Sweet ent-kaurene diterpene glycosides of *Stevia rebaudiana* Leaves Bertoni and Biological Evaluation

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Abstract: *Stevia rebaudiana*, Bertoni, a sweet, non-caloric natural source, perennial herb native to Paraguay and Brazil. A rapid better resolved HPLC separation of sweet glycosides of *Stevia rebaudiana* leaves and different biological activities were determined. Eight sweet ent-kaurene diterpene glycosides (SEDG) were quantitatively and qualitatively fractionated by HPLC from the butanol soluble fraction. Two major sweet glycosides were chromatographically isolated. Acid hydrolysis and chemical degradation of the glycosides were performed; their aglycones were also isolated and identified. Hypoglycemic effect of the glycosides on diabetic rats was estimated. Kidney function was revealed; by creatinine and serum urea. Significant decrease in the levels of serum fasting glucose, glycohemoglobin (HbA_1), urea, creatinine, total cholesterol, total lipids, alanine and aspartate aminotransferases enzyme (AST&ALT). In addition, remarkable improvement in vitamins C, and E levels and antioxidant enzymes, glutathione, glutathione oxidase, glutathione reductase and lipid peroxides levels were observed in the liver tissues compared to diabetic controls. Histopathological investigations of the control diabetic rats were characterized. The kidney of diabetic rats treated with sweet glycoside showed mild protective effects as compared to the diabetic control rats.
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Key words*: Stevia rebaudiana***,** glycosides, hypoglycemic**,** HbA, liver, kidney function antioxidant status.

1. Introduction:

Stevia rebaudiana Bertoni, belonging to the Asteraceae, is a perennial herb native to Brazil and Paraguay, is a sweet non-caloric natural source alternative to artificially produced sugar substitutes(1). *Stevia,* sweeteners extracts from the leaves of this herb, are commercially available in Japan, Korea, China, South-East Asia and South America and have been used for some decades to sweeten a variety of foods including beverages, confectionery and pickled vegetables. *Stevia* extracts have been extensively used as the dietary supplements in USA (2). Steviosid is the predominant sweetening approximately 3-8% of the dry leaves weight. Many researchers have demonstrated that *Stevia* has several therapeutic effects, such as hypoglycemic activity (3), as hypotensive (4) and as anti-inflammatory (5). In addition, it has been used for patients suffering from obesity and heart disease

In the present study, we report a more rapid, better resolved HPLC separation of sweet glycosides and also different biological studies were carried out for the cultivated *Stevia rebaudiana* leaves.

2. Material and Methods

Plant Materials

Stevia rebaudiana seeds were kindly secured by Prof. Dr Jose Walter Pedroza Carneiro, Maringa University, Brazil, to whom we appreciate very much. As *Stevia* seeds were poorly germinated and also establishment of the seedlings was slow, so tissue culture technique was conducted in order to obtain the plants which consequently give up the cuttings used for cultivation of appropriate area to provide the leaves material (6). The leaves were collected, air dried and powdered.

Spectral analysis

EI/MS, Finnigan mat SSQ 7000 (Thermo Inst., system Inc., USA), and mass mode, EI: 70 Ev. ^{1}H-NMR spectra were recorded in ($CDCl_3$) or (CD_3OD) at 300 MHz on a Varian Mercury (HPLC) of (SEDG) Shimadzu system Controller Scl-10AVP with UV detector, UV-Visible detector, and Shimadzu liquid chromatography pump. Conditions: Column: zorbax NH_2 Du pont (4.6m x 25 cm). Eluting solvent: 84-70% v/v acetonitrile- water (pH: 5) changed over a period of 15 min. Flow rate: 2ml/min. UV detector: 210 nm.

Preparation of Sweet glycosides:

The sweet glycoside was isolated from butanol soluble fraction. The dried butanol extract was dissolved in hot methanol (5 ml). The total crude (sweet ent-kaurene diterpene glycosides, SEDG) was precipitated by adding ether (500 ml) and the

precipitate was collected and purified by repeated precipitation with acetone The precipitate was collected, dried, weighed and kept in a desiccator over anhydrous calcium chloride; portions of isolated crude (SEDG) were dissolved in methanol and then subjected to fractionation on TLC, GF_{254} using solvent system , chloroform: methanol: water (6:3:1, v/v/v) plates were visualized by sulfuric acid (60%) kept at110˚C for10min and examined in daylight. Eight compounds were revealed from which two major compounds were isolated, each isolated compound was identified by spectral analyses, MS ^{1}H-NMR, IR and by HPLC.

HPLC of sweet glycoside:

100 mg of sweet glycoside and 2 mg of each isolated compound was dissolved in 1ml methanol, HPLC grade from which 25µl were injected to HPLC using previously mentioned condition (7), (Table 1)**.**

Acid hydrolysis:

Sweet glycoside and the isolated major compounds (0.5g each) were hydrolyzed using 20% sulfuric acid for 5hrs. The residue was applied on silica column chromatography using chloroform as eluting solvent and TLC using solvent system $CHCl_3$ –MeOH (9:1) then sprayed with p-anisaldehyde, isosteviol was revealed, crystallized with methanol and identified by spectral analyses.

Chemical degradation:

Sweet glycoside and the isolated major compounds (0.5g each) were stirred with sodium periodate (1g). The residue obtained after workup of the reaction mixture was chromatographed over a silica gel column for purification (particle size, 200-63µm; Merk 63µm) eluting with $CHCl_3$–MeOH (49:1) and crystallization from MeOH gave pure steviol which confirmed by spectral analyses (8). The sugar moiety in each case was identified as glucose by paper chromatography and confirmed by GLC after silaylation.

Determination of biological effects of Sweet glycoside:

The biochemical effects of sweet glycoside on diabetic rats were carried out. Induction of diabetes was performed by IP administration of alloxan monohydrate (150 mg/kg b.w) to adult male albino rats weighing from 100-140g used for one week (9). Rats were divided into three groups (6 rats each). Group I: Normal control rats. Group II: Control non-treated diabetic rats. Group III: Diabetic rats treated with sweet glycoside of *Stevia rebaudiana* leaves (2.5 g/kg b. w orally) over a period of 21 successive days. The blood samples were withdrawn from the retro-orbital venous plexus at zero, 10 and 21 days. The following biochemical parameters were determined including, $HbA_{1,}$ (10), serum fasting glucose (11) urea (12) creatinine (13) sodium, (14) potassium (15). Cholesterol (16), Total lipids (17) and the ALT, AST (18).

Ethics

Anesthetic Procedures and handling with animals were compiled with the ethical guidelines of medical Ethical Committee of the National Research Centre in Egypt and performed for being sure at any stage of the experiment.

Tissue samples:

The animals of the different groups were sacrificed at the end of the experiment, histopathological studies for kidney and liver tissues were investigated. (19). the following antioxidant parameters including vitamin C (20) vitamin E (21). Lipid peroxides (22) glutathione (23) glutathione peroxidase (24) and glutathione reductase (25) were estimated.

3. Results and Discussion

Sweet ent-kaurene diterpene glycosides reached 5.58% of dry weight which were detected and fractionated by TLC and HPLC, eight compounds were revealed (Table 1). Two major compounds were isolated; their structures were established by chemical and spectral analyses as follows:

Rebaudioside A:

Colorless needles were obtained from the methanol extract. It showed molecular ion peak at m/z 966 in EI/MS corresponding to molecular formula $C_{44}H_{70}O_{23}.3H_2O$. ^{1}H-NMR spectrum showed signals for the anomeric protons at δ 5.52 for β-glucosyl ester and δ 4.86, 5.20 and 5.4 for β-glucosidic linkage, these result were confirmed by Koha *et al.* (26).

Stevioside:

Colorless needles were obtained from methanol. EI/MS showed a molecular ion peak at m/z 804 corresponding to the molecular formula $C_{38}H_{60}O_{18}$, this result coincided with the results of Crosby *et al.* (27).

Isosteviol:

Acid hydrolysis of sweet glycoside, rebaudioside A and stevioside yielded isosteviol which was identified by TLC and spectral analyses that coincided with the reports of Starratt (28). Isosteviol has been isolated for the first time in free state from *Ceriops decandra*, (29). ^{1}H-NMR spectrum showed proton signals of three methyl group at δ 0.79, 0.98 and 1.25 ppm.

Mass spectrum of the substance showed a molecular ion peak at m/z 318 M^+ (80%) corresponding to the molecular formula C_{20} $H_{30}O_3$, another main ions were at m/z 300(40%), 273(42%), 259(25%), 203(22%), 165(25%), 152(22%), 109(30%) and 81(25%).

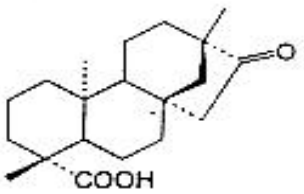
Table (1): HPLC of the SEDG in Stevia rebaudiana leaves.

Peak No	R_t (min)	Conc %	Identified compounds
1	1.31	1.78	Unidentified
2	1.69	2.10	Unidentified
3	2.60	0.83	Unidentified
4	3.42	1.55	Steviolbioside
5	3.89	0.76	Unidentified
6	4.19	1.21	Unidentified
7	4.57	2.46	Unidentified
8	5.45	2.86	Dulcoside A
9	6.13	4.27	RebaudiosideB
10	6.94	29.02	Stevioside
11	7.75	9.96	RebaudiosideC
12	8.96	31.21	RebaudiosideA
13	10.93	7.23	Rebaudioside E
14	12.28	4.26	Rebaudioside D
15	14.53	0.51	Unidentified

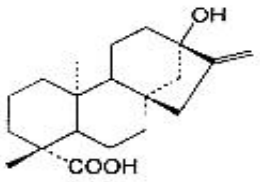
Steviol:

Chemical degradation of sweet glycoside, rebaudioside A and stevioside yielded steviol which was identified by TLC and spectral analyses, these analysis agreed with,Ogawa(30). Steviol was isolated in free state from the root bark of marine mangrove .

Many analytical methods have been applied for the separation and quantification of sweet ent-kaurene diterpene glycosides from the leaves of *Stevia rebaudiana*(31) Quantified only stevioside levels enzymatically (32) quantified total glycosides content by gas chromatography after acid hydrolysis(33). TLC was applied to identify four more abundant glycosides, stevioside, rebaudioside A, C and dulcoside A. Makapugay (34) quantified the diterpene glycosides by HPLC using soxhlet extraction. Mauri *et al* (35) Employed a capillary electrophoresis method to analyze *Stevia rebaudiana* glycosides where they obtained rebaudioside A and steviolbioside using a semi preparative HPLC.



Isosteviol



Steviol

Biochemical studies:

In the present study, the hypoglycemic effect of the sweet glycoside of *Stevia rebaudiana* leaves was evaluated on diabetic rats. The continuous treatment with crude stevioside (2.5g/kg b.w) for a period of 21 days caused a significant decrease in the blood glucose levels of diabetic rats (-32.98%) (Table 2) as compared with diabetic control rats. Jeppesen *et al*. (36) stated that, stevioside and steviol possess hypoglycemic effects by stimulation of insulin secretion from mouse islets, while Huber *et al.* (37) showed that stevioside has a stimulatory action on glycogen deposition in the rat liver.

The present work revealed an increase in HbA_1 percentage (19.66%) in diabetic control rats reflected as an increase in mean serum fasting glucose levels (160 mg/dl), group III treated with sweet glycoside restored normal level of HbA_1 percentages (7.05%) as compared to diabetic control rats (Table 3).

Concerning the kidney function tests, it was found that diabetic rats treated with sweet glycoside showed a slightly decrease in serum urea levels. (Table 4). Serum creatinine levels are frequently used as screening test for renal dysfunction (38). The level of serum creatinine was significantly decreased by (-26.88%) in comparison to diabetic control and reached normal values (0.68 mg/dl) after 21 days (Table 2).

The present results indicate that sweet glycoside had a significant decrease in serum sodium (139 mEq/L) and potassium levels (3.39mEq/L) after 21 days. This result may be due to increase the excretion of sodium and potassium in urine (Table 4).

Melis (39) showed that intravenous infusion of crude extract of Stevia leaves increase both sodium and potassium excretion however, insignificant difference was detected in mean arterial pressure in male Wister rats under antidiuresis or water diuresis. The author suggested that the extract may have a direct effect on salt and water transport in renal tubules.

Table (2) Effect of SEDG of *Stevia rebaudiana* leaves on serum creatinine levels in diabetic rats in comparison to normal and diabetic controls.

Groups / Time	blood glucose level (mg/dl)			Serum creatinine (mg/dl)		
	GI	GII	GIII	GI	GII	GIII
Zero Mean±S.E	86.66±2.47^{Aa}	160±3.89^{Ba}	159.00±2.00^{Ba}	0.65±0.05^{Aa}	0.86±0.06^{Ba}	0.89±0.05^{Ba}
Range	74-89	150 -177	150-165	0.47-0.70	0.6 -1.04	0.88-1.03
10th day Mean±S.E	88.33±3.32^{Aa}	156.50±2.18^{Ba}	112.00±2.14^{Cb}	0.68±0.08^{Aa}	0.83±0.04^{Aa}	0.72±0.04^{Aab}
Range	78-100	148 -164	104 -119	0.44 - 0.95	0.64 -1.0	0.56-0.88
% Change			-28.43			-13.25
21st day Mean±S.E	89.00±3.32^{Aa}	143.00±3.49^{Bb}	95.83±2.72^{Ac}	0.61±0.04^{Aa}	0.93±0.05^{Ba}	0.68±0.06^{Ab}
Range	78-93	133-152	90-108	0.45-0.80	0.77-1.09	0.42-0.80
% Change			-32.98			-26.88

Number of rats / group = 6 Means with different capital letters superscripts A, B... for rows and small letters a, b… for columns with a significant difference (P value) at ≤ 0.05. Percentage of change compared to diabetic rats**. GI**: normal control rats. **GII**: diabetic control rats. **GIII**: diabetic rats treated with SEDG.

Table (3) Effect of SEDG of *Stevia rebaudiana* leaves on Blood glycohemoglobin, Serum total lipids and Serum total cholesterol levels in diabetic rats in comparison to normal and diabetic controls.

Groups / Time	**Blood glycohemoglobin (HbA_1 %)**			**Serum total lipids (mg/dl)**			**Serum total cholesterol (mg/dl)**		
	G1	**GII**	**GIII**	**GI**	**GII**	**GIII**	**GI**	**GII**	**GIII**
Zero Mean±S.E	6.63±0. 10	11.00±0. 6	10.21±0.9^{B}	229.33±7.64^{A}	250.5±	257.66±2.92^{B}	45.33±1.33^{A}	54.66±	57.66±0.92^{B}
Range	6.30-7.0	10-12	9.5-11	200-246	4.28^{B}	245-260	39-47	1.77^{B}	55-60
					240-268			47-58	
21th day Mean±S.E	6.50±0. 25^{A}	19.66±0.60^{B}	7.05±0. 43^{A}	234.83±8.78^{A}	393± 9.13^{B}	200.66±5.52^{C}	48.33±1.77^{AC}	87.50± 2.67^{B}	45.33±1.77^{C}
Range	5.4 -7.2	17.7-22	5.6-8.4	200-257	351-418	188-218	44-56	76-94	38-50
% Change			**-64.14**			**-48.94**			**-48.19**

Number of rats / group = 6 Means with different capital letters superscripts A, B. for rows and small letters a, b… for columns with a significant difference (P value) at ≤ 0.05. Percentage of change compared to diabetic rats. GI: normal control rats. GII: diabetic control rats. GIII: diabetic rats treated with S.

Table (4): Effect of SEDG of *Stevia rebaudiana* leaves on serum sodium, serum potassium and serum urea levels in diabetic rats in comparison to normal and diabetic controls.

Groups / Time	Serum sodium levels (mEq/L)			Serum potassium levels (mEq/L)			Serum urea levels (mg/dl)		
	G1	GII	CIII	GI	GII	CIII	GI	GII	CIII
Zero Mean ±S.E	152.66±4.43^{Aa}	157.83±2.45^{Aa}	159.66±4.82^{Aa}	5.06±0.13^{Aa}	5.73±0.31^{Aa}	5.58±0.44^{Aa}	19.68±2.42^{Aa}	27.66±0.06^{Ba}	28.78±2.68^{Ba}
range	143-165	146-163	143-177	4.7-5.5	4.4-5.9	4.5-7.0	11-20	22-30	22-39
10th day Mean ±S.E	145.33±1.66^{Aa}	172.16±5.20^{Bb}	144.00±3.89^{Ab}	4.93±0.25^{Bb}	3.98±0.23^{Bb}	4.03±0.33^{B}	21.4±1.51^{Aa}	28.66±0.91^{Ba}	22.13±2.00^{Ab}
range	140-150	156-190	130-155- 16.35	4 -5.8	2.9-4.6	3.4 -5.6	16-26	27-33	16 -29
% change						1.25			-22.78
21st days Mean ±S.E	148.85±4.59^{Aa}	176.00±5.10^{Bb}	139.00±1.46^{Ab}	4.71±0.21^{Bb}	3.43±0.18^{Bb}	3.39±0.26^{Bb}	21.71±1.48^{Aa}	30±1.46^{Ba}	24.25±1.15^{ADab}
range	136-164	163-190	135-145	3.7-5.2	2.8-4	3.2- 4.8	15 -26	25-35	20 -28
% change			-21.02			-1.16			-19.16

Number of rats / group = 6 Means with different capital letters superscripts A, B... for rows and small letters a, b… for columns with a significant difference (P value) at ≤ 0.05. Percentage of change compared to diabetic rats. GI: normal control rats. GII: diabetic control rats. GIII: diabetic rats treated with SEDG.

From these results it is concluded that sweet glycoside have natriuresis and kaliuresis effect, and acts as a vasodilator. The fact that water and sodium excretion increased following sweet glycoside infusion in spite of an unchanged glomerular filtration rate. This may be explained by decreased water and sodium reabsorption in the proximal tubules. The greater enhancement of renal blood flow caused more marked changes in the startling forces around the proximal convolution, resulting in a substantial decrease of the proximal tubular reabsorption and an increase in urinary flow and sodium excretion(**39).**Treatment of diabetic rats with sweet glycoside not only caused blood glucose homeostasis but also reversed changes in lipid metabolism

In the alloxan-induced control diabetic rats, the rise in blood glucose is accompanied by an increase in serum total cholesterol (87.5mg/dl) and total lipids (393 mg/dl) levels after 21 days as compared to the normal control. The treatment with sweet glycoside caused decrease in total cholesterol and total lipids level (45.3 mg/dl and 200.7 mg/dl, P≤0.05 respectively) as compared to diabetic control rats (Table 3). It has been suggested that the increase in the total cholesterol and triglycerides levels in diabetes mellitus was the result of resistance to insulin dependent glucose uptake, which consequently increase the serum glucose levels, leading to an increase in hepatic total cholesterol and triglycerides synthesis and secretion.

The aminotransferases constitute a group of enzymes that catalyze the interconversion of amino acids and α-oxo-acids by transfer of amino groups. In liver diseases, serum ALT and AST levels are elevated, where ALT level is characteristically higher than AST level. Elevated serum AST activity with no ALT elevation indicates muscle necrosis or myocardial infarction.

In diabetic control rats, serum ALT and AST levels (30.5 U/ml and 66 U/ml, P≤0.05, respectively) were significantly higher than normal rats (19.33 U/ml and 16.16 U/ml, respectively) but treatment with sweet glycoside caused significant decrease in both enzymes (-1.63 and -41.42% P≤0.05, respectively) as compared to diabetic control rats (Table 5).

Free radicals formation and oxidative stress may act as a common pathway to diabetes itself as well as, to its later complications (40). Hyperglycaemia is thought to be associated with increased oxidative stress via glucose autoxidation which produces superoxide radicals and free radicals generated from glycosylated proteins (41).

In the present study it was found that lipid peroxidation level was increased (0.34µmole/g tissue) in diabetic rats compared to normal ones (0.28 µmole/g tissue). On the other hand, diabetic rats treated with sweet glycoside(Table 6) showed significantly decreased levels of lipid peroxidation (-11.7% P≤0.05).

Peroxidation reactions and oxidative stress occurring in disease states can be controlled by supplementing the diet with antioxidants such as vitamin C, E, flavonoids, caffeic acid, chlorogenic acid, anthocyanins. Vitamin C is a strong reducing agent, it is a naturally occurring suppressor of free radicals also it enhances vitamin E efficiency in reducing lipid peroxidation**.** Vitamin E is the major chain breaking antioxidants in plasma, red cells and tissues .It represents first line of defense against peroxidation of polyunsaturated fatty acids in cellular and subcellular membrane phospholipids (42).

In this study, it could be concluded that *Stevia* sweet glycoside were used to control diabetes and scavenging agents for control free radicals associated with diabetes. So, the effect of administered *Stevia* to diabetic rats was also undertaken as antioxidant agent.

The present results showed that diabetic rats treated with sweet glycoside led to a significant increase in vitamin C, E (80.99 and 75.68% P≤ 0.05, respectively) as compared to diabetic control rats(Table 6).

In this study, a significant increase in glutathione level was found in both diabetic rats treated with sweet glycoside (19.11 %,), in addition to glutathione peroxidase and reductase activities which were significantly increased (28.84 and 38.49% P≤0.05, respectively) in comparison to diabetic control rats (Table 6), it could be also concluded that sweet glycoside may have potential use as antidiabetic agents, and could be used as antioxidant agents, controlling free radicals in diabetes.

Histopathological studies

The present study showed that the kidney tissues of diabetic control rats were characterized by vacuolar degeneration, fatty changes in tubular epithelial cells and glomerular degeneration. The kidney of diabetic rats treated with sweet glycoside showed mild protective effects as compared to the diabetic control rats. These changes appeared in the form of marked diminution of fats. In the present work, the pathological changes observed in liver and kidney of diabetic rats may be attributed to lipid peroxidation and free radical causing damage to cells, these side effects may be neutralized by treatment of diabetic rats with sweet glycoside Figs (1 and 2).

It is realized that the treatment of diabetic rats with sweet glycoside alleviated the deleterious effect of alloxan on liver and kidney tissues.

Stevia rebaudiana leaves might be recommended as natural alternative to chemical sugar substitutes; in addition, the sweet glycosides have many biological effects.

Table (5): Effect of SEDG of *Stevia rebaudiana* leaves on serum AST and ALT levels in diabetic rats in comparison to normal and diabetic controls.

Groups	GI	GII	GIII	GI	GII	GIII
Time	AST (u/ml)			ALT (u/ml)		
Zero Mean±S.E Range	15.50±1.60^{Aa} 11-21	34.00±3.67^{Ba} 27-47	34.50±0.88^{Ba} 31-37	21.33±2.17$^{A\ a}$ 15-22	20.83±2.30^{Aa} 15-28	22.16±0.70^{Aa} 20-25
10th day Mean±S.E Range % Change	15.83±1.30^{Aa} 11-19	69.66±6.50^{Cb} 41-88	37.33±2.67^{Ba} 30-47 - 46. 4	15.66±3.29^{Aa} 10-20	23.33±2.26^{Ba} 13-28	22.0±1.12^{Ba} 19-26 -5.7
21st day Mean±S.E Range % Change	16.16±2.79^{Aa} 11-27	66.00±6.00^{Bb} 52-85	38.66±3.07^{Ca} 30-49 - 41.42	19.33±2.37^{Aa} 11-29	30.5±1.82^{Bb} 22-35	30.00±1.23^{Bb} 26-34 -1.63

Number of rats / group = 6 Means with different capital letters superscripts A, B... for rows and small letters a, b… for columns with a significant difference (P value) at ≤ 0.05. Percentage of change compared to diabetic rats.
GI: normal control rats. GII: diabetic control rats. GIII: diabetic rats treated with SEDG.

Table (6): Effect of (SEDG) of *Stevia rebaudiana* leaves on liver, glutathione, glutathione peroxides, glutathione reducatase Vitamin C, vitamin E and lipid peroxides levels in diabetic rats in comparison to normal and diabetic controls.

Parameters	Glutathione mg/g tissue	Glutathione peroxidase µmole/g tissue	Glutathioe reductase µmole/g tissue	Vitamin C mg/g tissue	Vitamin E mg/g tissue	Lipid peroxides µmole/g tissue
Group I Mean±S.E	31.69±0.022^{A}	2.98±0.07^{A}	14.05±0.64^{A}	6.31±0.06^{A}	7.27±0.08^{A}	0.28±0.02^{A}
Group II Mean±S.E	20.62±0.39^{B}	2.03±0.04^{B}	9.43±0.29^{B}	4.63±0.04^{B}	4.77±0.04^{B}	0.34±0.01^{B}
Groups III Mean±S.E	24.56±0.33^{D}	2.96±0.04^{A}	13.06±0.17^{A}	8.38±0.07^{D}	8.38±0.08^{D}	0.30±0.01^{A}
% of change	19.11	45.81	38.49	80.99	75.68	-11.7

Number of rats / group = 6 Means with different capital letters superscripts A, B... for rows and small letters a, b… for columns with a significant difference (P value) at ≤ 0.05. Percentage of change compared to diabetic rats.
GI: normal control rats. GII: diabetic control rats. GIII: diabetic rats treated with SEDG.

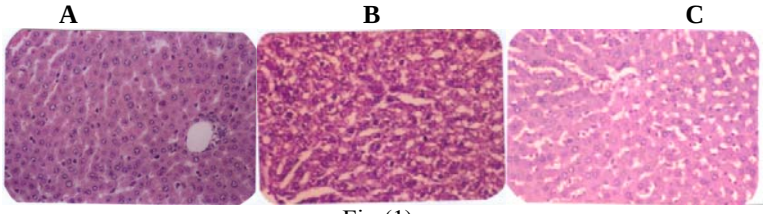


Fig (1):
A. Liver tissue of control rat.
B. Liver tissue of diabetic rat.
C. Liver tissue of diabetic rat treated with crude stevioside

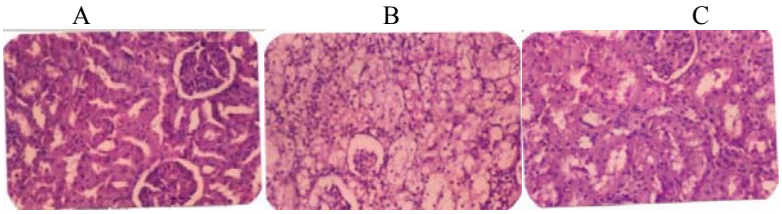


Fig (2):
A. Kidney of control rat.
B. kidney of diabetic rat
C. kidney of diabetic rat treated with crude stevioside

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