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Original Article

Investigating the effect of royal jelly and nanosilver particles on kidney and liver cytokines

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Abstract

Background and aims: Cytokines play crucial roles in immune responses. Nanoparticles, such as nanosilver (NS), are widely used to improve drug delivery. It has been reported that NS may affect immune cell functions. Royal jelly (RJ) has protective roles against several molecules, including free radicals and other harmful molecules. This project was designed to explore the influential role of RJ and NS in the immune response in the livers and kidneys of rats.

Methods: In this study, 40 rats were divided into control, RJ, NS, and RJ-NS-treated groups. The rats were treated with RJ, NS, and their combination. Further, levels of interleukin-10 (IL-10), IL-13, tumor necrosis factor-α (TNF-α), and C-C motif chemokine ligand 3 (CCL3) in the liver and kidney were evaluated using the enzyme-linked immunosorbent assay technique.

Results: The results demonstrated that NS reduced the CCL3 and TNF-α levels in the liver and kidney, respectively. In addition, RJ decreased IL-13 levels in both kidney and liver tissues. In the kidney, IL-13 levels were significantly reduced in the RJ group compared to the control ($P=0.043$) and NS-RJ ($P=0.002$) groups. In the liver, IL-13 levels also decreased in the RJ group compared to the control group ($P=0.050$).

Conclusion: Based on the results, RJ significantly protects macrophage functions, the main cells that produce TNF-α and respond to CCL3. RJ may also improve the pathogenesis of IL-13-related diseases. RJ could neutralize the effects of NS on CCL3 and TNF-α levels.

Keywords: Cytokine, Nanosilver, Royal jelly

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Introduction

Cytokines are small, unstable molecules that participate in several intracellular pathways of immune and non-immune cells, including the induction of appropriate immune responses against microbes. Accordingly, they were divided into two essential categories, including pro- and anti-inflammatory cytokines (1). Tumor necrosis factor-α (TNF-α) is the most important innate immunity cytokine with several pro-inflammatory properties and consequently participates in the first immune responses against microbes (2). In contrast to TNF-α, interleukin 10 (IL-10) is the most important anti-inflammatory cytokine produced by innate or adaptive macrophages, T regulatory, and T helper 2 (Th2) immune cells. TNF-α and IL-10 have antagonist effects on each other (3). Additionally, some Th2 cytokines play critical roles in the induction of Th2-related inflammation, although they neutralize Th1-related inflammation. IL-13 is a Th2 cytokine that plays a

crucial role in the pathogenesis of several Th2-related pro-inflammatory diseases, such as asthma (4). Chemokines are also a category of cytokines and have both pro- and anti-inflammatory functions (5). Chemokine (C-C motif) ligand 3 (CCL3) is a pro-inflammatory chemokine and plays a vital role in the chemotaxis of macrophages to the infected sites. Hence, it is also called macrophage inflammatory protein 1-alpha (6).

Due to the introduction, factors inhibiting the production of TNF-α and CCL3 and increasing the expression of IL-10 may be considered the immunosuppressor agents that inhibit appropriate immune responses against the microbes. Nanoparticles are crucial molecules and are used to increase drug delivery through elevating the interaction of drugs with their target molecules (7). Nanosilver (NS) is a famous example of nanoparticles utilized in several drug delivery strategies. However, some reports documented its severe side effects, from the oxidation of DNA (8) and cell cytotoxicity (9) to the induction of damage to human tissues (10). Therefore, NS may be associated with the dysfunction of immune responses against the microbes via alterations in cytokine production. For example, it has been reported that NS significantly reduces the expression of pro-inflammatory cytokines, including TNF-α, by peripheral blood mononuclear cells (11). Due to the suitable effects of NS in the drug delivery process, combination materials that neutralize the effects of NS may be worthwhile to use in human disease NS-based therapy. Recently, investigators have reported the antiviral (12), antibacterial (13), immunomodulatory (14), and antioxidant (15) properties of royal jelly (RJ). RJ is a worker bee *(Apis mellifera)* viscous macromolecule-produced molecule, a critical food for the queens. Therefore, RJ is widely utilized to produce various human dietary supplements (16). Due to these properties, it has been hypothesized that RJ may be considered a protective material to suppress the effects of NS on immune responses. The liver and kidney are two critical mammalian tissues for detoxification and the disappearance of toxins; thus, NS may target these tissues more than other mammalian tissues. In this project, the effects of the NS, RJ, and a combination of both NS and RJ on the levels of IL-10, IL-13, TNF-α, and CCL3 in the kidney and liver have been explored using rats as animal models.

Materials and Methods

The NS solution (8000 ppm, size less than 100 nm, and 99.98% purity) was purchased from Nanophishgaman Company, Mashhad, Iran. Similarly, RJ was provided by Pars-Asal Company, Shiraz, Iran. Due to previous investigations, RJ was prepared by dissolving 20 g of RJ in 1 L of sterilized double-distilled water (17). The rats were manipulated in standard conditions, including standard light, monitoring nutritional status, and standard temperature and humidity conditions. According to the same previous studies (18,19), 40 male rats weighing 200-250 g were used to explore the effects of NS, RJ, and a combination of both NS and RJ on the levels of IL-10, IL-13, TNF-α, and CCL3 in the kidney and liver. Therefore, the Wistar rats were divided into four groups. The control group received no treatment with any materials. The RJ group was treated with 100 mg/kg RJ, and animals in the NS-RJ group underwent treatment with both NS (30 mg/kg) and RJ (100 mg/kg). Finally, the NS group received 30 mg/kg NS. The NS, NS-RJ, and RJ solutions were prepared in fresh format and gavaged for 28 days (18, 19). The levels of IL-10, IL-13, TNF-α, and CCL3 in the liver and kidney were measured after 28 days of treatment with NS, NS-RJ, and RJ through killing the animals and extracting the kidney and liver under sterile, ethical, and standard conditions. To estimate the levels of IL-10, IL-13, TNF-α, and CCL3 in the kidney and liver, the tissues were suspended in the phosphate buffer saline buffer, and the cytokine levels were evaluated using enzyme-linked immunosorbent assay commercial kits from Karmania Pars Gene Company (Kerman, Iran). Eventually, a one-way analysis of variance and the post hoc Tukey test were used to analyze the results by SPSS, version 18. A P value less than 0.05 was considered statistically significant. The GraphPad Prism software was employed to draw the figures.

Results

The required data were obtained from four groups (control, RL, NS, and NS-RJ), each containing ten rats. Then, the levels of IL-10, IL-13, TNF-α, and CCL3 in the liver and kidney underwent analysis. IL-10 levels in the kidney were 37.36 ± 0.68, 38.56 ± 0.71, 36.70 ± 0.61, and 35.45 ± 1.94 in the control, NS-RJ, NS, and RJ groups, respectively. Thus, IL-10 levels did not change in the kidney ($P = 0.286$) and liver ($P = 0.067$) among all four groups (Figure 1). The changes in the level of IL13 in both the kidney ($P = 0.009$) and liver ($P < 0.001$) were statistically meaningful. In the kidney, IL-13 levels were significantly decreased in the RJ group compared to the control ($P = 0.043$) and NS-RJ ($P = 0.002$) groups. In the liver, IL-13 levels also decreased

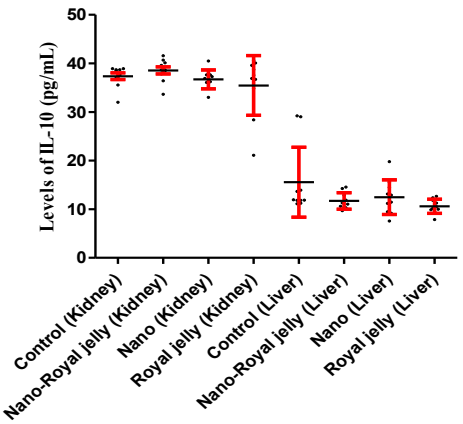

Figure 1. Changes in IL-10 kidney and liver levels among the groups. *Note.* IL: Interleukin. Based on the data, IL-10 in kidney and liver levels did not change among the groups

in the RJ group in comparison with controls ($P=0.050$, Figure 2). TNF-α levels in the kidney demonstrated a decrease in RJ and NS compared to the control ($P<0.001$ and $P<0.001$, respectively). Moreover, the combination of RJ and NS could decrease kidney TNF-α levels compared to the control group ($P<0.001$ and $P<0.049$, respectively). However, the RJ, NS, and NS-RJ of the liver failed to change TNF-α levels ($P=0.1$, Figure 3). The CCL3 levels in the kidney did not differ among the four groups ($P=0.060$). Nonetheless, its level significantly differed in the liver ($P=0.001$), and NS significantly decreased liver CCL3 levels compared to the control ($P=0.020$, Figure 4). Briefly, the results revealed that NS significantly decreased levels of TNF-α and CCL3 in the kidney and liver, respectively. Based on the results, NS reduced the levels of CCL3 and TNF-α in the liver and kidney, respectively. Further, RJ decreased IL-13 levels in both kidney and liver tissues. In the kidney, IL-13 levels represented a significant reduction in the RJ group compared to the control ($P=0.043$) and NS-RJ ($P=0.002$) groups. In the liver, IL-13 levels were also decreased in the RJ group in comparison with controls ($P=0.050$).

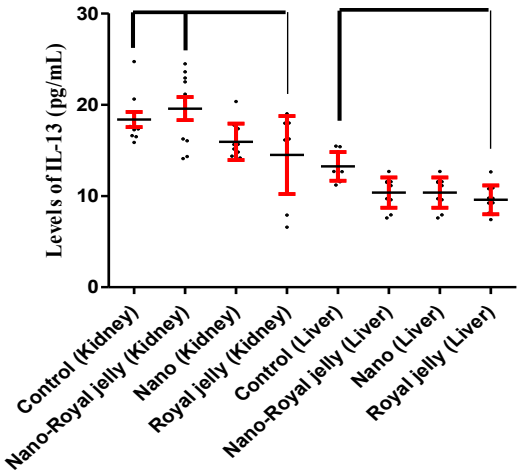

Figure 2. Comparison of IL-13 in kidney and liver tissues in the control and NS-RJ groups. *Note*. IL: Interleukin; NS: Nanosilver; RJ: Royal Jelly. Based on the results, RJ significantly decreased IL-13 in kidney and liver tissues compared to the control and NS-RJ groups

Discussion

Our result showed that IL-10 levels did not change in the kidney and liver. As a result, moderate concentrations of NS have no severe effects on producing IL-10, an anti-inflammatory molecule. Additionally, because the concentration of IL-10 was not altered following NS therapy, RJ could not change the cytokine levels because they were in the normal range. CCL3 is the chemotactic factor for macrophages, and TNF-α is the main cytokine produced by the cells (6). Accordingly, it appears that NS has the highest effect on macrophages and suppresses their functions. Interestingly, although RJ significantly decreased the level of TNF-α in the kidney, its combination with NS led to no decrease in cytokine production in the kidney. Thus, it seems that RJ can neutralize the cytotoxic effects of NS on macrophages in the kidney tissue. RJ may have had the same effect on the liver because the results demonstrated that although NS significantly decreases the level of CCL3 in the liver, the combination of RJ and NS suppresses NS effects; hence, there were no significant differences between the control and NS-RJ groups regarding the level of CCL3 in the liver. Furthermore, the findings revealed that RJ decreased the level of IL-13 in the kidney and liver compared to the control group. Considering that IL-13 plays a critical role in the induction of alternative functions of macrophages (18), the pathologic feature of macrophages, it may be hypothesized that RJ may be considered an essential factor in decreasing macrophage-associated liver and kidney disorders. IL-13 is an important cytokine to induce Th2-related inflammation (4). Accordingly, RJ can be a potential molecule that can improve kidney and liver function. Our previous investigation revealed that RJ could reduce the level of IL-6 in the kidney, a pleiotropic pro-inflammatory

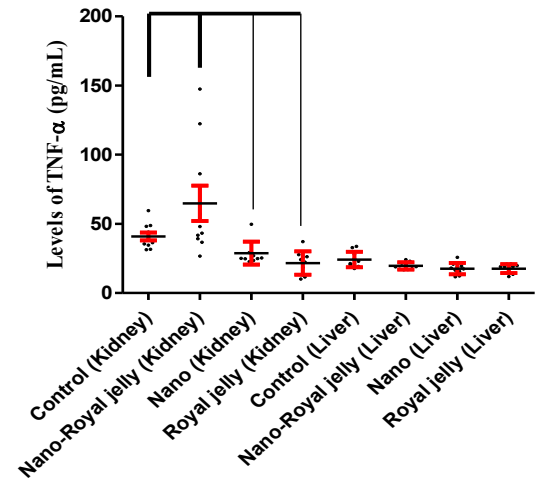

Figure 3. Comparison of the level of TNF-α in the control and NS-RJ groups. *Note*. TNF-α: Tumor necrosis factor-α; NS: Nanosilver; RJ: Royal Jelly. NS-RJ significantly increased the level of TNF-α in the kidney, while NS and RJ significantly decreased its level in the kidney in both control and NS-RJ groups

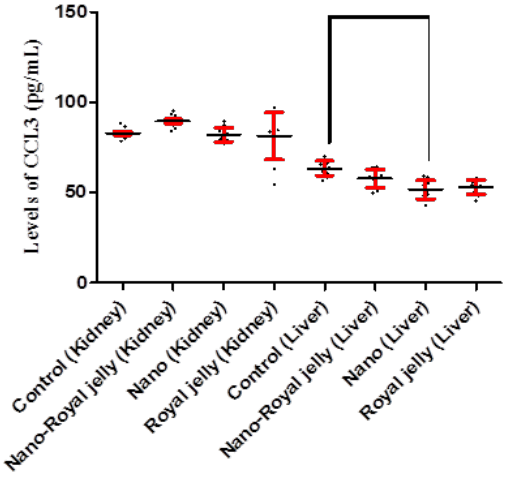

Figure 4. Comparison of the Level of CCL3 in the kidneys of NS and control groups. *Note*. CCL3: C-C motif chemokine ligand 3; NS: Nanosilver; The level of CCL3 was not altered in the kidneys of the groups; it was significantly decreased in the NS group compared to the control group

cytokine, in rats treated with RJ and NS (19). Additionally, in line with our results, Vukovic et al reported that NS is a potential cytotoxic agent against peripheral blood mononuclear cells and reduces the production of TNF-α by the cells (11). Nakkala et al also noted that, at high concentrations, NS induces liver cell swelling and vascular degeneration in the rat model (20). The cytotoxicity effects of NS in animal models have also been reported previously (21). However, chronic exposures to NS were associated with neuroinflammation and progressive brain tissue loss (22). Hence, it may be hypothesized that using RJ may also reduce neuroinflammation in either humans or animals, which needs to be explored using clinical trials and animal modeling. Another study revealed that high concentrations of NS induce the expression of pro-inflammatory cytokines and inflammatory activity in human monocytes in *in vitro* conditions. Therefore, at moderate concentrations, like in the current study and *in vivo* conditions, NS seems to show different effects on the immune cells. Additionally, the protective role of RJ in animal tissues, including skeletal muscles, adipose tissue (23), testis (24), and immune cells such as macrophages (25), has been reported by several investigations. Moreover, the results demonstrated that neither RJ nor NS and NS-RJ had any effects on the IL-10 levels in both the liver and kidney. Given that IL-10 is produced by several cells (3), it appears that although macrophages may be affected by NS, other cells replace the IL-10 tissue levels.

Conclusion

It was revealed that RJ plays a critical role in protecting against the side effects of NS on the immune cells, especially macrophages, and inducing the appropriate immune response. However, the effects of RJ and its combination with NS on the immune cells must be explored in both *in vitro* and *in vivo* conditions through further investigations.

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Competing Interests

The authors declare that there is no conflict of interests.

Ethical Approval

Ethical considerations in this study included obtaining permission from the Ethics Committee of Rafsanjan University of Medical Sciences (ethical code: IR.RUMS.REC.1397.093) and written consent from the participants.

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