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Immunomodulatory effects of *Stevia rebaudiana* leaves and commercial stevia on rats: a comparative study

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Abstract

Stevia is herbal native to South-America that is renowned for its natural sweet leaves containing stevia glycosides. Our study aimed at examining and comparing the immunomodulatory effects of the *Stevia rebaudiana* leaf extract and of commercially-available stevia products in rats. Our experiment involved the preparation of *Stevia rebaudiana* leaf extract and the use of market-available stevia obtained from the local-market. *Stevia rebaudiana* leaf extraction was meticulously performed, and 60 healthy adult male rats were randomly separated into three groups: untreated control, commercial stevia treatment (25 mg/kg), and *Stevia rebaudiana* leaf extract treatment (25 mg/kg). The rats were orally administered the treatments for 60 days, after which blood samples were collected for analysis. Our results revealed a significant rise in interleukin-1 beta (IL-1β) levels in rats treated with the *Stevia rebaudiana* leaf extract and the commercially-available stevia when compared to the control group. Additionally, immunoglobulin A (IgA) levels exhibited a notable increase in both stevia-treated groups, with the *Stevia rebaudiana* leaf extract-receiving group showing higher IgA levels than the commercially-available stevia-treated one. Our findings suggest that stevia may influence the immune response, particularly the regulation of the pro-inflammatory cytokine IL-1β and the IgA levels. This study contributes valuable insights into stevia's effects on the immune system.

KEYWORDS
cytokine, extraction, immunoglobulin A, interleukin-1 beta, stevia

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1. INTRODUCTION

Stevia rebaudiana belongs to the *Asteraceae* family, which includes sunflowers and daisies. These glycosides are responsible for stevia's heavy-duty sweetness, which makes it a popular sweetener. The stevia sweeteners are sugar alternatives with zero calories and widely used as sugar substitutes, high-intensity sweeteners, non-nutritive sweeteners, or low-calorie sweeteners, as they offer a satisfying taste of sweetness without the added calories [1]. The US Food and Drug

Administration (FDA) authorized stevia as a sweetener in January 2022. The FDA has also recognized as safe the use of high purity stevia glycosides, notably rebaudioside A (GRAS).

Some researchers suggest that stevia's components, such as stevioside and rebaudioside A, may have anti-inflammatory properties [2]. Inflammation is a component of the immune response, and drugs having anti-inflammatory properties could potentially affect the immune system's function. Stevia has been shown to exert antioxidant activity. Antioxidants help neutralizing free radicals that are responsible for causing oxidative-stress and inflammation, while antioxidants also contribute to overall health, and their specific impact on the immune function is nuanced and context-dependent [3]. This study aimed at investigating and comparing the potential differences in the immunomodulatory effects of the *Stevia rebaudiana* leaf extract and the commercially-available stevia products in rats.

2. MATERIALS AND METHODS

This study were conducted from April 2023 to January 2024 in Babylon city, Iraq. *Stevia rebaudiana* leaves and commercially-available stevia (market-available stevia) were obtained from the local market.

For the preparation of the extraction of the *Stevia rebaudiana* leaves, the leaves were ground into a fine powder by using a mortar. Subsequently, 500 g of this fine powder were placed in a beaker with warm water, and the mixture was continuously stirred for 24 h. The extraction was filtered by using a Whatman No.1 filter paper. The supernatant was then concentrated by using a rotary-evaporator under vacuum, was dried, and the percentage weight of the dried extraction was determined [4].

Our experiments included 60 rats that were randomly separated into three groups as follows: (i) the first group (n=20) served as the untreated "control", (ii) the second group (n=20) received a 25 mg/kg dose of the commercially-available stevia, and (iii) the third group was treated with a 25 mg/kg dose of the *Stevia rebaudiana* leaf extract. The rats were treated for 60 days, orally, by using stevia dissolved in distilled water. Following the 60-day treatment period, the animals underwent anesthesia using ether, and cardiac-puncture was performed in order to collect 1 mL of blood [5]. The assessment of the plasma levels of interleukin-1 beta (IL-1β) and immunoglobulin A (IgA) was performed by using an ELISA kit (Elabscience) as previously described [6].

All statistical analyses were done by using GraphPad Prism (version 6), including the performance of Student's *t*-test, analysis of variance (ANOVA), regression analysis, and non-parametric tests.

3. RESULTS AND DISCUSSION

The extraction weight was 329 mg our of 500 g of leaves of *Stevia rebaudiana* powered, and the percentage weight was 0.0658% of 500 g of the *Stevia rebaudiana* leaf powder. The amount of the extracted compounds is somewhat low, as indicated by the small percentage (0.0658%). Numerous factors can affect the extraction efficiency, including the extraction method used. Different extraction methods (e.g., solvent extraction, water extraction) can yield different results in relation to both the quantity and the quality of the extracted compounds.

The plasma IL-1β levels exhibited a significant increase (P<0.0001) in rats treated with the leaf extract when compared with those of the control group (Table 1). In addition, the rats treated with the commercial stevia also displayed a significant increase in their IL-1β levels (P=0.0036) when compared to those of the control group (Table 1). The significantly higher levels of IL-1β in the group treated with *Stevia rebaudiana* leaf extract when compared to those of the group treated with the market-available stevia may indicate that the extraction technique or specific components in the *Stevia rebaudiana* leaves may contribute to a more dramatic effect on IL-1β levels. *Stevia rebaudiana* or its components might stimulate the immune system, leading to an increase in IL-1β production that might be a reaction to perceived threats, even if those threats are not harmful [7].

The mean plasma IgA levels were 568.1 ± 55.92 ng/mL in the rats treated with market-available stevia, showing a significant difference (P=0.0022) when compared to those of the control group (Table 1). Rats treated with the *Stevia rebaudiana* leaf extract exhibited an extremely significant difference (P<0.0001) in terms of their IgA levels (605.4 ± 45.60 ng/mL) when compared to those of the control group (Table 1). Moreover, there was a significant difference observed (P=0.0263) in the IgA levels between the two stevia-treated groups (Table 1).

The rise in IgA levels, as observed in this study, could be influenced by various factors. IgA is an antibody that plays a crucial role in the immune-system, mainly in mucosal immunity and the defense against infections in mucosal membranes such as those in the digestive and the respiratory tracts. IgA is also essential for keeping a balance between the host and the gut microbiota [8]. Stevia

might influence the gut microbiota, leading to an increase in IgA as a response to the need of maintaining homeostasis in the gastrointestinal tract. These are immunomodulatory effects of stevia or of its components, as they stimulate the immune system, leading to an elevated IgA production.

Stevia has been reported to possess antioxidant properties [9]. If there is oxidative stress or inflammation in the body, the immune response, including IgA production, may be heightened so as to stabilize these phenomena. The dose and the period of treatment with stevia might also play a role in our findings. Different doses or extensive treatment periods could lead to varying immune responses.

Table 1. Comparison of interleukin-1 beta (IL-1β) and immunoglobulin A (IgA) levels between rats receiving different treatments. Notes: [a], comparison between the control group and the market-available stevia group; [b], comparison between the control group and the *Stevia rebaudiana* leaf extract group; [c], comparison between the market-available stevia group and the *Stevia rebaudiana* leaf extract group.

IL-1β levels in rats under different treatments			
Groups / treatments	**n**	**Mean ± SD (pg/mL)**	***P*-value**
Control	20	51.10 ± 7.953	--
Market-available stevia	20	77.70 ± 37.45	0.0036[a]
Stevia rebaudiana leaf extract	20	233.5 ± 81.93	< 0.0001[b,c]
IgA levels in rats under different treatments			
Groups / treatments	**n**	**Mean ± SD (ng/mL)**	***P*-value**
Control	20	568.1 ± 28.52	--
Market-available stevia	20	568.1 ± 55.92	0.0022[a]
Stevia rebaudiana leaf extract	20	605.4 ± 45.60	< 0.0001[b]; 0.0263[c]

4. CONCLUSION

This study contributes to the increasing research interest on stevia's potential health-effects, especially on the immune system. Both rat groups treated with *Stevia rebaudiana* leaf extract and market-available stevia exhibited a significantly increased IL-1β, and IgA levels compared to the control group. This finding suggests that stevia may influence the immune response, particularly the pro-inflammatory cytokine IL-1β and IgA.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

REFERENCES

1. Orellana-Paucar A.M.: Steviol glycosides from *Stevia rebaudiana*: an updated overview of their sweetening activity, pharmacological properties, and safety aspects. *Molecules* 28(3): 1258 (2023).
DOI: **10.3390/molecules28031258**
PMID: **36770924**

2. Muñoz-Labrador A., Hernandez-Hernandez O., Moreno F.J.: A review of the state of sweeteners science: the natural *versus* artificial non-caloric sweeteners debate. *Stevia rebaudiana* and *Siraitia grosvenorii* into the spotlight. *Crit. Rev. Biotechnol*. *in press* (2023).
DOI: **10.1080/07388551.2023.2254929**

3. Papaefthimiou M., Kontou P.I., Bagos P.G., Braliou G.G.: Antioxidant activity of leaf extracts from *Stevia rebaudiana* Bertoni exerts attenuating effect on diseased experimental rats: a systematic review and meta-analysis. *Nutrients* 15(15): 3325 (2023).
DOI: **10.3390/nu15153325**
PMID: **37571265**

4. Abed H.H., Ali A.M., Al-Ziaydi A.G., Nayif E.M.: Evaluation toxic effect of bisphenol A in reproductive system of male mice and amelioration its effect by green tea extract. *Plant Arch*. 19(s2): 880-885 (2019).

5. Abed H.H., Al-Ziaydi A.G., Taher I.A., Al Dulaimi A.K.: Comparison of some hematological parameters between male and female patients infected with COVID-19. *Hum. Antibodies* 30(3): 151-155 (2022).
DOI: **10.3233/HAB-220006**
PMID: **35786649**

6. Fan J., Wu L., Li G., Tao S., Sheng Z., Meng Q., *et al.*: Effects of enteral nutrition with parenteral glutamine supplementation on the immunological function in septic rats. *Br. J. Nutr*. 113(11): 1712-1722 (2015).
DOI: **10.1017/S0007114515001099**
PMID: **26067806**

7. Kurek J.M., Krejpcio Z.: The functional and health-promoting properties of *Stevia rebaudiana* Bertoni and its glycosides with special focus on the antidiabetic potential – a review. *J. Funct. Foods* 61: 103465 (2019). DOI: **10.1016/j.jff.2019.103465**

8. Guo J., Ren C., Han X., Huang W., You Y., Zhan J.: Role of IgA in the early-life establishment of the gut microbiota and immunity: implications for constructing a healthy start. *Gut Microbes* 13(1): 1-21 (2021). DOI: **10.1080/19490976.2021.1908101** PMID: **33870860**

9. Iddir M., Brito A., Dingeo G., Fernandez Del Campo S.S., Samouda H., La Frano M.R., *et al.*: Strengthening the immune system and reducing inflammation and oxidative stress through diet and nutrition: considerations during the COVID-19 crisis. *Nutrients* 12(6): 1562 (2020). DOI: **10.3390/nu12061562** PMID: **32471251**