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## Impacts of *Stevia rebaudiana* leaf aqueous extract on Some Physiological Parameters and Fertility in Male Albino Mice

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## RESEARCH ARTICLE

# Impacts of *Stevia rebaudiana* leaf aqueous extract on Some Physiological Parameters and Fertility in Male Albino Mice

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## ABSTRACT

The objective of the work is to examine the impacts of *Stevia* leaf aqueous extract, after chronic administration (60 days), on some physiological aspects and fertility in male albino mice. Twenty-four adult mice were categorized into four groups, each involving six animals. Three treatment groups were separately exposed to three respective concentrations of the extract (25, 50, and 100 mg/kg), the remainder is a control group. The animals were given daily oral doses of 0.1 ml/10 gm body weight of the group-respective extract concentration for 60 consecutive days. Several physiological and fertility-related parameters were tested based on the serum's activities or levels of selected functional enzymes. Enzymatic activities of AST, ALT, and ALP were significantly elevated in animals that received treatments of 50 and 100 mg/kg compared to those that received 25 mg/kg and control doses. A similar elevation was observed in the average concentration of MDA, whereas those of CAT and GST were significantly reduced in all treated groups in the average concentrations of the enzymes CAT and GST in all concentrations of *Stevia* extract, compared with the control groups. The serum levels of FSH, LH or ICSH, and T in response to all studied concentrations of *Stevia* extract were significantly decreased. Also, our data indicated that the number of epididymal sperm was significantly decreased in the experimental animals, a result also observed in the average percentage of normal and deformed sperms. Morphological sperm examination demonstrated several forms of deformation in response to the leaf extract, where sperms with two tails, broken tails, and curled tails appeared, in addition to tailless sperms and those having cytoplasmic droplets. These results show consistency with the potentiality of *Stevia* aqueous extract in reducing fertility in male mice.

**Keywords:** Albino mice, Fertility, Liver enzymes, *Stevia rebaudiana*

## Introduction

*Stevia* (stevioside) is a shrub plant that is native to South America. Among the 230 species that belong to the family Asteraceae, *Stevia rebaudiana* Bertonii is unique in terms of the production of sweet steviol glycosides. Previous proposals that *S. phlebophylla* shares this feature as well were dismissed by recent data.<sup>1</sup> The consumption of *Stevia* might lead to a reduction in caloric content in diets and drinks, with other reported side effects, which possibly involve nausea, bloating, hypotension, and hormonal disruption. *Stevia* is a sweetener with

non-nutritional, non-caloric properties attributed to steviol glycosides. Its sweetness is about 10 to 15 times higher as compared to that of sucrose.<sup>2</sup> This type of glycosides cannot be metabolized by human physiological mechanisms; hence is considered as zero caloric nutrients. Opposite to its impact on unnatural sweeteners, heating does not lead to the decomposition of these glycosides, rendering it favorable to cook and bake.<sup>3</sup> Previous research revealed anti-hypertension properties of *Stevia*. The Acceptable Daily Intake (ADI) is 4 mg/kg body weight expressed as steviol equivalents based on Generally Recognized as Safe (GRAS) notification. *Stevia*

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additionally confers improvement to the nutritional status of people with diabetes.<sup>4</sup> The Food and Drug Administration (FDA) issued safety approval to stevia, but only in its pure form, known as stevioside. The safety of such products can be observed when their ingredient list includes items like stevia extract or *Stevia rebaudiana*. When found in natural food markets, products like stevia leaves, as well as crude stevia extracts, are considered unsafe to purchase. The FDA states a lack of information on the possible health influences of these products; e.g. renal and cardiovascular issues.<sup>5</sup> Previous work reported that treatment with *stevia* and sucralose is correlated with the activity of hepatic enzymes in rats, Nevertheless higher level of data sufficiency is required to reveal a correlation to levels of the serum markers of fatty liver disease.<sup>6</sup> An association between *stevia* and a reduced number of sperms and serum concentration of testosterone was revealed. In parallel, no relationship was found between the commonest sucralose and sperm quality and glycolysis.<sup>7</sup>

The present investigation aimed at determines the effects of chronically administered leaf extract from *Stevia rebaudiana* on some physiological aspects and fertility in male albino mice.

## Materials and methods

**Stevia leaf extract:** Steviosides (500 mg/1.5 ml) was purchased as an aqueous product from Sigma-Aldrich/Chemical manufacturer/Germany (<https://www.sigmaaldrich.com/US/en>), which was subjected to dilution with distilled water until achieving the desired three concentrations (25, 50 and 100 mg/kg).

### Laboratory animals

The experiments involved twenty-four male adult albino Swiss mice (16 to 18 Wks. old; 25 to 30 g weight) provided by the Animal House, Biotechnology Research Center/Nahrain University and kept in the animal experimental laboratory, the Research Center, Baghdad, Iraq. Housing conditions involved 12-hr light/dark cycle,  $25 \pm 1^\circ\text{C}$  *ad libitum* feeding and watering, and 1 Wk. pre-experimental acclimatization time.

### Experimental design

Mice (Twenty four male adult albino mice) were categorized into four groups, a treatment group, which was treated orally with *Stevia* leaves aqueous extract with concentrations of 25, 50 and 10 mg/kg,

as well as a control group, which was given water. as follows:

Group I: Control; Daily single oral gavage administration of water for 60 days.

Group II: 25 mg/kg Daily single oral dosage of *Stevia* leaves aqueous extract for 60 days.

Group III: 50 mg/kg Daily single oral dosage of *Stevia* leaves aqueous extract for 60 days.

Group IV: Daily single oral dosage of 100 mg/kg *Stevia* leaves aqueous extract for 60 days.

Following the treatment, the animals were subjected to sedative (pentobarbital; 80 mg/kg body weight), followed by blood collection via direct heart puncture<sup>8</sup> using appropriate syringes.<sup>9</sup> Serum was separated via centrifugation at a rate of 2000 cycles / min for 10 minutes, and kept at  $-4^\circ\text{C}$  for analysis of the markers related to the biochemistry and Physiological parameters in the blood. Markers related to the hematology were examined in heparinized blood.

### Biochemical analysis

Determination of the serum enzymatic activities of alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (ALP) was achieved by utilizing specific kits (Labtest, Lagoa Santa, Brazil). For the expression of results, the UI/L unit was employed.

Determination of the serum enzymatic activities of malondialdehyde, glutathione- S- transferase (MDA), catalase (CAT), and glutathione- S- transferase (GST) was achieved as described earlier.<sup>10</sup>

### Epididymal sperm preparation

After 60 days, a small portion of the cauda epididymis was excised, moved into 1 mL of pre-warmed Ham's F10 medium, and subjected to incubation at  $37^\circ\text{C}$  and 5%  $\text{CO}_2$ . The tissue was sliced carefully for the release of spermatozoa swim-out within the medium. The solution was homogenized and a drop was smeared onto a Hemocytometer (Neubauer, Germany).<sup>11</sup>

The percentage of normal and abnormal sperms was calculated according to the following equation.<sup>12</sup>

$$\begin{aligned} &\text{Percentage of sperm (normal and abnormal)} \\ &= (\text{Normal and abnormal sperms}) \\ &\quad / (\text{sperm total number}) \times 100. \quad (1) \end{aligned}$$

### Sperm analysis and micro-photography

To analyze sperm properties, removal of the epididymis tail was first performed in a clean petri-dish and a solution with 2 ml of saline solution was made (NaCl 0.9), of which one drop was homogenized and smeared on a glass slide with one drop of eosin and one drop of necrosin dyes and the slide was left to dry, followed by incubation (37 °C). Slide fields were randomly selected for microscopic examination to determine the abnormal sperm concentration, which was photographed with a camera (Camera C35) connected to a compound microscope (Olympus BH2). Several cases of abnormalities appeared in the examined sperms, which included alterations in the head, tail, and the position of the cytoplasm.

### Hormonal analysis

Blood was drawn from the hearts of the animals directly (by heart Puncture) and collected in special packages placed in a centrifuge at a rate of 2000 cycles/min for 10 minutes to obtain serum. The serum was isolated from the rest of the blood components and kept at -4°C until used. Hormonal examinations were performed using specialized kits and Minividas hormone measuring device. The serum concentrations of Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH) or Interstitial cell-Stimulating Hormone (ICSH), and Testosterone (T) hormones were conducted.

### Statistical analysis

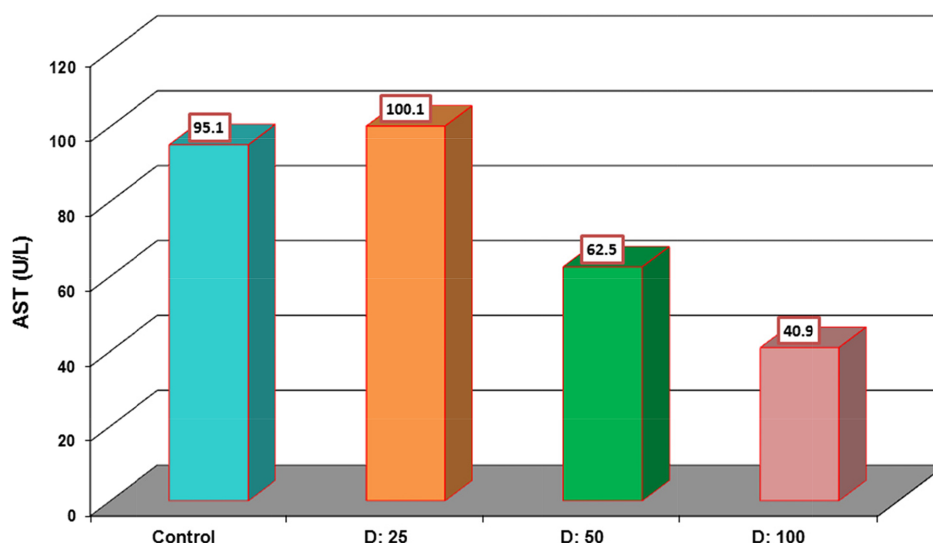
One-way ANOVA was adopted for data comparisons in addition to Tukey-Kramer Multiple Comparisons Test. Results were expressed as mean  $\pm$  standard deviation (SD) whereas a probability limit of  $P < 0.05$  was adopted for the significance of differences among data.

## Results and discussion

### Biochemical parameters

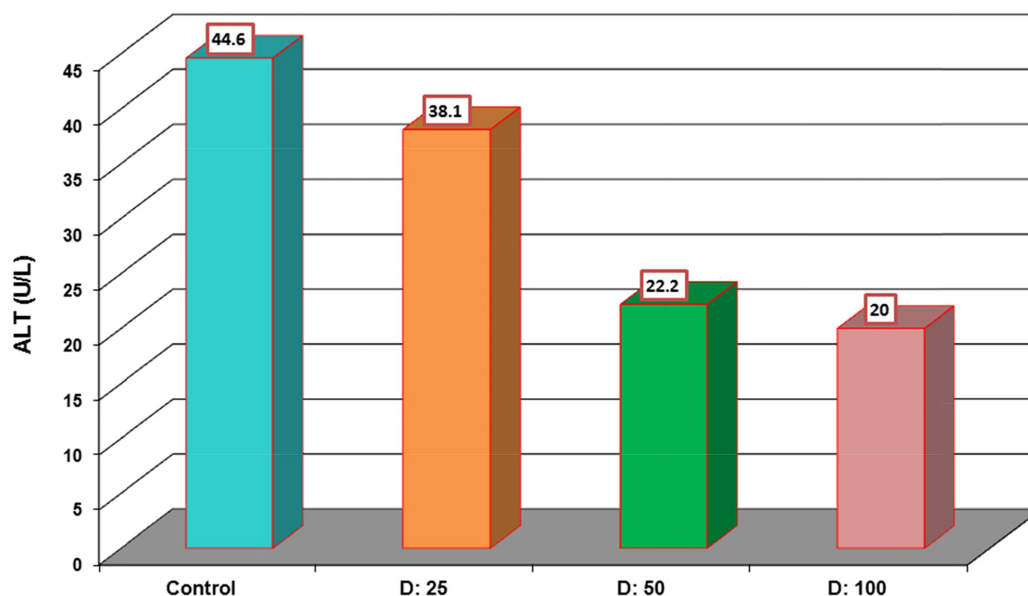
*Stevia rebaudiana* leaf aqueous extract's administration resulted in a significant increase ( $p < 0.05$ ) in the serum concentration of AST, ALT, and ALP in mice groups subjected to treatment with concentrations of (50 mg/kg) ( $62.5 \pm 1.94$ ) ( $22.2 \pm 3.1$ ) ( $25.8 \pm 3.3$ ) and (100 mg/kg) ( $40.9 \pm 6.1$ ) ( $20.0 \pm 5.7$ ) ( $20.2 \pm 4.5$ ) respectively. However, group I revealed only insignificant alterations ( $95.1 \pm 21.2$ ) ( $44.6 \pm 7.7$ ) ( $54.43 \pm 1.36$ ) respectively. In addition, the chronic treatment resulted in significant alterations in the levels of ALT, AST, and ALP in comparison with the control and groups II (25 mg/kg) ( $100.1 \pm 18.3$ ) ( $38.1 \pm 10.4$ ) ( $53.1 \pm 4.09$ ) respectively, Figs. 1 to 3.

Hepatic malondialdehyde (MDA), Catalase (CAT), and Glutathione-S-Transferase (GST) levels were assessed, in addition to the achievement of biochemical examination. The results showed that, after oral dosing male mice with any of the concentrations

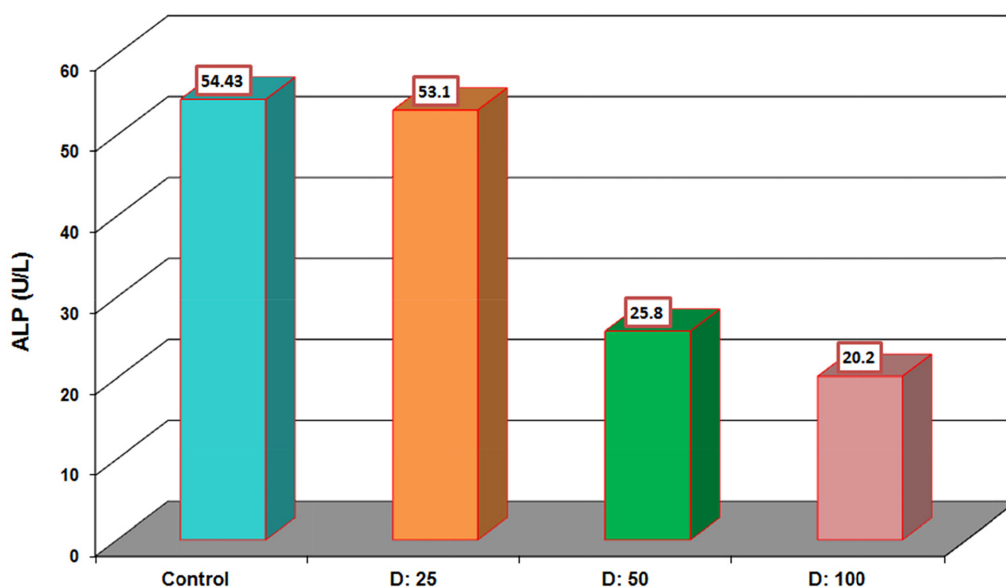


**Fig. 1.** Serum concentration of AST(U/L) in mice groups after oral single dose administration of *Stevia rebaudiana* leaves aqueous extract. Group 1 = ( $95.1 \pm 21.2$ ), group 2 = ( $100.1 \pm 18.3$ ), group 3 = ( $62.5 \pm 1.94$ ), group 4 = ( $40.9 \pm 6.1$ ). The data is presented in mean  $\pm$  SD. Statistical analysis by one-way ANOVA with significant difference  $p < 0.05$ .





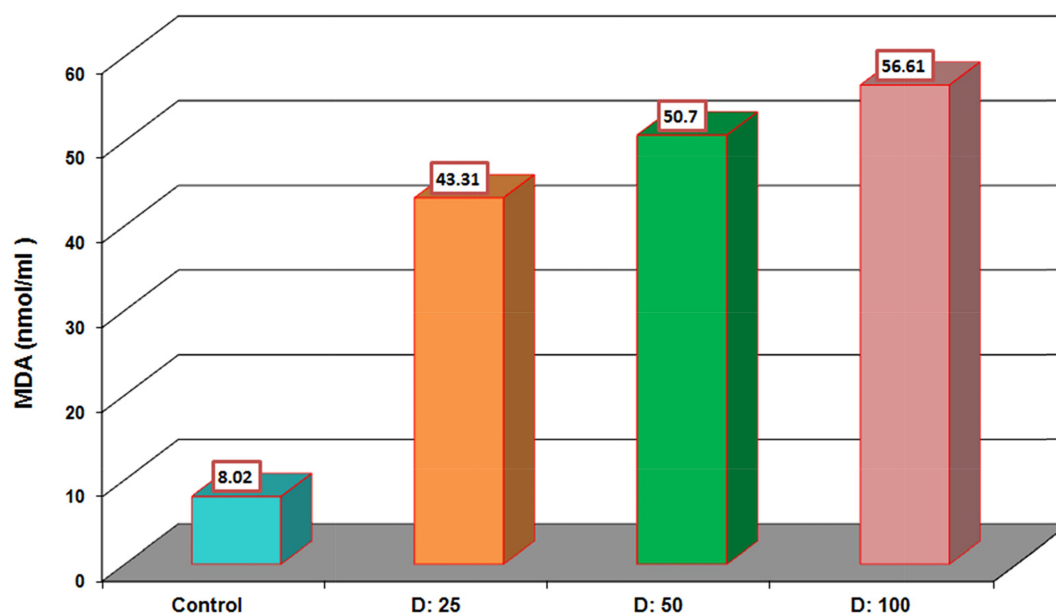
**Fig. 2.** Serum concentration of ALT (U/L) in mice groups after oral single dose administration of *Stevia rebaudiana* leaves aqueous extract. Group 1 = (44.6 ± 7.7), group 2 = (38.1 ± 10.4), group 3 = (22.2 ± 3.1), group 4 = (20.0 ± 5.7). The data is presented in mean ± SD. Statistical analysis by one-way ANOVA with significant difference  $p < 0.05$ .



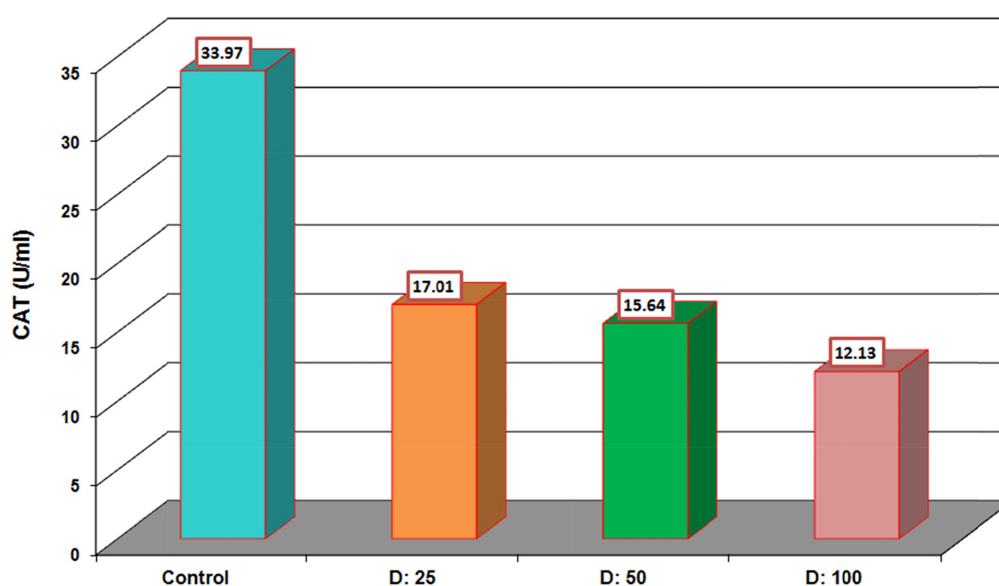
**Fig. 3.** Serum concentration of ALP (U/L) in mice groups after oral single dose administration of *Stevia rebaudiana* leaves aqueous extract. Group 1 = (54.43 ± 1.36), group 2 = (53.1 ± 4.09), group 3 = (25.8 ± 3.3), group 4 = (20.2 ± 4.5). The data is presented in mean ± SD. Statistical analysis by one-way ANOVA with significant difference  $p < 0.05$ .

(25, 50, and 100 mg/kg) of the aqueous extract of stevia leaves for 60 days, there were significant differences in the levels of MDA and anti-oxidant enzymes represented by CAT and GST in the serum of treated mice, in comparison with mice of the control group (dosed with distilled water), as shown in Figs. 4 to 6.

Our data also revealed a significantly elevated average concentration of MDA after administering mice with any of the three concentrations of the plant extract ( $43.31 \pm 3.84$ ), ( $50.70 \pm 3.07$ ) ( $56.61 \pm 1.86$ ), compared with the control group ( $8.02 \pm 0.57$ ), in addition to a significant decrease in of CAT



**Fig. 4.** Serum Concentration of MDA (nmol/ml) in mice groups after oral single dose administration of *Stevia rebaudiana* leaves aqueous extract. Group 1 = (8.02 ± 0.57), group 2 = (43.31 ± 3.84), group 3 = (50.70 ± 3.07), group 4 = (56.61 ± 1.86). The data is presented in mean ± SD. Statistical analysis by One-way ANOVA with significant difference  $p < 0.05$ .



**Fig. 5.** Serum concentration of CAT (U/ml) in mice groups after oral single dose administration of *Stevia rebaudiana* leaves aqueous extract. Group 1 = (33.97 ± 1.46), group 2 = (17.01 ± 0.20), group 3 = (15.64 ± 0.61), group 4 = (12.13 ± 0.40). The data is presented in mean ± SD. Statistical analysis by one-way ANOVA with significant difference  $p < 0.05$ .

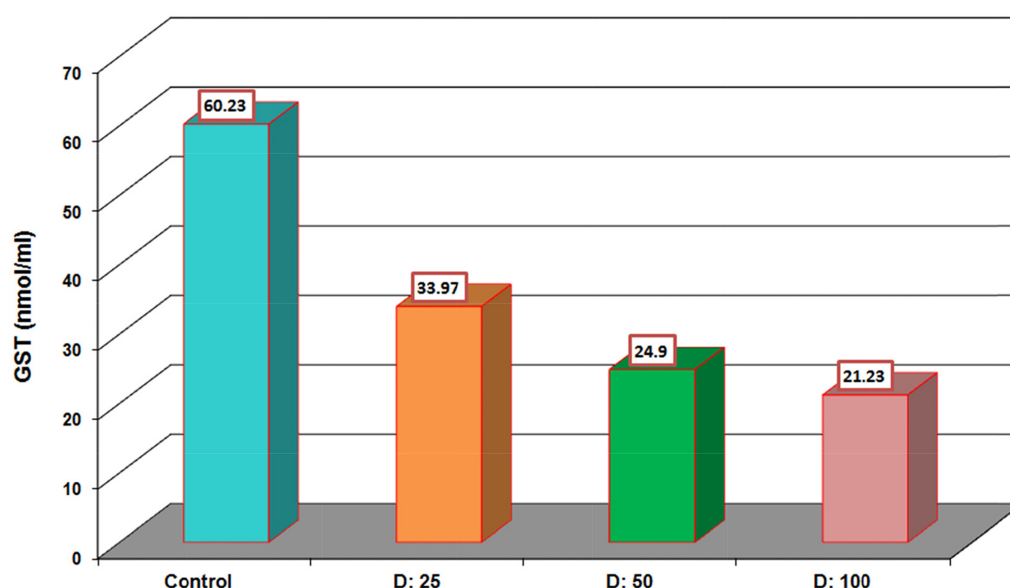
(17.01 ± 0.20) (15.64 ± 0.61) (12.13 ± 0.40) and GST (33.97 ± 0.62) (24.90 ± 2.20) (21.23 ± 0.26) concentrations, as shown in Figs. 4 to 6.

#### Fertility parameters

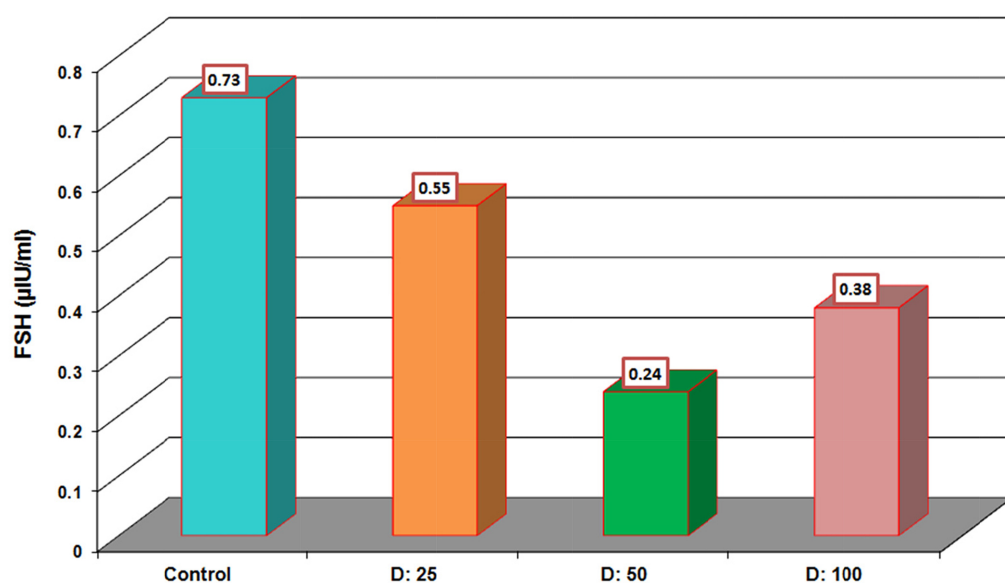
The outcomes of the present investigation also indicated significant reductions ( $P \leq 0.05$ ) in the

mean serum levels of hormones includes, Follicle Stimulating Hormone (FSH), Lutenizing Hormone or Interstitial cell-Stimulating Hormone (ICSH) and Testosterone (T) in male mice treated with *Stevia* leaf extract at all treatment the three concentrations in comparison with the control group Figs. 7 to 9.

Our data also revealed a significant decrease ( $P < 0.05$ ) in sperm concentration in the caudal



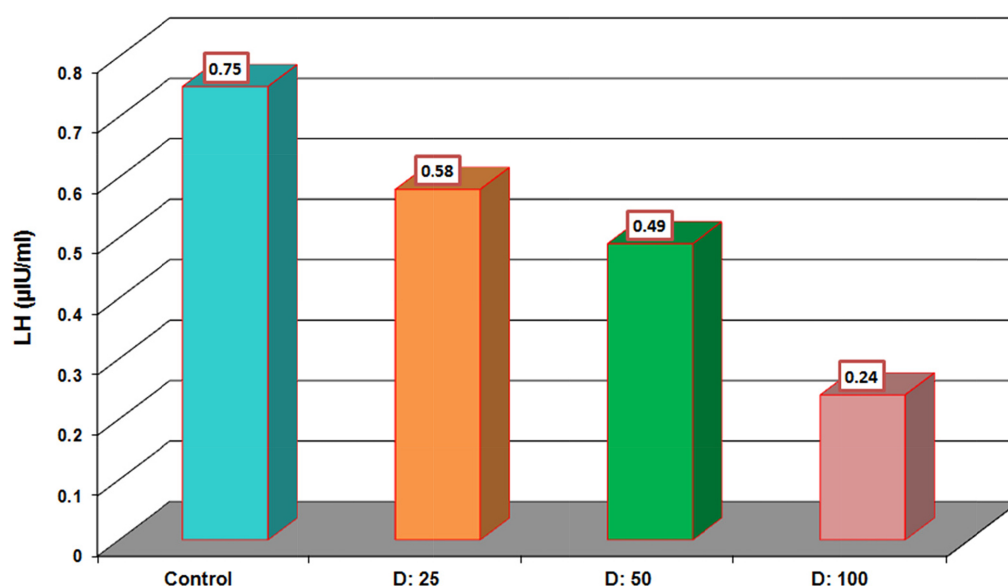
**Fig. 6.** Serum concentration of GST (nmol/ml) in mice groups after oral single dose administration of *Stevia rebaudiana* leaves aqueous extract. Group 1 =  $(60.23 \pm 2.20)$ , group 2 =  $(33.97 \pm 0.62)$ , group 3 =  $(24.90 \pm 2.20)$ , group 4 =  $(21.23 \pm 0.26)$ . The data is presented in mean  $\pm$  SD. Statistical analysis by one-way ANOVA with significant difference  $p < 0.05$ .



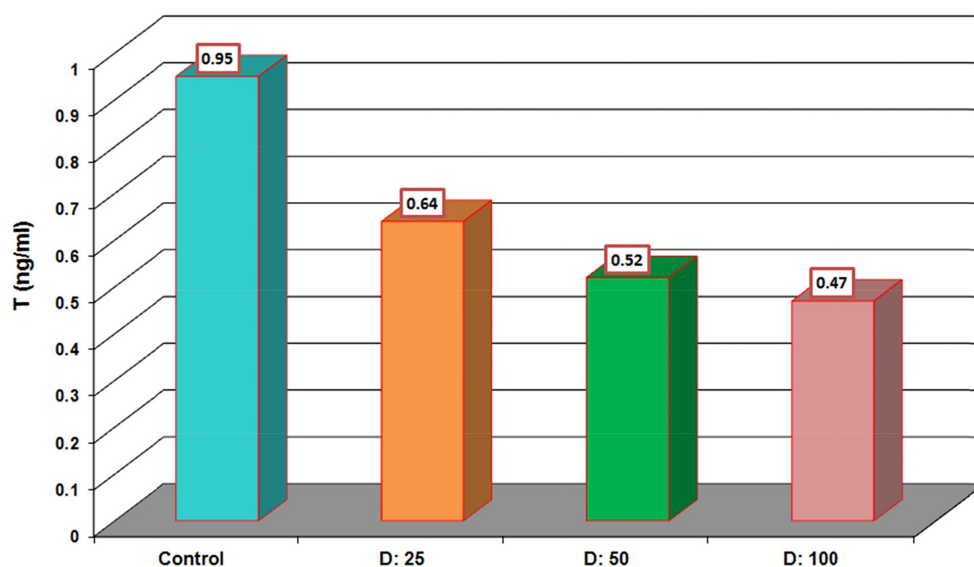
**Fig. 7.** Serum hormonal concentration of FSH ( $\mu$ U/ml) in mice groups after oral single dose administration of *Stevia rebaudiana* leaves aqueous extract. Group 1 =  $(0.73 \pm 0.09)$ , group 2 =  $(0.55 \pm 0.02)$ , group 3 =  $(0.24 \pm 0.05)$ , group 4 =  $(0.38 \pm 0.02)$ . The data is presented in mean  $\pm$  SD. Statistical analysis by one-way ANOVA with significant difference  $p < 0.05$ .

epididymis of animals treated with *Stevia* aqueous extract at all concentrations (25, 50, 100 mg/kg)  $(200.0 \pm 33.06)$   $(123.33 \pm 17.4)$   $(90.0 \pm 43.65)$  respectively, when compared to the control  $(586.6 \pm 40.22)$  Fig. 10. Significant differences were also observed in sperm concentrations, which were found to be decreased with increasing *Stevia* extract concentration, especially at the concentration of 100 mg/kg.

Significant differences ( $p < 0.05$ ) were also recorded in the average values of normal sperms, which included live and dead sperms, as well as deformed sperms, both live and dead, Figs. 11 and 12 as the percentage of normal sperms decreased in the group of animals treated with different concentrations (25,50,100 mg/kg)  $(81.28 \pm 2.28)$   $(78.98 \pm 2.17)$   $(75.32 \pm 8.22)$  respectively, in



**Fig. 8.** Serum hormonal concentration of LH ( $\mu\text{IU/ml}$ ) in mice groups after oral single dose administration of *Stevia rebaudiana* leaves aqueous extract. Group 1 =  $(0.75 \pm 0.05)$ , group 2 =  $(0.58 \pm 0.04)$ , group 3 =  $(0.49 \pm 0.02)$ , group 4 =  $(0.24 \pm 0.03)$ . The data is presented in mean  $\pm$  SD. Statistical analysis by one-way ANOVA with significant difference  $p < 0.05$ .



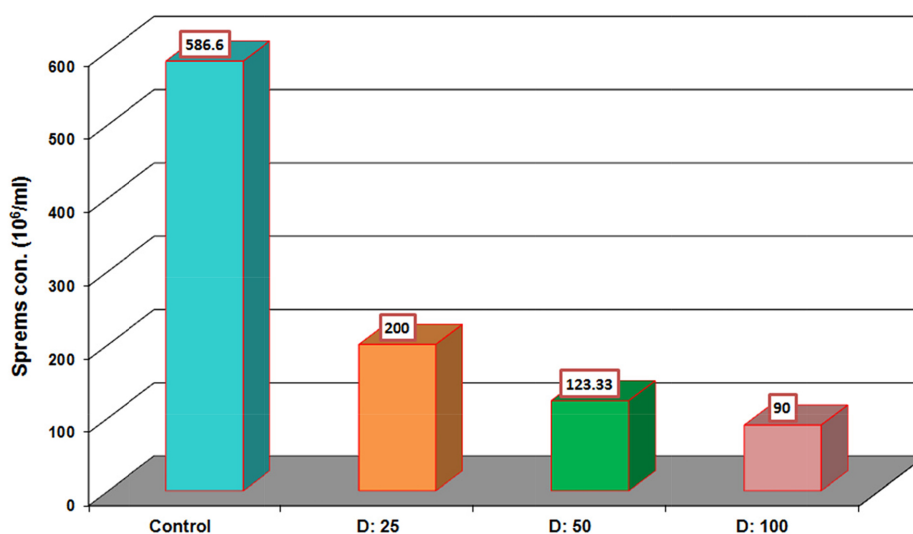
**Fig. 9.** Serum hormonal concentration of testosterone (ng/ml) in mice groups after oral single dose administration of *Stevia rebaudiana* leaves aqueous extract. Group 1 =  $(0.95 \pm 0.03)$ , group 2 =  $(0.64 \pm 0.07)$ , group 3 =  $(0.52 \pm 0.04)$ , group 4 =  $(0.47 \pm 0.05)$ . The data is presented in mean  $\pm$  SD. Statistical analysis by one-way ANOVA with significant difference  $p < 0.05$ .

comparison with the control ( $93.95 \pm 0.73$ ). As for the percentage of deformed sperms, it elevated significantly ( $p < 0.05$ ) in all groups of animals treated with *Stevia* aqueous extract concentrations (25, 50, 100 mg/kg) ( $2.28 \pm 18.72$ ) ( $2.15 \pm 21.01$ ) ( $8.21 \pm 24.66$ ) respectively, than in the control group of animals ( $0.73 \pm 4.07$ ) Figs. 11 and 12.

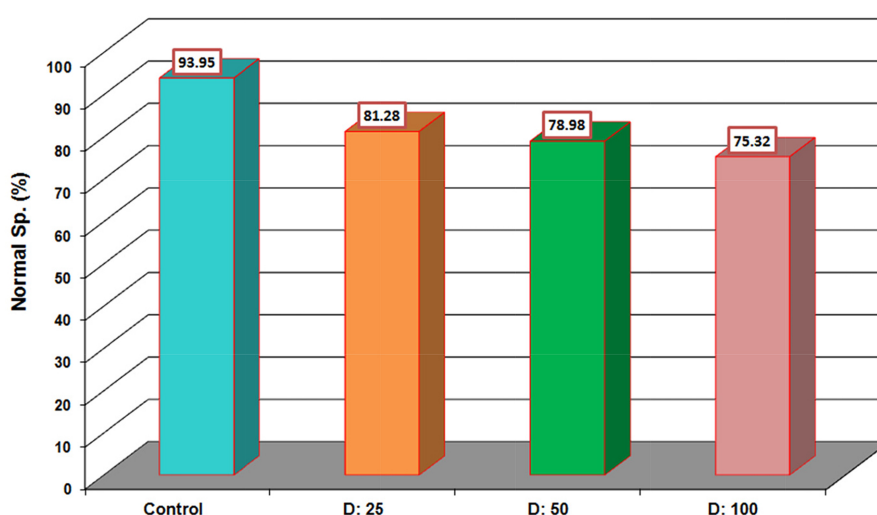
In addition, when comparing the sperms of the control group animals Fig. 13 with the sperms of the animals treated with *Stevia rebaudiana* leaves aqueous

extract, it was observed that abnormal (distorted) cases appeared in all concentrations (25, 50, 100), represented by Head with two tails sperm, curled-tailed and cytoplasmic droplet sperm, sperm with a broken tail, atrophic head sperm, headless & hooked tail sperm, and tailless and short-tailed sperm Figs. 14 to 19.

Studies addressing the influences of *Stevia* on hepatic function are still premature and indecisive. Earlier work demonstrated that treatment



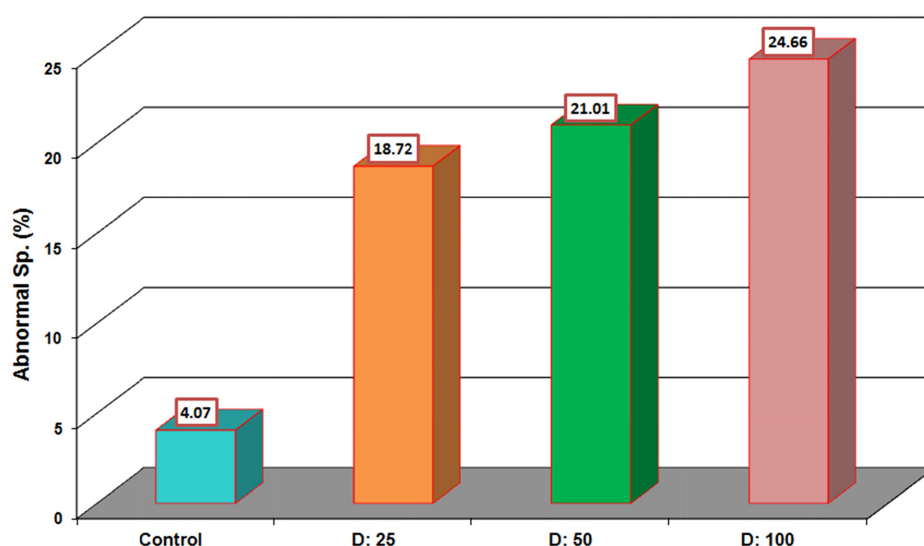
**Fig. 10.** Sperm concentrations ( $10^6/\text{ml}$ ) of male mice after oral single dose administration of *Stevia rebaudiana* leaves aqueous extract. Group 1 = ( $586.6 \pm 40.22$ ), group 2 = ( $200.0 \pm 33.06$ ), group 3 = ( $123.33 \pm 17.4$ ), group 4 = ( $90.0 \pm 43.65$ ). The data is presented in mean  $\pm$  SD. Statistical analysis by one-way ANOVA with significant difference  $p < 0.05$ .



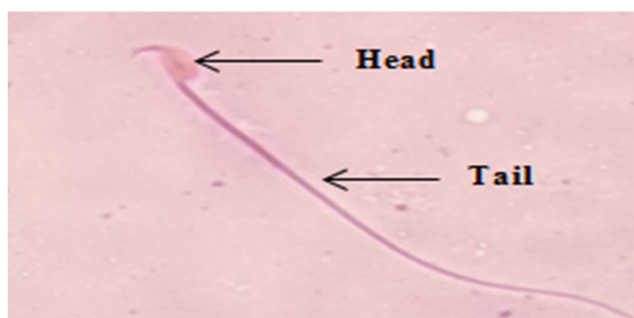
**Fig. 11.** Normal percentage (%) of male mice after oral single dose administration of *Stevia rebaudiana* leaves aqueous extract. Group 1 = ( $93.95 \pm 0.73$ ), group 2 = ( $81.28 \pm 2.28$ ), group 3 = ( $78.98 \pm 2.17$ ), group 4 = ( $75.32 \pm 8.22$ ). The data is presented in mean  $\pm$  SD. Statistical analysis by one-way ANOVA with significant difference  $p < 0.05$ .

with *Stevia* and sucralose is correlated to increased murine liver enzymes (uridine diphosphate-glycosyltransferase UGT76G1 and Catalase), because sucralose might bind to taste receptor subunits to the detection of sweet stimuli T1R3 to activate the downstream signals and further promote lipogenesis in the liver. Sucralose induced reactive oxygen species generation and lipogenesis through T1R3, The enzyme is a uridine diphosphate-dependent glucosyltransferase, UGT76G1, and it catalyzes the addition of branched glucosides to compounds in stevia—primarily two diterpenoids called stevioside and rebaudioside, nevertheless additional investigations

in human models proposed a role for *Stevia* in causing a reduction in the levels of the markers of fatty liver disease.<sup>13</sup> The *Stevia rebaudiana* leaf extract-treated group also exhibited increased ALT levels. Moreover, the aspartate aminotransferase (AST) levels were found significantly increased. Alkaline phosphatase levels and histological examinations, despite 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 to elucidate the specific



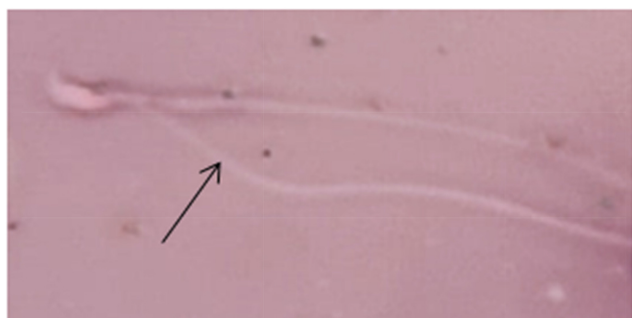
**Fig. 12.** Abnormal percentage (%) of male mice after oral single dose administration of *Stevia rebaudiana* leaves aqueous extract. Group 1 =  $(0.73 \pm 4.07)$ , group 2 =  $(2.28 \pm 18.72)$ , group 3 =  $(2.15 \pm 21.01)$ , group 4 =  $(8.21 \pm 24.66)$ . The data is presented in mean  $\pm$  SD. Statistical analysis by one-way ANOVA with significant difference  $p < 0.05$ .



**Fig. 13.** Normal sperm of control group.

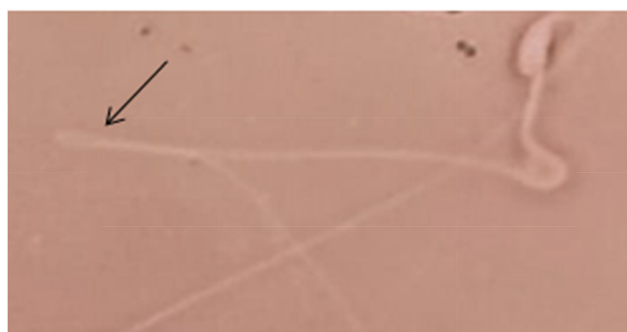


**Fig. 15.** Sperm with a cytoplasmic droplet in right & curled-tailed sperm in left with *Stevia* group (25 mg/kg).



**Fig. 14.** Head with two tails sperm of *Stevia* group (25 mg/kg).

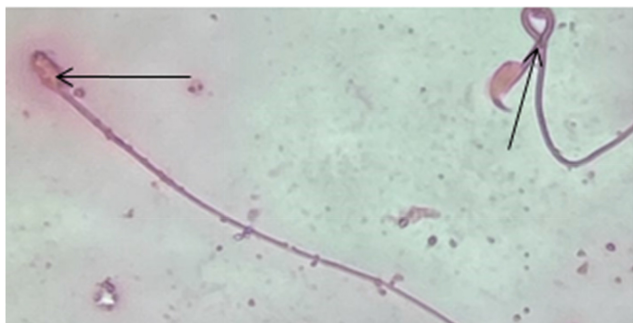
components and mechanisms responsible for the observed variations in liver enzymes.<sup>14</sup> Treatment with stevia caused significant prevention of such changes, revealing powerful antioxidant activity. GPx is among the vital enzymes with anti-ROS activity, utilizing GSH to detoxify  $H_2O_2$ .<sup>15</sup> The chronic consumption of artificial sweeteners (stevia) in beverages contributes



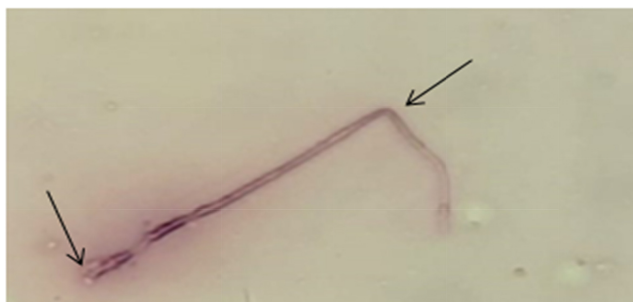
**Fig. 16.** Sperm with a broken tail in mice treated with *Stevia* group (50 mg/kg).

to depression and alters the metabolism, due to an increase in the level of MDA enzyme and alteration in female mice after the long-term consumption of novel natural sweeteners.<sup>16</sup>

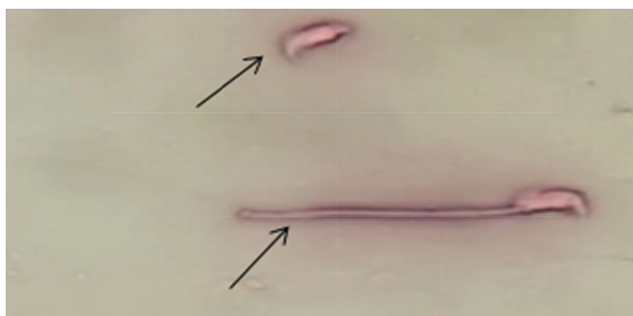




**Fig. 17.** Curled-tailed sperm in right & atrophic Head sperm in left with *Stevia* group (50 mg/kg).



**Fig. 18.** Headless & hooked tail sperm in the *Stevia* group (100 mg/kg).



**Fig. 19.** Tailless sperm (upper) & short-tailed sperm (lower) in *Stevia* group (100 mg/kg).

*Stevia* administration for 8 weeks significantly increased oxidative stress that results from high levels of NO and low levels of the anti-oxidant enzyme SOD, also that *stevia* chronic consumption can decrease the beneficial intestinal bacteria and promotes the unhealthy one.<sup>17</sup> The antioxidant properties of *stevia* leaf extract probably have a biological basis. Polyphenolic compounds from abundant plant sources have often been demonstrated to have antioxidant activities.<sup>18</sup> *Stevia* treatment tended to reduce testosterone levels in the serum, potentially through a presumed affinity of glycosides found in the extract for a

specific androgen receptor, whereas luteinizing hormone levels remained unchanged. These results show consistency with the possible role of *Stevia* extract in reducing the fertility of male rats, *stevia* significantly decreased blood testosterone levels, *Stevia*, the natural sweetener native to South America, was also associated with decreased sperm count and lowered testosterone level, on the other hand, it was reported that the most common sucralose does not impair sperm quality and has no effect on sperm glycolysis,<sup>19</sup> manifested in terms of the functions of accessory sex glands and androgen levels. Such information can confer insights into the degree to which androgens are inhibited in male rats exposed to treatment with *stevia* extract, thereby assisting in interpreting the results of future research on the functions of the reproduction system.<sup>20</sup>

## Conclusion

Chronically administered leaf extract from *Stevia rebaudiana* caused significantly elevated Enzymatic activities of AST, ALT, and ALP. A similar elevation was observed in the average concentration of MDA, whereas those of CAT and GST were significantly reduced in all treated groups in the average concentrations of the enzymes CAT and GST in all concentrations of *Stevia* extract serum. The serum levels of FSH, ICSH, and Testosterone in response to all studied concentrations of *stevia* extract were significantly decreased, and *Stevia* aqueous extract has potential to reduce fertility in male mice.

## Acknowledgment

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## Authors' declaration

- Conflicts of Interest: None.
- I hereby confirm that all the Figures in the manuscript are mine. Furthermore, any Figures and images, that are not mine, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Authors sign on ethical consideration's approval.
- No animal studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

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# تأثير المستخلص المائي لأوراق نبات على بعض الصفات الفسيولوجية والخصوبة في ذكور الفئران البيض *Stevia* *rebaudiana*

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## المستخلص

هدفت الدراسة إلى معرفة التأثيرات المحتملة للمستخلص المائي لأوراق نبات الستيفيا بعد تناوله بشكل مزمن (60 يوماً) على بعض الجوانب الفسيولوجية والخصوبة في ذكور الفئران . تم تقسيم أربعة وعشرين من الفئران البالغة إلى أربع مجموعات ، خمسة ذكور لكل مجموعة ، وتم اختيار ثلاثة تراكيز من الستيفيا على المجموعات المعنوية (25 ملغم/كغم، 50 ملغم/كغم، 100 ملغم/كغم) والباقية منها مجموعة سيطرة . أعطيت الحيوانات عن طريق الفم 0.1 مل لكل 10 جرام من وزن الجسم لكل تركيز من مستخلص أوراق ستيفيا مرة واحدة يومياً لمدة 60 يوماً متتالية وأظهرت النتائج زيادة معنوية في تركيز AST و ALT و ALP للمجموعة المعاملة بجرعة 50 ملغم/كغم و 100 ملغم/كغم من مستخلص الستيفيا، مقارنة مع المجموعة المعاملة بمستخلص ستيفيا تركيز 25 ملغم/كغم ومجموعة السيطرة. أشارت النتائج إلى حدوث زيادة معنوية في متوسط تركيز MDA في جميع تراكيز مستخلص الستيفيا، وانخفاض معنوي في متوسط تركيز الانزيمات CAT و GST في جميع تراكيز مستخلص الستيفيا ، مقارنة مع مجموعة السيطرة . كما لوحظ انخفاضاً معنوياً في تركيز الهرمون المحفز للحويصلات واللويتيني والتستوستيرون في جميع تراكيز مستخلص الستيفيا . كما أظهرت النتائج انخفاضاً معنوياً في تركيز الحيوانات المنوية في البربخ ، و فروقاً معنوية في متوسط النسبة المئوية للحيوانات المنوية الطبيعية والمشوهة ، مقارنة مع مجموعة السيطرة. كشفت فحوصات شكل الحيوانات المنوية عن وجود عدد من التشوهات نتيجة المعاملة ، منها وجود حيمن بذيلين ، حيوانات منوية مكسورة وملتوية الذيل ، حيوانات منوية فاقدة للذيل ، فضلاً عن وجود حيوانات منوية تحوي القطرات السيتوبلازمية. هذه النتائج تتفق مع احتمال أن المستخلصات المائية للستيفيا قد تقلل من خصوبة ذكور الفئران.

**الكلمات المفتاحية:** فئران ألبينو، الخصوبة، إنزيمات الكبد، ستيفيا ريبيديانا.