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

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 pro-
duce steviol glycosides, which can be up to 300
times sweeter than table sugar (sucrose). More-
over, stevia has been suggested to possess anti-
flammatory, 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 un-
derstand 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 mod-
els, 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, shed-
ding light on possible changes in liver enzyme re-
sponses 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 dis-
tilled 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 al-
binor Wistar rats weighing between 240 and 280 g.
Before beginning the experiment, all rats were ac-
climatized 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 (mar-
ket stevia), and the third group was treated with a
25 mg/kg dose of the Stevia rebaudiana leaf ex-
tract. 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 deter-
mined 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-nitrophenolphosphate [5]. The rat liver was re-
moved in order to histologically assess it by stain-
ing 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 cor-
responded 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 pro-
cessing.

As far as the ALT levels are concerned, a sta-
tistically 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 de-
tected between the groups treated with commer-
cial stevia and those treated with the Stevia rebau-
diana 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 func-
tional disorders. The significant increase in ALT
levels in the group treated with commercial stevia
suggests that the material may have a negative ef-
fect on liver function. The significant difference ob-
served between the two treatment groups sug-
gests that the type or form of stevia used may also
affect the ALT levels. This can be due to differ-
ences in the chemical compounds, the processing
processes, or the concentrations of active compo-
nents between the commercial stevia and the Ste-
via 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 different 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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