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Hepatotoxic effects of *Stevia rebaudiana* leaf extract and commercial stevia on rats: a comparative study

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Abstract

The popularity of stevia is high, especially among diabetics and those looking to reduce their calorie-intake. The aim of this study was to compare the effects of a commercially-available stevia and of a *Stevia rebaudiana* leaf extract on the liver function and histology of rats. After preparing the *Stevia rebaudiana* leaf extract, 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). Our results show that after 60 days of treatment (oral administration), a significant elevation of the alanine aminotransferase (ALT) levels was observed in the commercial stevia-treated group, suggesting potential effects on liver function. The *Stevia rebaudiana* leaf extract-treated group also exhibited increased ALT levels. Moreover, the aspartate aminotransferase (AST) levels were found significantly increased in both of these treatment groups (when compared to the control group). Alkaline phosphatase levels were not found altered between groups. Histological-examinations, in spite of the elevated ALT and AST levels, exhibited no abnormalities in the liver. Although stevia is generally regarded as safe, this study underlines the importance of considering the type and form of stevia when evaluating its effects on liver health. Further study is warranted so as to elucidate the specific components and mechanisms responsible for the observed variations in liver enzymes, and to confirm the overall safety of stevia products.

KEYWORDS

Stevia rebaudiana, steviol glycosides, rat model, liver enzymes, hepatotoxicity

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1. INTRODUCTION

Stevia has become popular in recent years as a natural, zero or low-calorie alternative to typical sweeteners like sugar and artificial-sweeteners. The leaves of *Stevia rebaudiana* are the main source of these pleasing chemicals [1]. They are



usually harvested, dried, and processed to produce steviol glycosides, which can be up to 300 times sweeter than table sugar (sucrose). Moreover, stevia has been suggested to possess anti-inflammatory, antioxidant, and antihypertensive properties [2]. However, it is necessary to note that, while stevia is typically considered harmless, individual-reactions to sweeteners might vary, and more research is required in order to properly understand its long-term effects.

It is important to note that many aspects can influence liver health, including an individual's overall diet, lifestyle, and any pre-existing medical issues. Few studies have explored the potential hepatoprotective effects of stevia in animal models, suggesting that it may exert antioxidant and anti-inflammatory effects [3]. The aim of this study was to compare the effects of a commercially-available stevia and of a *Stevia rebaudiana* leaf extract on the liver function and histology of rats. Our study tried to provide insights into the impact of different forms of stevia on liver function, shedding light on possible changes in liver enzyme responses and histological abnormalities.

2. MATERIALS AND METHODS

The *Stevia rebaudiana* leaf extract was prepared by drying the leaves in the dark, and then grinding them with a mortar. Subsequently, 500 g of the leaves were placed in a flask with 500 mL of distilled water, on a hot plate, and were left stirring for 24 h at 70°C. The *Stevia rebaudiana* extract was filtered through a Whatman No. 1 filter paper, and the supernatant was concentrated with a rotary vacuum evaporator [4].

This study involved 60 healthy adult male albino Wistar rats weighing between 240 and 280 g. Before beginning the experiment, all rats were acclimatized to the normal conditions of 12 h of light and 12 h of darkness, at 25°C±4°C. The rats were randomly separated into three groups; the first group (n=20) served as the untreated control group, the second-group (n=20) received a 25 mg/kg dose of commercially-available stevia (market stevia), and the third group was treated with a 25 mg/kg dose of the *Stevia rebaudiana* leaf extract. Stevia was liquefied in distilled water and was administered orally to the rats for 60 days. Following the 60-day treatment period, the animals received anaesthesia by using diethyl ether, and a heart puncture was performed in order to collect 1 mL of blood from each rat [4]. Blood test determined the alanine aminotransferase (ALT) levels based on the Wroblewski and Ladue methods, the aspartate aminotransferase (AST) levels on the method described by Karmen, and the alkaline

phosphatase (ALP) levels based on the hydrolysis of *p*-nitrophenylphosphate [5]. The rat liver was removed in order to histologically assess it by staining its sections with haematoxylin and eosin. All statistical analyses were performed by using Microsoft Excel 2020 and the GraphPad Prism software (version 6).

3. RESULTS AND DISCUSSION

The weight of the extracted material from 500 g of *Stevia rebaudiana* leaves' powder was determined to be 411 mg, whereas the percentage weight corresponded to 0.0822% of the 500 g of the *Stevia rebaudiana* leaf extract. The extraction weight of the *Stevia rebaudiana* leaf powder is an important quantitative measure that shows the yield of the extraction process; it reveals the amount of the material extracted from the leaves during processing.

As far as the ALT levels are concerned, a statistically significant difference ($P<0.0001$) appears to exist between the control group (27.50±4.286 U/L) and the group treated with commercial stevia (68.80±8.205 U/L). Additionally, our results reveal a significant difference ($P=0.0002$) between the control group and the group treated with the *Stevia rebaudiana* leaf extract ALT levels (56.40±10.72 U/L), while a significant difference was also detected between the groups treated with commercial stevia and those treated with the *Stevia rebaudiana* leaf extract ($P=0.0002$). ALT is an enzyme found mainly in the liver, and variations in its levels frequently indicate liver health issues and functional disorders. The significant increase in ALT levels in the group treated with commercial stevia suggests that the material may have a negative effect on liver function. The significant difference observed between the two treatment groups suggests that the type or form of stevia used may also affect the ALT levels. This can be due to differences in the chemical compounds, the processing processes, or the concentrations of active components between the commercial stevia and the *Stevia rebaudiana* leaf extract [6].

The assessment of the AST levels revealed substantial differences between the study's groups. The rats treated with commercial stevia had significantly greater AST levels (50.05±12.23 U/L) than those of the control group (40.50±6.501 U/L; $P=0.0038$). Moreover, our study found that rats treated with the *Stevia rebaudiana* leaf extract also had significantly greater AST levels (50.55±14.71 U/L) when compared to those of the control group ($P=0.0081$). Remarkably, no significant difference was observed between the treatment groups. These findings reveal that both forms of stevia

cause a significant and similar increase in AST levels. These findings reveal the potential impact of stevia products on liver function, and underline the need for further research into the exact compounds and mechanisms affecting the AST levels [7].

The assessment of the ALP levels revealed no significant difference among the studied groups. The ALP levels were 206.0 ± 81.89 , 208.3 ± 73.54 , and 217.9 ± 82.51 U/L in the control, the commercial stevia-treated, and *Stevia rebaudiana* leaf extract-treated group, respectively. The absence of substantial differences in terms of the ALP levels between the studied groups implies a degree of

safety from potential hepatotoxic consequences.

Finally, in spite of the significant differences in ALT and AST levels, the undertaken haematoxylin and eosin staining of liver sections revealed no significant changes in the cellular architecture and morphology of the liver in any of the studied rat groups. These histological findings could be attributed to the liver's remarkable ability to employ compensatory mechanisms. Even in the presence of elevated enzyme levels, the liver can sometimes maintain its structural integrity and functionality, thereby exhibiting a seemingly normal histological appearance [8].

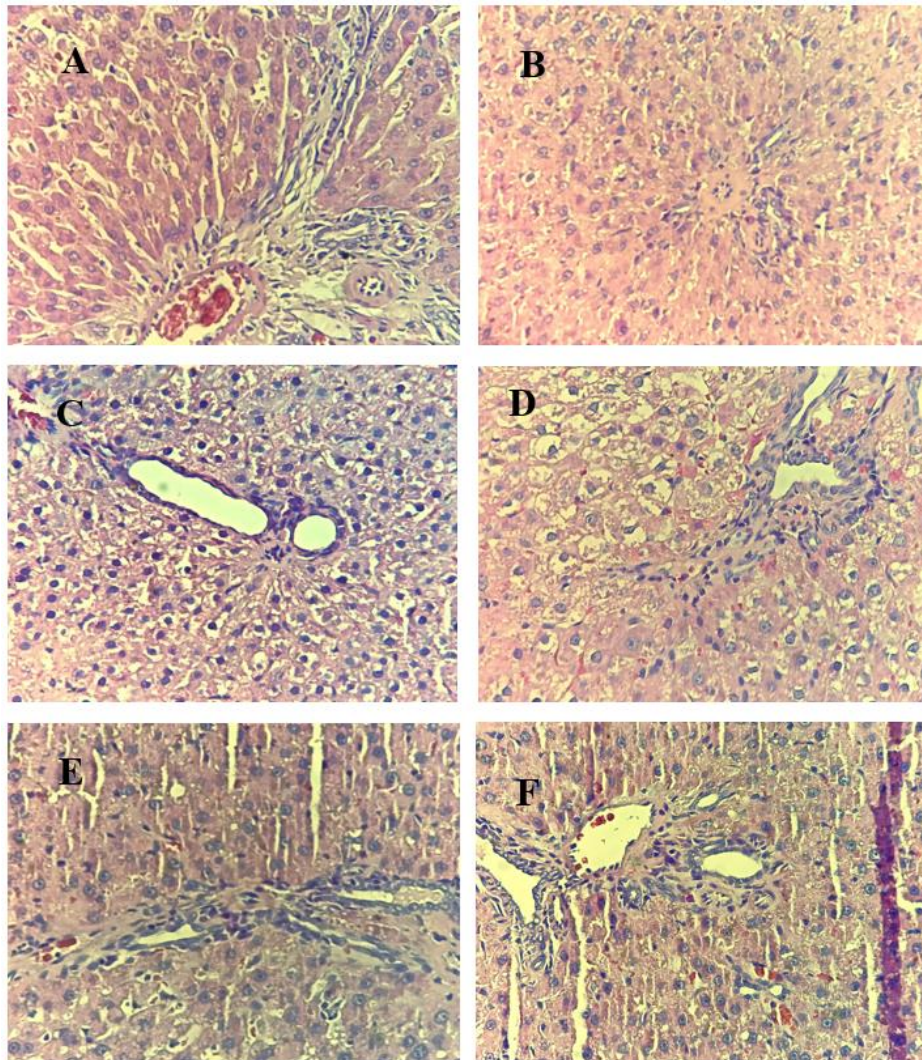


Figure 1. Representative light microscopy captions of the haematoxylin and eosin staining for the histological evaluation of the rat livers of the untreated control group (captions **A** and **B**), the commercial stevia-treated group (captions **C** and **D**), and the *Stevia rebaudiana* leaf extract group (captions **E** and **F**) showing no significant histopathological changes in all cases.

4. CONCLUSION

This study underlines the importance of considering the type and form of stevia when evaluating its effects on liver health. Further study is warranted so as to elucidate the specific components and mechanisms responsible for the herein observed variations in liver enzymes, and to confirm the overall safety of stevia products.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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