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Exploring the impact of stevioside and *stevia Rebaudiana* Bertoni (SRB) extract on the TyG index in male rats

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Abstract
Stevia rebaudiana (Bertoni) and its components such as stevioside has become an important economic plant for its commercial use as a sweetener. Nowadays, stevia plays a significant role in the healthcare practice of the many cultures in different populations. This study aimed to evaluate the effects of *Stevia rebaudiana* (St) extract and stevioside (Sd) oral administration (cavage) on the hepatic functions in male rats and to estimate the relationship between consumption of high dose of Sd on TyG index. Thirty (30) adult rats were randomly selected and divided equally into three experimental groups and treated for one and two months as the follows, CONT: 10 rats in this group served as control, 10 rats in St group were given 250 mg/kg B.W. of stevia extract, and 10 rats in Sd groups were given 250 mg/kg b.w. Sd solution. At the conclusion of the tests, fasting blood samples were obtained using the heart puncture technique, and serum samples were obtained for the determination of the serum lipid profile, liver enzymes, and FBG and all rats equally drinking water during the period of two month. The results shows significantly increase levels of TC, TG, LDL, VLDL, and decreasing levels of HDL (mg/dl) (p-value<0.05) in Sd group compare to St and CONT groups. The results also showing statistical differences in TSB, ALT, AST, and ALP (U/l) levels between all study groups (p-value< 0.05). TyG index were calculated in all study groups and also showing significant variables in Sd group (5.5) compare to St and CONT groups. In conclusion. The results of current study showing Sd is more risk for hyperlipidemia (elevated in lipid profile) more than St and more risk to increase insulin resistance (elevated in TyG index) and metabolic disorders and liver diseases (elevated in liver enzymes).

Keywords: *Stevia rebaundiana*, stevioside, lipid profile, liver enzymes, TyG index

Introduction
A little perennial herb native to South America is called Stevia rebaudiana bertoni (Asteraceae). Traditional bitter beverages like mate tea have been sweetened with dry stevia leaves [1]. This work expands upon the body of scientific knowledge regarding stevia and its glycosides as naturally occurring sweeteners and potentially medicinal compounds. This study also addresses the safety issues with human intake. There are 230 species in the Asteraceae family's genus Stevia, including Stevia rebaudiana Bertoni, which yields sweet steviol glycosides [2]. Growing throughout South America, especially in Brazil and Paraguay, S. rebaudiana is a perennial shrub sometimes referred to as "Honey Leaf," "Sweet-Leaf," or "Sweet-Herb." It has been demonstrated that stevia preparations are utilized in a variety of forms, including dry and fresh S*tevia* leaves, powdered stevia leaf, liquid concentrations and extracts. Since stevia extract is 200-300 times sweeter than sugar, it's a fantastic substitute for artificial sweeteners [3]. A natural sweetener called stevioside is taken from the leaves of the Stevia rebaudiana plant [4]. Stevia sweeteners are derived from the leaves of the Stevia plant and undergo a purification process to reduce bitterness, with steviol glycosides like stevioside being the key components [5]. Stevioside, alongside rebaudiosides A, is the primary sweetener present in the Stevia plant's leaves, historically used for its sweetening properties in various regions worldwide. Research indicates that stevioside, a glycoside found in Stevia, shows potential in promoting cancer cell death and impacting mitochondrial pathways, hinting at possible anticancer properties [6]. A notable method involves converting stevioside into rebaudiosides A and B to enhance taste, hinting at the versatility and application of these compounds [7].

Sweeteners, sometimes known as sugar replacements, are compounds like aspartame, sucralose, and substances derived from stevia that are used to sweeten food and sometimes even improve its flavor [8]. Studies, both preclinical and clinical, have shown that stevioside is not degraded by stomach-related enzymes or gastric juice [9]. Furthermore, oral stevioside is not ingested at the level of the upper small intestine, most likely because to its high subatomic weight [10]. Bacterial digestive plants (Bacteroides class) can contaminate stevioside in the lower gastrointestinal tracts of rodents, mice, pigs, and humans, converting it to free steviol, an aglycone of steviol glycosides [11]. Following 750 mg/day of stevioside use, an analysis including human subjects revealed no detectable levels of stevioside, free steviol, or any other steviol metabolite in the blood. It explained in detail that steviol might be found in the waste [12]. The aim of this study to evaluate the effects of *Stevia rebaudiana* bertoni (St) extract and stevioside (Sd) oral administration on the hepatic functions and lipid profile of male rats.

Materials and Methods

Collection and identification of *stevia* and stevioside

Fresh *stevia* were collected from market in Babylon province and stevioside were brushed from China. The process was done daily to get freshly prepared extract for daily administration.

Preparation of the plant extract

Stevia rebaudiana Bertoni leaves were ground in a grinding mill, and the resulting dried stevia leaf powder was passed through a 1mm mesh sieve. After that, 500g of this powder was dispersed in 1000 ml of water and incubated at 90°C for 30min to obtain the suspension. Daily, 1ml of this suspension was adjusted to be containing 250 mg/kg/day of *stevia* leaf powder was orally administered to the rats by gavage. Stevioside solution was prepared by dissolving of 150 mg in 100 ml of water and the administration was 150 mg/kg/day.

Animal care handling

For this study, (30) adult male wistar rats weighing 100-120g were bought from the animal house of Babylon university, Hilla city, Iraq. The test animals were kept for fourteen days in iron cages under standard conditions following the procedures of Ezejiofor and Orisakwe (2015). The protocol for the experiment was approved by the Al-Qasim Green University under Research Ethics Committee no.2023/2024. The animals were given standard feed and water.

Experimental design

Weight-matched rats were divided into three groups of ten rats of each, group 1 was maintained as the normal control and was given only feed and water for one month, while group 2 was maintained as the negative control and given 1ml of 500 mg/kg.bw stevia extract (St) for one months, and group 3 was administrated 1ml of stevioside (Sd) (250 mg/kg.bw) for one and two months. All groups were harvested to assessment of liver functions and lipid profile measurement in the end of 1st and 2nd month of this study. The volume of administration was calculated according to the average weight of the animals in each treatment group.

Sample Collection and Sacrifice

At the end of the treatment (1st month and 2nd month), the rats were sacrificed by cervical dislocation (blood was collected by ophthalmic puncture). The liver was quickly removed and fixed in 10% of formaldehyde. After centrifugation of the homogenates (3000 rpm, 15 min), the supernatants were used for biochemical assays related to liver function damage and lipid profile as described below.

Biochemical Analysis

Evaluation of liver function

Heparinized blood was centrifuged at 4000 rpm for 10 min for biochemical analysis. We measured the activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP) and total bilirubin (TB). All parameters level were estimated by colorimetric method by used specific kits depending on manufacture instructions.

Assessment of lipid profile

Lipid profile were assessed by spectrophotometric analysis for estimation of TC, TG, LDL, VLDL, and HDL by used kits and depending on manufacture instructions.

TyG index estimation:

Depending on the following equation (12).

ln (TG [mg/dL]×glucose [mg/dL]/2).

Statistical Analysis

Statistical analysis will be performed using the Excel and the data were expressed as means ± S.D. The effects of treatments were evaluated statistically using the one-way analysis of variance (one-way ANOVA) followed by multiple comparison treatments. Statistical significance is set at the $p < 0.05$ level.

Results

In the Baseline investigation, out of 30 rats including in this study, 10 rats were included in the CONT group, 10 rats were in St extract group and 10 rats were in the Sd group. The Mean ±SD of weight of all rats in CONT group was 250±13.13 gm, in St group was 245±10.76 gm and in Sd group was 242±9.66gm, respectively. Table 1 shows the blood parameters of all rats in all groups after two months of experiment that showing statistically decrease in WBC of Sd group (2.73 compare to CONT group (5.47) and increasing in Lym% (36.6) in CONT group compare to (86.9) in Sd group (p-value 0.001).

Table 1: Blood parameters in study groups at end of experiment

2nd Month	WBC	Lym%	Hb	PCV
CONT	5.49	36.6	13.2	40.7
St	5.05	66	13.8	38.6
Sd	2.73	86.9	14.8	32.3
p-value	0.001*	0.001*	0.044	0.012*

Figures 1, 2, and 3 shows the levels of TC, TG, LDL, VLDL, and HDL (mg/dl) in different study groups.

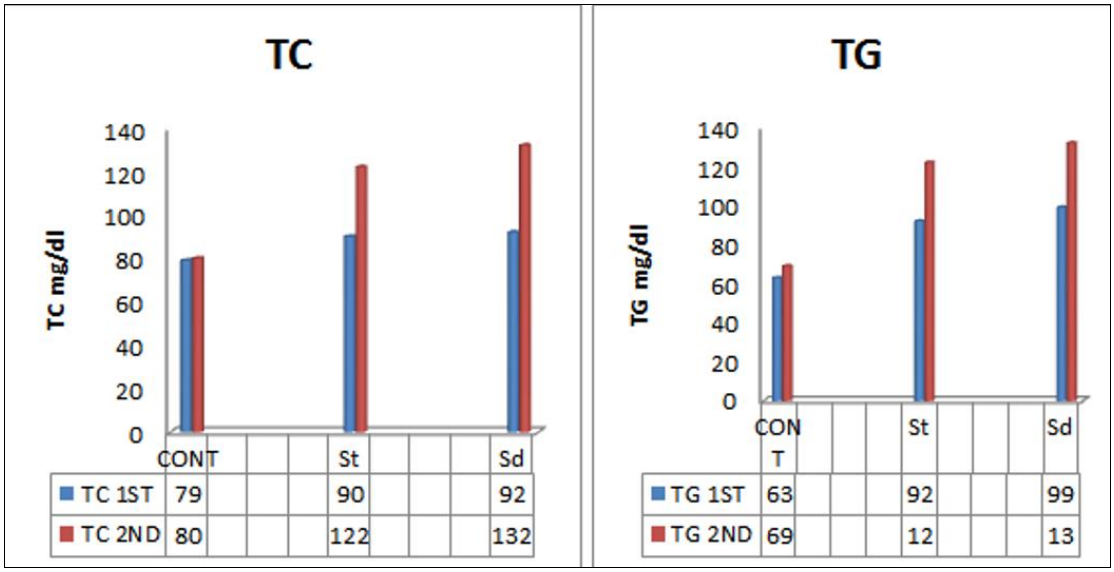

Fig 1: Levels of TC and TG (mg/dl) in study groups aftet one and two months of experiment

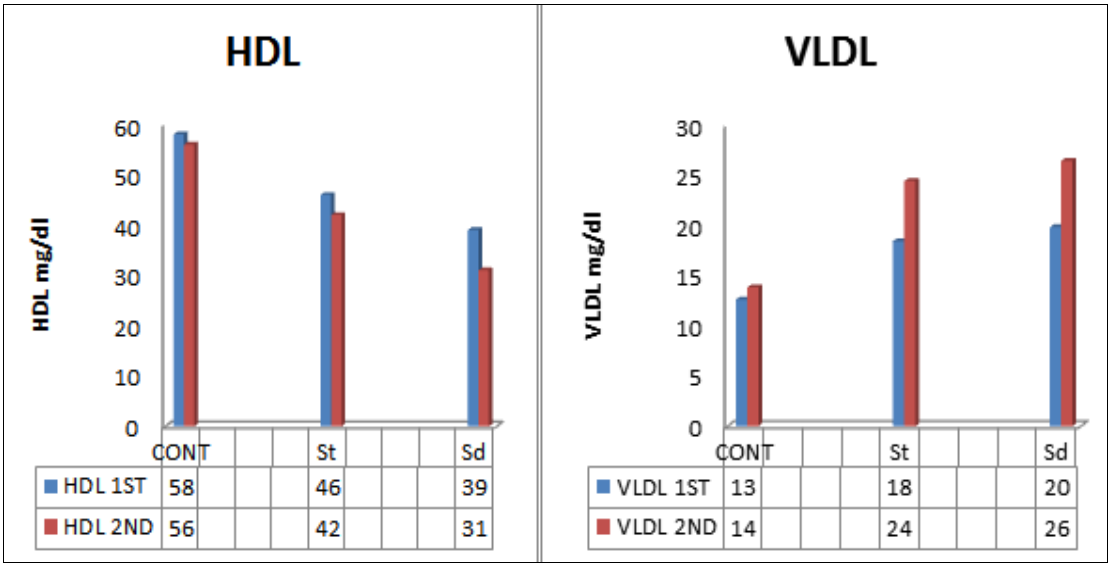

Fig 2: Levels of HDL and LDL (mg/dl) in study groups after one and two months of experiment

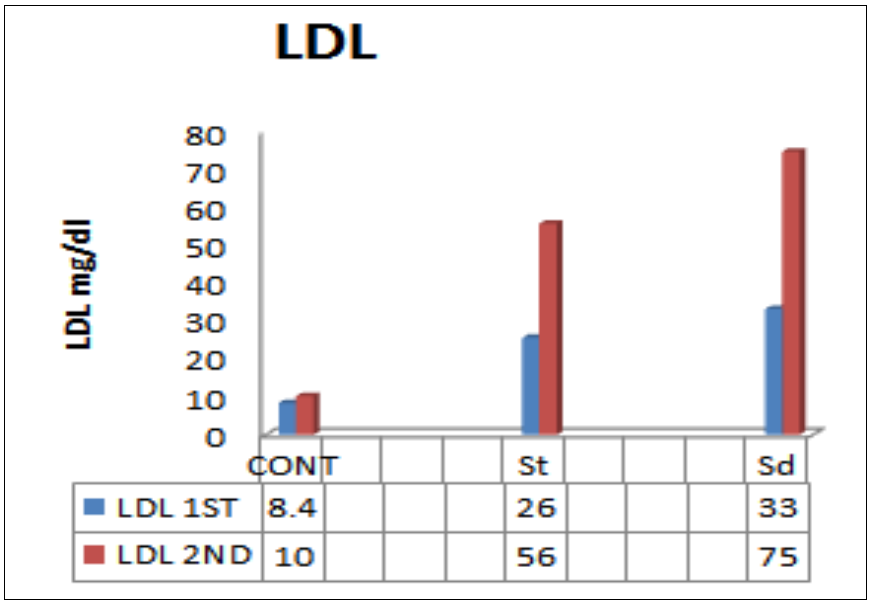

Fig 3: Levels of TC and TG (mg/dl) in study groups after one and two months of experiment

Tables1, 2, 3, and 4 illustrated the levels TSB (mg/dl), AST, ALT, and ALP(U/I) at the end of 1st and 2nd month of the study in all groups.

Table 2: TSB levels (mg/dl) at the end of 1st and 2nd month of the study in all groups

Groups	1st month TSB (mg/dl) mean ± SD	2nd month TSB (mg/dl) mean ± SD	P-Value
CONT	0.13±0.001	0.14±0.0011	0.676
St	0.3±0.001	0.45±0.0013	0.001*
Sd	0.6±0.003	0.75±0.0032	0.001*
P-Value	0.0001*	0.0001*	-

Table 3: AST levels (U/I) at the end of 1st and 2nd month of the study in all groups

Groups	1st month AST (U/l) mean ± SD	2nd month AST (U/l) mean ± SD	P-Value
CONT	65±2	67±2	0.211
St	120±4	149±3	0.001*
Sd	201±12	212±8	0.001*
p-value	0.0001*	0.0001*	-

Table 4: ALT levels (U/I) at the end of 1st and 2nd month of the study in all groups

Groups	1st month ALT (U/l) mean ± SD	2nd month ALT (U/l) mean ± SD	P-Value
CONT	45±2.4	51±1.5	0.113
St	145±10	176±4.7	0.001*
Sd	199±11	228±12	0.001*
p-value	0.0001*	0.0001*	-

Table 5: ALP levels (U/I) at the end of 1st and 2nd month of the study in all groups

Groups	1st month ALP (U/l) mean ± SD	2nd month ALP (U/l) mean ± SD	P-Value
CONT	159±11	159±11	0.676
St	110±7.8	107±10	0.001*
Sd	91±6.2	80±5.9	0.001*
p-value	0.0001*	0.0001*	-

Figure 4 and 5, shows the TyG index of all study groups after end of 1st and 2nd months of this experiment.

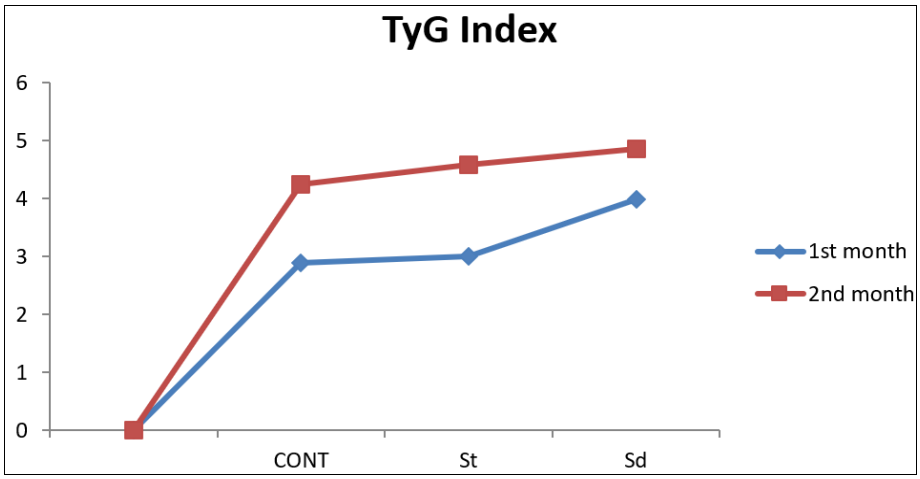

Fig 4: Shows the TyG index of all study groups 1st and 2nd months

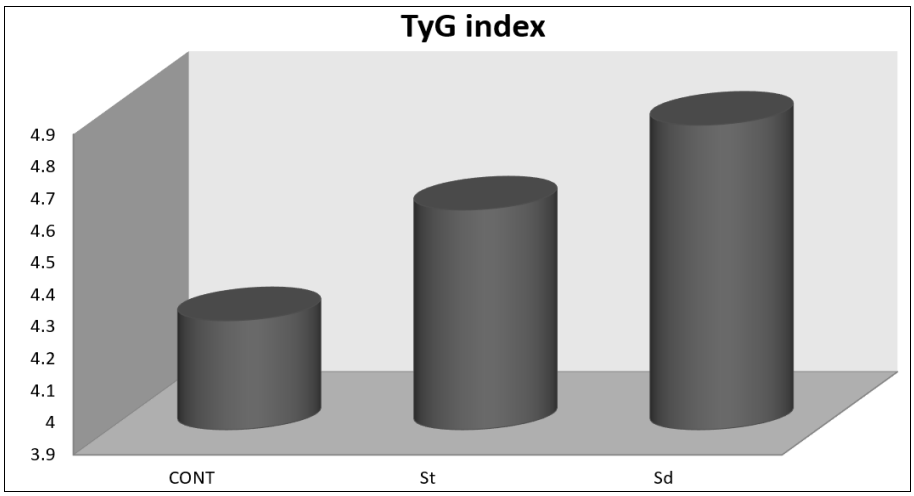

Fig 5: TyG index of study groups after two month of experiment

Discussion

Due to their distinct clinical circumstances and dose dependence, the bioactivities of stevia and its glycosides warrant particular consideration. In light of the numerous health hazards connected to the oral ingestion of stevia and its derivatives, such as stevioside, we examined the effects of stevia aqueous extract and stevioside against liver function in male rats in this study [13]. This study focus was on evaluating the protective or non-protective action of this sweetness on the liver function and structure using hepatic enzymes and lipid profile as a marker. This study investigates the impact of Stevia rebaudiana leaves (St) and its extracted compound, stevioside (Sd) on blood lipid profile regulation and correlation with TyG index. The research likely employed animal models, potentially rats, divided into groups recieving St, Sd, and no treatment (control) group [14]. Studies have shown that oral administration of stevioside is not absorbed by the human body or only in trace amounts, and the digestive enzymes in the gastrointestinal tracts of various animals and humans cannot break down stevioside into steviol, the aglycone of stevioside. However, in feeding experiments with rats and hamsters, steviol glycosides were metabolized by cecal flora to steviol. Steviol was found in the blood of the animals, with concentrations reaching their highest levels after 8 hours. The studies cited do not show that coprophagy in rodents is preventable. Therefore, it is unclear whether steviol contained in the blood is absorbed directly from the large intestine or indirectly through ingested feces (after passing through the intestine again) [15, 16, 17]. Stevia and its subsidiary Stevioside have been broadly read up for their possible poisonousness and defensive impacts. A few investigations have shown that stevia and stevioside display low harmfulness, making them ok for utilization. For example, intense and subacute poisonousness studies have uncovered exceptionally low harmfulness of stevia and stevioside [18]. Furthermore, research has shown that steviol glycosides found in stevia are not teratogenic, mutagenic, or cancer-causing, and cause no intense and subacute poisonousness [19]. Moreover, studies have exhibited the defensive impact of stevioside on the corruption of ascorbic corrosive, demonstrating its capability to safeguard against oxidative harm [20].

Besides, the non-poisonous and non-mutagenic impacts of stevia sugars on human well-being have been affirmed by toxicological investigations. Also, stevia organization has been found to safeguard testicular cell films from receptive oxygen species (ROS) assaults and to give assurance against harmful impacts initiated by specific substances [21]. Moreover, stevioside has been accounted for to defensively affect beta cells in lipotoxic conditions, recommending its possible job in safeguarding against beta-cell passing. These discoveries are reliable with the statement that stevia isn't just non-harmful yet in addition good for long-haul utilization [22].

Conclusion

The current study showing that using of high doses of Stevioside is more risk for hyperlipidemia and more than Stevia and high risk to increase insulin resistance, metabolic disorders, and liver diseases. From this it is clear that the use of high concentrations and doses of artificial sweeteners in food products or as substitutes for table sugar may pose future risks, and companies must deal with it with caution in the future.

Conflict of interest: Authors declare no conflict of interest recorded in this study.

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Author contributions

Conceptualization, methodology, software, and validation by AHA and HHK and formal analysis, investigation, and resources by HKA; After reading the this version of the manuscript, each author gave their approval.

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