

Figure 4. Effect of RES and VE on the viability of RAW264.7 cells. (a) Effect of RES on RAW264.7's cell viability; (b) Effect of VE on RAW264.7's cell viability. **P* < 0.05, relative to control group; ***P* < 0.01, relative to control group.

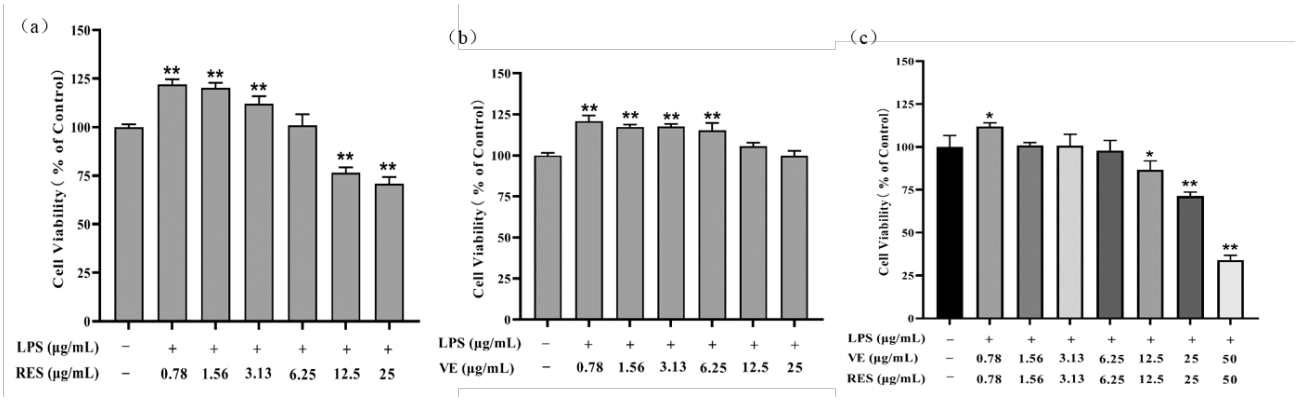

Figure 5. Effect of RES and VE on the viability of LPS-induced RAW264.7 cells. (a) Effect of RES on LPS-induced RAW264.7's cell viability; (b) Effect of VE on LPS-induced RAW264.7's cell viability; (c) Effect of RES combined with VE on LPS-induced RAW264.7's cell viability. *$P < 0.05$, relative to control group; **$P < 0.01$, relative to control group.

3.13 μg/mL ($P < 0.01$). Similarly, RES treatments also resulted in a significant inhibition on NO production by 56% at 3.13 μg/mL ($P < 0.01$). To determine the combinational effects of RES and VE, LPS-induced RAW264.7 cells were treated with low dosage (0.78 μg/mL RES + 0.78 μg/mL VE) and high dosage (3.13 μg/mL RES + 3.13 μg/mL VE). The results showed that combinations of RES with VE resulted in stronger a dose-dependent inhibition on NO production than the individual treatments with RES or VE. And there was no significant difference between DEX group and combinational treatments group ($P > 0.05$). It is noteworthy that the combined treatment group could greatly improve the anti-inflammatory ability on the NO level and had a certain synergistic effect, which was similar to the positive drug (DEX).

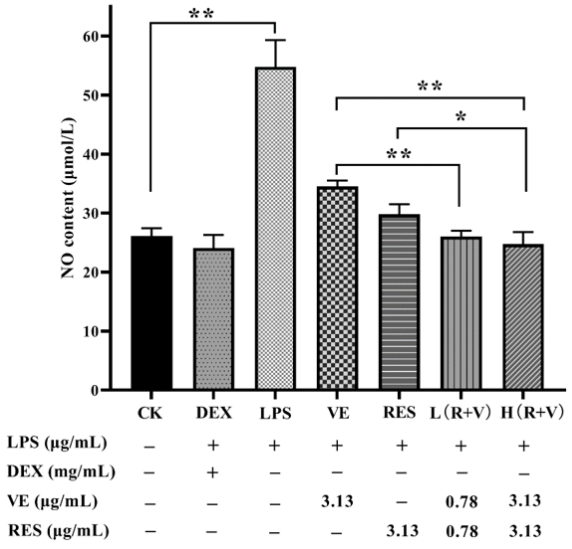

Figure 6. Effects of RES and VE on Nitrogen Oxide (NO) production in LPS-treated different treatment RAW264.7 cells. *$P < 0.05$, **$P < 0.01$.

3.4 Effects of the combined RES and VE on LDH, MPO and GSH activity in LPS-induced RAW264.7 cells

As shown in Figure 7, compared to the CK group, LPS-induced RAW264.7 cells demonstrated a significant increase in activity of LDH and MPO ($P < 0.01$) (Figure 7a-7b) and decrease in activity of GSH (Figure 7c). Compared to the LPS group, cells in both the single groups (VE and RES groups) and combined groups [L(R + V) and H(R + V) groups] showed a significant decrease in activity of LDH and MPO and decrease in activity of GSH ($P < 0.01$). Especially, in contrast to single RES group and VE group, treatment with high concentration of the combined RES and VE to induce RAW264.7 cells all significantly demonstrated curative activity by decreasing the activity of LDH ($P < 0.01$) and MPO ($P < 0.05$), and increasing GSH ($P < 0.01$) activity. However, there were no significant difference in the effects on the activity of LDH, MPO and GSH between the H(R + V) group and DEX group. These figures suggested that RES combined with VE has a good anti-inflammatory synergistic effect on oxidative stress in LPS-stimulated RAW264.7 cells.

3.5 Effects of the combined RES and VE on the pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) in LPS-induced RAW264.7 cells

In this study, cytokines released from cell culture supernatant were detected by ELISA. After incubation with LPS for 24 h, the production of TNF-α, IL-6, and IL-1β was significantly increased (Figure 8), indicating that the cellular inflammatory model was successfully established. Either treatment with single RES and VE or the combined RES and VE all effectively suppressed LPS-induced synthesis of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6). Meanwhile, in contrast to RES group and VE group, there were a significant ($P < 0.01$) decrease in the level of TNF-α, IL-6 in H(R + V) group and IL-1β in L(R + V) and H(R + V) group. This indicated that the combined RES and VE could considerably enhanced immune activity by inhibiting the synthesis of pro-inflammatory cytokinest in contrast to the single RES and VE, respectively. The pro-inflammatory cytokines in the combined treatment group were significantly increased, and there was no significant difference between the H(R + V) group and DEX group in reducing LPS-induced RAW264.7 cell inflammation ($P > 0.05$). This indicates that the combination therapy has a good synergistic effect, and its inhibitory effect on LPS-induced RAW264.7 cell inflammation is similar to that of the positive control.

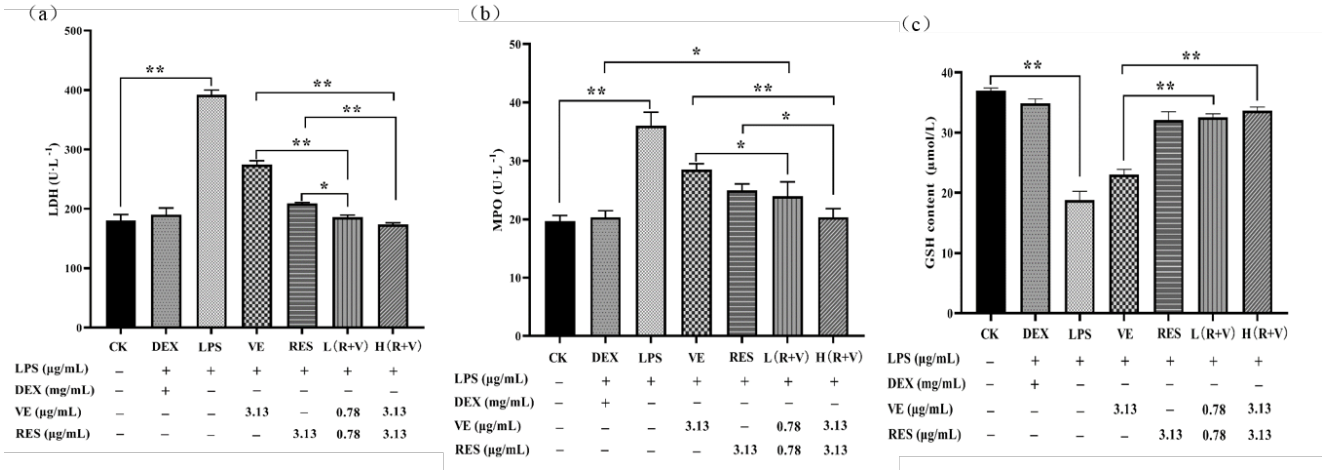

Figure 7. Effects of RES and VE on LDH, MPO and GSH activity in LPS-treated RAW264.7 cells. The figure shows that effects on the activity of LDH (a), MPO (b) and GSH (c) in different groups treatment. *$P < 0.05$, **$P < 0.01$.

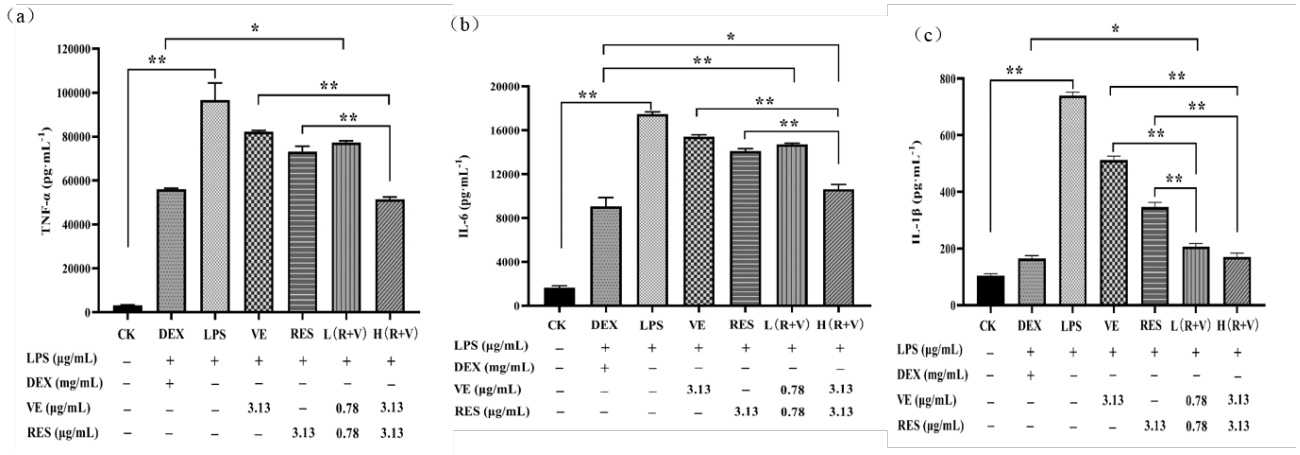

Figure 8. Effects of RES and VE on proinflammatory cytokines expression levels of TNF-α (a) and IL-6 (b) and IL-1β (c) in LPS-stimulated RAW264.7 cells by ELISA assay. *$P < 0.05$, **$P < 0.01$.

3.6 Effect of the combined RES and VE on gene express of key inflammatory markers (TNF-α, IL-6, IL-1β, TLR4, NF-κB) in LPS-induced RAW264.7 cells

To further evaluate the anti-inflammatory synergistic effect of RES and VE on LPS-induced RAW264.7 cells, we used qRT-PCR to detect the expression of inflammation-related genes TNF-α, IL-6, IL-1β, TLR4, NF-κB (Lan-Fang et al., 2022). The expression levels of inflammation-related genes were relative quantified by β-actin internal reference genes. As shown in Figure 9, LPS (1 μg/mL) significantly increased the mRNA expression levels of TNF-α, IL-6, IL-1β, TLR4 and NF-κB by about 13.5, 6.3, 16, 2.3 and 2-fold compared with normal cells. Compared with the LPS group, both the monotherapy group and the low-dose combination group had lower key indicators of inflammation. The expression levels of TNF-α, IL-6, IL-1β, TLR4 and NF-κB were significantly decreased by high dose combination therapy, which was the same as DEX treatment. The results demonstrated that the combined RES and VE could considerably inhibit the expression of multiple inflammatory markers at the transcriptional level, thus achieving the effect of inhibiting inflammation.

3.7 Effect of the combined RES and VE on inflammation-associated proteins (TLR4, p-NF-κBp65, p-IκBα) in LPS-induced RAW264.7 cells

To investigate the effects of combined RES and VE in inhibiting the expression of inflammation-associated proteins, such as TLR4, p-NF-κBp65, p-IκBα, we performed a western blotting analysis on the production of LPS-stimulated RAW264.7 cells after treatment with RES and VE. The results showed that there were greatly considerable difference between the RES and VE treatment groups and LPS group ($P < 0.05$) (Figure 10). However, in contrast to the single treatment group, the combined treatments significantly down-regulated the expression of the anti-inflammation protein TLR4, p-NF-κBp65, p-IκBα in L (R + V) group ($p < 0.05$) and H (R + V) group ($P < 0.01$). Meanwhile, compared with the DEX group, the expression of the anti-inflammation protein in the combined treatment groups were significantly decreased ($P < 0.05$).

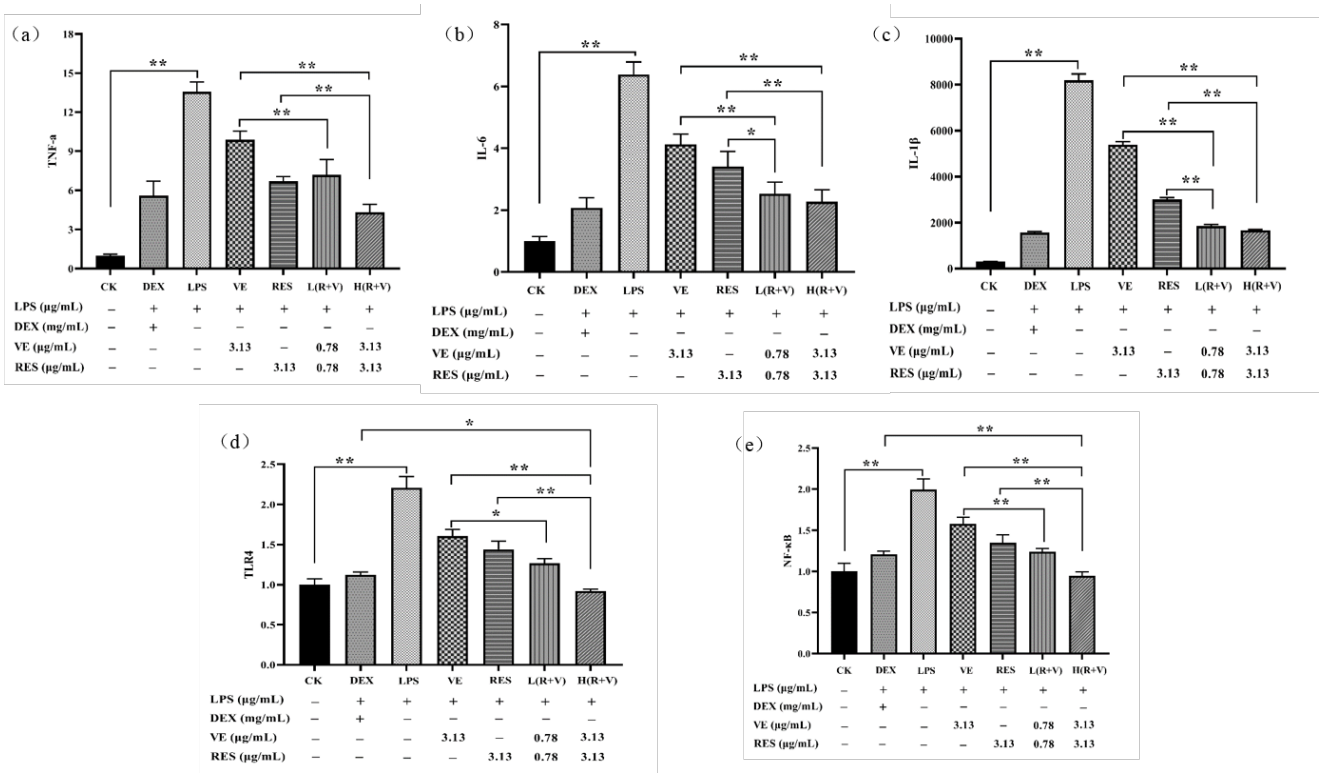

Figure 9. Effects of RES and VE on LPS-treated TNF-α (a) and IL-6 (b) and IL-1β (c) TLR4 (d) and NF-κB (e) mRNA expression in RAW264.7 cells. *$P < 0.05$, **$P < 0.01$.

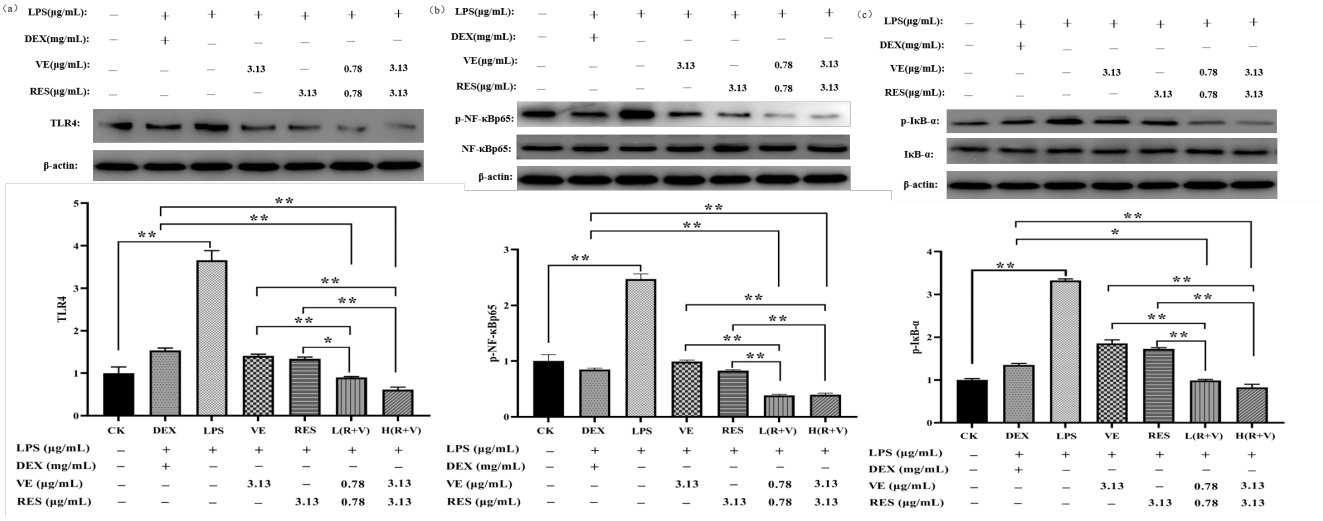

Figure 10. Inhibitory effects of RES and VE on LPS-induced TLR4 (a) and NF-κBp65 (b), IL-1β and (c) IκBα protein expression in RAW264.7 cells. *$P < 0.05$, **$P < 0.01$.

Above results indicated that RES combined VE through effectively down-regulate the expression of TLR4, p-NF-κBp65, p-IκBα, thus play a good anti-inflammatory effect.

4 Discussion

Nowadays, natural compounds especially in foods have been arresting considerable attention in the field of pharmaceuticals and cosmetics due to their extensive bioactivities such as anti-oxidant and anti-inflammatory effects (Luo et al., 2022b). RES or VE have been shown to have anti-inflammatory effects as different natural products. Zhou et al. (2018) reported that the anti-inflammatory effect of RES is through inhibition of NF-κB pathway, thus reducing the inflammatory effect of acute pharyngitis model animals. Moreover, VE has good characteristics and applications in many diseases as an

antioxidant. Recently, the anti-inflammatory effects of VE have been studied in atherosclerosis and other diseases (Muñoz & Munné-Bosch, 2019). Yang & Jiang (2019) indicated that VE had anti-inflammatory activities by inhibition of NF-κB and anti-cancer effects in animal models.

The main purpose of this study was to identify the extent to which RES and VE enhance each other and produce enhanced inflammatory inhibition. Our results indicated that the combination of RES and VE could significantly inhibit NO level in LPS-induced macrophage cells. It is important to note that all doses used in monotherapy and combination therapy are non-toxic to macrophages. Meanwhile, the combined effects of RES and VE at different doses on LPS-induced RAW264.7 cells significantly inhibited LDH and MPO enzyme activities and increased GSH enzyme activity, and the combined effects were significantly better than that of single effect. Zhao et al. (2017a) showed that RES could enhance endogenous antioxidant capacity, reduce inflammatory response, and have anti-apoptotic effect, which was expected to be an effective drug for the treatment of cadmium-induced testicular injury.

To reveal the molecular mechanisms of RES and VE interactions, their effects on several important pro-inflammatory proteins were investigated. The results demonstrated that both RES and VE inhibited LPS-induced upregulation of TLR4, NF-κBp65, IL-1β and p-IκBα, while the high dose of RES and VE combined leading to the upregulation of these inflammatory factor proteins had a stronger inhibition. We further studied the effects of RES, VE and their combined effects on mRNA levels of TNF-α, IL-6, IL-1β, TLR4 and NF-κB in RAW264.7 cells. TNF-α, IL-6 and IL-1β are pro-inflammatory factors that play an important role in inflammatory manifestations (Luo et al., 2022a). The results indicated that the combination of RES and VE to inhibit inflammation was accompanied by a decrease in pro-inflammatory cytokines, and the combination of high dose RES and VE significantly reduced the mRNA levels of these pro-inflammatory cytokines compared with RES or VE alone. These results demonstrates that TNF-α, IL-6 and IL-1β may be affected by the synergistic effect of RES and VE. In addition, the protein expression levels of TLR4, p-NF-κBp65 and p-IκBα were significantly decreased by the combination of RES and VE, suggesting that the synergistic anti-inflammatory mechanisms of RES and VE were involved multi-pathways. Zhao et al. (2017b) showed that the co-treatment of RES and quercetin could reduce the inflammatory effects through the AMPKα1/SIRT1 signaling pathway, and regulate intestinal flora to reduce obesity in high-fat diet-induced obesity rats. Hayes (2011) found that curcumin in combination with RES had better in vivo anti-inflammatory effects than curcumin alone. Zhou et al. (2018) and Liu et al. (2019) researched that the combination of RES and eriocitrin inhibited NO, IL-1β, TNF-α and NF-κB in LPS-stimulated RAW264.7 cells stronger than that separately. On the basis of the information, the combination of RES and VE plays an important role in inhibiting lPS-induced RAW264.7 cell inflammatory response. These results provide new knowledge on the interactions between different bioactive ingredients in diets, which is an important but under-researched area.

5 Conclusions

In summary, Resveratrol combined with vitamin E have a great synergistic anti-inflammatory effect. The combination of high dose RES and VE (3.13 μg/mL RES + 3.13 μg/mL VE) could significantly decrease the contents of inflammatory mediators NO, IL-6, TNF-α and IL-1β, and effectively inhibit the expression of TNF-α, IL-6, IL-1β, TLR4, p-NF-κBp65 and p-IκBα. Especially, the combined anti-inflammatory effect is more effective than the using one alone. These findings indicates that RES combined with VE could inhibit the production of inflammatory mediators and further suppress the activation of TLR4, p-NF-κBp65 and p-IκBα signaling pathway in LPS-induced RAW264.7 cell. The synergistic effect of RES and VE on inflammation was revealed, which provided a new effective way for inflammation treatment.

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