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The effect of natural sweeter stevia leaves on liver function tests in rats

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ)

صدق الله العليُ العظيم

سورة يونس (ايه 10)

الاهداء

الى من سهَّل لنا الخطوات واخرجنا من العثرات وأنقذنا في أصعب الخطوات أبنُ من دنا فتدلّى فكانَ قابَ قوسينِ او أدنى سيد الدنيا و الآخرة الامام الحسين "عليه السلام"

Summary

Stevia is medical herbs, utilized thousands of years for its sweetening and beneficial properties. Steviol-glycosides, mostly (stevioside and rebaudioside A), are thought to be responsible for stevia sweetness. The popularity of stevia, usually among diabetics and those looking to reduce their calorie-intake. This study Aim to evaluate and investigate the potential influence of Stevia-rebaudiana and focuses on comparing the effects of a commercial-stevia product with those of a stevia leaf extract on specific liver enzymes in a rat model. Commercial stevia and stevia leaves were processed, and three groups of rats were orally treated 25mg/kg for 60 days: (control group, a commercial stevia-treated group, and a stevia-leaf herbal treated group). The results revealed a significant elevated in alanine-aminotransferase level in the commercial-stevia-treated group, suggesting potential effects on liver function. The stevia-leaf-herbal-treated group also showed increased in ALT level, referred to specific compounds in the extract inducing liver enzyme activity. Aspartate-aminotransferase level increased significantly in both treatment groups compared to the control. Alkaline phosphatase enzyme showed no notable changes between groups. This study has concluded, although stevia is generally regarded as safe in limited dose, this study underscores the importance of considering the type and form of stevia in evaluating its effects on liver-health .

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Introduction & Literature Review

1.1. Introduction:-

Stevia-rebaudiana" is consider as herbal native to South-America that indigenous people have used for ages as a sweetener and medicinal herbs. Stevia's high sweetness is due to the presence of sweet molecules known as steviol-glycosides. The most notable glycosides are "stevioside and rebaudioside A" Stevia herbs has grown popularity in last years as a natural, zero or low-calorie alternative to typical sweeteners like sugar and artificial-sweeteners. The plant's leaves are the main source of these pleasing chemicals. They are usually harvested, dried, and processed to produce steviol glycosides, which can be up to 300 times sweeter than table sugar (sucrose).The most benefits of stevia herbs is its potential as a sugar-alternative for diabetic's patients or anyone seeking to reduce their calorie-intake. Steviol-glycosides do not add calories or carbohydrates to the diet and have tiny effect on blood glucose levels. Stevia has also been examined for possible health advantages. Some research show that it may have anti-inflammatory, antioxidant, and antihypertensive-properties . However, it is necessary to note that, while stevia is typically considered harmless, individual-reactions to sweeteners might vary, and more research is required to properly understand its long-term effect and large amount ingestion.

Research on commercial-stevia products, mainly those approved for use as sweeteners, have naturally shown that they are safe to consume. The "U.S. Food and Drug Administration (FDA)" and the "European Food Safety Authority (EFSA)" have determined acceptable daily consumption amounts for specific steviol-glycosides

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. some research concerns the potential hepatoprotective (liver-protective) special effects of stevia in animal models

1.2. Aim of study:

This study Aim to evaluate and investigate the potential influence of Stevia-rebaudiana and focuses on comparing the effects of a commercial-stevia product with those of a stevia leaf herbal on specific liver enzymes in a rat model

1.3. Literature Review

1.3.1. Stevia's sweetness

The sweet-tasting components of stevia are called steviol glycosides, which are naturally present in the stevia leaf There are 11 major steviol glycosides of which rebaudioside A and stevioside are the most abundant [1].

1.3.2. Stevia production

The process of purifying stevia into high-purity stevia leaf extract is similar to how other plant-based ingredients, such as cane sugar or natural vanilla extract, are made through a series of steps beginning with the harvested, raw plant material through to the end product. The process begins by drying the leaves and then steeping the min hot water. Next, the liquid extract is filtered and purified with water or in some cases in combination with food-grade alcohol. If food-grade alcohol is used, it is later removed and no significant amount of alcohol remains in the end product. Other processes may be used in high purity stevia, or rebiana. Only high-purity stevia extracts meeting this specification are approved by major regulatory agencies, the Joint Food and Agriculture Organization/WHO Expert Committee on Food Additives 4 and Codex, for use in foods and beverages. For simplicity, the term ‘‘stevia’’ as used in this article refers to purified steviol glycosides [2].

1.3.3. Stevia Metabolism

The backbone of all steviol glycosides is steviol, to which various glycoside (glucose) groups attach to form the variety of sweet compounds in stevia. Steviol glycosides pass through the upper gastrointestinal tract fully intact. Gut bacteria in the colon hydrolyze steviol glycosides into steviol by snipping off their glucose units. Steviol is then absorbed via the portal vein and primarily metabolized by the liver, forming steviol glucuronide, which is primarily excreted in the urine.

Research shows that there is no accumulation of stevia (or any component or byproduct of stevia) in the body and that it passes through the body during metabolism. Energy from fermentation of glucose units (usually assessed as 2 kcal/g) is so low that it is minimal, and so, effectively, stevia can be said to provide zero calories "High-purify stevia leaf extract is not metabolized, so it provides zero calories." [3].

1.3.4 .The effect on the liver-health

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. some research concerns the potential hepatoprotective (liver-protective) special effects of stevia in animal models, indicating that it may have antioxidant and anti-inflammatory effective [4].

The last decade saw an intensive elevation in the number of food products that contains non-caloric sweeteners in order to overcome health problems associated with obesity and diabetes. And many research studies have focused on sweetener consumption in obese and diabetic patients. In both cases, the main purpose was to reduce the caloric intake in their usual diet The use of artificial sweeteners, as sucralose, has been increased due to the health problems related to sucrose. Sucralose, 1,6- dichloro-1,6-dideoxy-b-D-fructofuranosyl-4-chloro-4-deoxy-a-Dgalactopyranoside, is a synthetic disaccharide that is produced from sucrose when three hydroxyl groups are replaced by three chlorine atoms. Sucralose has 385–650 more sweetness than sucrose depending upon the specific food application [5].

According to U.S. Food and Drug Administration, the acceptable daily intake (ADI) level for sucralose is 5 mg/kg per day (U.S. FDA, 1998); and according to Scientific Committee on Food of the European Commission, DAI is 15 mg/kg/d (SCF, 2000).

Also, sucralose usage is permitted in pregnancy, nursing, with children and patients with medical conditions [5].

1.3.5 .The hazardous effects of artificial sweeteners

The hazardous effects of artificial sweeteners have directed the consumers

towards natural sweeteners as stevia. This approach suggests that anatomic, physiologic and biochemical mechanisms between species have some special characteristic when an allometric scale is used for potential differences in the pharmacokinetic /physiological time [6].

1.3.6.Enzymes used as Liver function tests

Transaminases (Aminotranferases) Aminotransferases The aminotransferases (ALT and AST) are enzymes involved in the transfer of an amino group from a2-amino acid to a 2-oxoacid; they need the cofactor pyridoxal phosphate for optimal activity. They are widely distributed in the body(AST, formerly known as SGOT [serum glutamic-oxaloacetic transaminase]; also called aspartate aminotransferase) and alanine transaminase (ALT, formerly SGPT[serum glutamic pyruvictransaminase]; also alanine aminotransferase) are participants in gluconeogenesis. Both enzymes are plentiful in the cytosol of the hepatocyte, and an AST isozyme is present in the mitochondria as well. AST is also found in a variety of tissues, including heart, brain, and skeletal muscle; ALT is more specific to the liver. These enzymes are elevated in many forms of liver disease, presumably as a result of leakage from damaged cells. Substantial hepatic necrosis as found in chemical and ischemic injury appears to be particularly associated with elevation of these enzymes. Advanced cirrhosis can exist without significant elevations if active cell injury is absent or minimal at the time of evaluation [7].

The relative increase in AST compared with ALT can be useful in supporting a diagnosis of alcohol injury (AST/ALT > 2) versus most other acute liver injuries (AST/ALT $\leq$ 1), although cirrhosis is also associated with AST/ALT ratio greater than 1. Absolute levels can be diagnostic when extreme and helpful when moderately elevated Aspartate transaminase (AST), which may also be referred to as aspartate aminotransferase or serum glutamic-oxaloacetic transaminase (SGOT) in other literature, catalyzes the reversible transfer of amino group between aspartate and glutamate. Like ALT, AST is found in the cytoplasm of hepatocytes and other tissues, including skeletal muscle [8].

Injury to hepatocytes causes leakage of AST into the extracellular compartment with subsequent elevation in serum AST activity The magnitude of ALT elevation is usually greater than AST when both are elevated due to hepatocellular injury because of the longer half-life of ALT and the greater fraction of AST that is bound to the mitochondria . The practice of considering a high ratio of AST/ALT to be more indicative of skeletal muscle injury has been suggested and may aid in distinguishing muscle versus liver injury [9].

Chapter two

Chapter two

Material and methods

2.1 Material and methods:

Study was carried out at "Babylon University" in Babylon, Iraq, in period from December 2023 to March 2024. Stevia-leaves and commercial-stevia were obtained from the local-market in Iraq. This study involved thirty six healthy-adult male rats (albino Wistar rats) weighing between (240 and 250 gm). Before beginning the research, all rats were acclimatized to the normal-circumstances of 12 hours of light and 12 hours of darkness at 25 ± 4 ºC.

2.1.1 Preparation of Stevia Leaf solution

Stevia-leaf solution was prepared by drying the stevia leaves in the dark, grinding with a mortar, 20 g were measured on a sensitive-balance, and washing them three times with distilled-water. The leaves were placed in a flask with 1000 ml of distilled water. The stevia solution was filtered through Whatman No.1 filter paper, and the supernatant make as stoke solution. From the stoke solution was prepared the solution concentration 2mg/1ml by taken 100ml of stoke solution to completed to 1 liter . from this solution was given to rat orally 3ml daily that insurance the 25mg/1kg ingestion daily.

While the commercial-stevia product was prepared by dissolving two sachet equal 2g in the 1 litter. from this solution was given to rat orally 3ml daily that insurance the 25mg/1kg ingestion daily .

2.1.2 Chemicals and Instruments

Chemicals used in this study, is tabled below in the table (2-1).

Table 2-1 :Chemicals ,Instruments and equipments used in this study

Instruments and equipment's		Chemicals	
1	Beaker	1	Diethyl ether
2	Centrifuge	2	Distilled water
3	Electronic Balance	3	Alcohol
4	Eppendorf tube	4	ALT- kit
5	Micropipette	5	AST- kit
6	Oral gavage	6	ALP- kit
7	Spectrophotometer	7	Stevia commercial
8	Syringe 1ml ,3ml ,5ml	8	Stevia –leaf –herbal
9	Water bath		
10	Gel Tubes		

2.2 Experimental designs

Animals rats (n=36) were randomly separated into three groups; the first group (n=12) served as the untreated rats group "control", the second-group (n=12) received a 25mg/kg dosage of commercial-Stevia (market-stevia), and the third-group (n=12) was treated with a 25mg/kg dosage of Stevia-leaf-extraction. Stevia was liquefied in distilled-water and administered orally to the rats for 60 days. Following the 60-day treatment period, the animals received anaesthesia using “Di ethyl ether" and a heart-puncture was directed to collect (1 ml) of blood. The

acquired blood samples were stored in gel tubes and keep it 30 min at room temperature . Following that, the blood samples were centrifuged at 1800 rpm for (15 minutes), and the serum was cautiously transferred into new-tubes. The serum-samples were kept at -20 ºC until the analysis .

2.2.1 Alanine aminotransferase test:

This test depend on Wrobleski and Ladue methods for testing alanine aminotransferase (ALT), which converts alanine to pyruvate. Through this reaction, NAD^+(Nicotinamide Adenine Dinucleotide) is transformed into "NADH". The reduction of NAD^+ to NADH is normally associated with a co-substrate or co-enzyme in enzymatic processes and is proportional to the samples ALT activity level. This change in absorbance is detected by a spectrophotometer at 340 nm

2.2.2. Asparate aminotransferase (AST) test

This test is based on an enzymatic-reactions described by Karmen that includes the transfer of an amino-group from "aspartate to alpha-ketoglutarate". The change of NAD^+ to NADH, results in a measurable change in light-absorption at a particular wavelength. This change in absorbance is detected by a spectrophotometer at 340 nm.

2.2.3 Alkaline phosphatase (ALP) test

Alkaline-phosphatase enzyme (ALP) calculated depend on catalyzes the hydrolysis of *p*-nitrophenylphosphate(p-NPP), resulting in the synthesis of free *p*-nitrophenol and inorganic-phosphate, while the alkaline buffer serves as a "phosphate-group acceptor". The rate of production of *p*-nitrophenol at wavelength 405 nm is used to monitor the reaction-kinetically, and it is proportional to the activity of ALP in the specimen.

2.4 Statistical Analysis:

All statistical results, Figures and Tables were done using Microsoft Excel 2020 and GraphPad-prism program software (version 6) using independent t-tests, analysis of variance (ANOVA), regression analysis, and non-parametric tests.

Chapter three

Chapter three

Results and Discussion

3.1. Result and Discussion:

In the table 3-1 was showen the result Statistically Analysis

Table (3-1): Statistical analysis of enzymes in the rats groups under different treatment with commercial stevia and stevia leaf extraction and control, *p* value ≤0.05

Groups	N	Mean of ± SD (U/l)	*P* value	Significant in *p* value ≤ 0.05
ALT enzyme level of the rats groups				
Control	12	27.50 ± 4.286		-
Commercial Stevia	12	68.80 ± 8.205	$< 0.0001^{a,c}$	Yes (S****)
Stevia Leaf Extraction	12	56.40 ± 10.72	0.0002^{b}	Yes (S***)
AST enzyme level of the rats groups				
Control	12	40.50 ± 6.501		-
Commercial Stevia	12	50.05 ± 12.23	0.0038^{a}	Yes (S**)
Stevia Leaf Extraction	12	50.55 ± 14.71	0.0081^{b} 0.9076^{c}	Yes (S**) NS
ALP enzyme level of the rats groups				
Control	12	206.0± 81.89		-
Commercial Stevia	12	208.3± 73.54	0.303^{a}	NS
Stevia Leaf Extraction	12	217.9± 82.51	0.5081^{b} 1.2076^{c}	NS NS

*S: significant, NS: non-significant, a: *p* value between Control and commercial stevia groups, b: *p* value between control and stevia leaf extract groups, c: *p* value between commercial stevia and stevia leaf extraction groups. Significant in p value ≤ 0.05

The data obtainable in Table 1, precisely detailing the alanine aminotransferase enzyme (ALT) test results, a statistically significant distinction appears between the control-group (mean value 27.50 ± 4.286 U/l) and the group treated with commercial-stevia (mean: 68.80 ± 8.205 U/l), with a highly significant *p*-value of <0.0001. Additionally, the results reveal a significant difference between the control-group and the group treated with stevia-leaf-extract (mean value 56.40 ± 10.72 U/l), representing statistical significance with a *p*-value of 0.0002. Additionally, a significant difference is detected between the groups treated with commercial-stevia and stevia-leaf-extract, showing a *p*-value of 0.0002.

The experiential differences in ALT (alanine aminotransferase) levels between groups can be recognized to the effects of numerous treatments with stevia and its derivatives. ALT is an enzyme found mainly in the liver, and variations in its levels frequently indicate liver-health and function disorder. 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. This could be attributed to additives, processing, or "other components" included in commercial stevia. The rat group treated with stevia-leaf-extract also exhibited a significant difference in ALT enzyme levels. This suggests that there could be specific chemicals or quantities in the stevia-leaf-extract that affect liver-function.

The significant difference gotten between the two treatment rats groups (commercial stevia and stevia-leaf-extract) proposes that the type or form of stevia used may effect the observed level of ALT enzyme. This can be due to variances in the chemical compounds, processing-processes, or concentrations of active-components between the commercial stevia and the stevia leaf-extract.

Aspartate aminotransferase (AST) level enzyme results in Table 1 show substantial differences in this enzyme values between the groups. The rats group treated with commercial-stevia had significantly greater AST levels (mean 50.05 ± 12.23 U/l) than the control-group (mean 40.50 ± 6.501 U/l), with a *p*-value of 0.0038. The study found that rats group treated with Stevia-Leaf-extract had

significantly greater AST enzyme levels (mean 50.55 ± 14.71 U/l) compared to the control group (*p*-value = 0.0081). Remarkably, there is non-significant difference between the groups treated with commercial-stevia and stevia-leaf-extract. These findings reveal that both cause a significant increase in AST levels, with similar effects regardless of the accurate "stevia formulation used".

The elevation in AST enzyme level of rat shows that the commercial-stevia treatment may have an effect on liver function. Other factors can causing this elevation, such as specific components in the commercial stevia or dosage. Likewise, rats treated with Stevia-Leaf-extract show a significant elevated in AST enzyme levels; this result suggests that the impact on AST levels is not limited to commercial-stevia and can also be seen with the use of stevia-leaf-extract. The results illustration that both the commercial-stevia and the stevia-leaf-extract treatment groups had significantly higher AST enzyme levels than the control-group. Result show the potential impact of stevia products on liver function and demands for more research into the exact components and mechanisms affecting these alterations in AST enzymes level.

The results affecting to ALP level enzymes in rats (Table 3-1) revealed no statistically-significant-differences between the serum samples from the control-group, the group treated with commercial-stevia, and the group treated with stevia-leaf-extract. The mean values were 206.0 ± 81.89 U/l, 208.3 ± 73.54 U/l, and 217.9 ± 82.51 U/l, respectively.

Since ALP is an enzyme found in numerous tissues, including the liver, bone, and intestines, the consistent ALP levels across the groups may indicate that the therapies had no negative impact on these specific tissues. The absence of substantial-differences in ALP enzyme levels between the groups implies a degree of safety for potential hepatotoxic consequences. Still, a more comprehensive investigation incorporating additional-parameters and longer term explanations is necessary to determine the broader physiological-impact of the treatments on different-tissues and organs

Conclusion

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

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خِتاماً لهذه المرحلة من المسيرة العلميّة نقول:

بسمه تعالى:

وفوقَ كلّ ذي علمٍ عليم.

يُشرّفنا ان نعتمرَ قبّعاتِ التخرّج، والتي كانَ مسلمي الأندلس الأصلَ في ابتكارها لغرضِ وضع المُصحفِ فوقها تطبيقاً للقول الشريف آنِفِ الذِكر.

ووفقنا الله وايّاكم في اكمال مسيرة طلب العلم من ذويه