UTILIZATION OF STEVIA EXTRACT IN BISCUITS

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Abstract

Effect of adding stevioside (the extract of stevia rebaudiana) during biscuit preparation on rheological properties and organoleptic properties of produced biscuits, as well as biological evaluation including rats body weight gain, organs weight, biochemical parameters [glucose, triglycerides, urea, cholesterol, and kidney functions] and histological examination were investigated. Obtained results declared that no significant difference on rheological or organoleptic properties between control and treated samples when adding stevioside at levels of (0.025 , 0.050 , 0.075 and 0.1%) to wheat flour. Serum glucose for rats fed on stevioside added before or after baking showed non-significant values from the beginning till the end of the experiment compared to normal control. On the other hand, rats fed on stevioside added befor or after baking showed significant decrease after three weeks from starting compared with diabetic control. Serum triglycerides and serum total cholesterol showed non-significant changes between control and tested groups. No significant changes in all the histological parameters are showed when adding steviosoide.

INTRODUCTION

Stevia [*Stevia rebaudiana*] is composed of several natural, heat-stable, give 100 to 300 times the sweeteners of sucrose. Since 1950 the Japanese have developed its production and have overcome problems of refining to eliminate undesirable flvours. A slow start in the 1970, but stevia is now used in a wide range of food applications in Japan. *(Keith Phillips and Associates, 1975).* Stevia might have beneficial effects on glucose tolerance (and therefore potentially help with diabetes). Even if stevia did not have direct effects on diabetic, its use as sweetener could reduce intake of sugar with in regard to such patients *Curi et al, (1979).* To study the safety of the sweetening component of stevia, *Lee et al.(1979)* analyses of total blood (red blood cells, white blood cells, Hb and Hct), blood serum components including total protein, glucose,

cholesterol, GOT, and eleven parameters of liver tissues including nuclear deterioration of liver cells, proliferation of kupffer cells fibrosis of portal area, showed no significant difference between control and treatments except for lactate dehydrogenase activity after 56 day-oral deterministration, extract from the results obtained, it was concluded that the stevia extract has no acute toxic effects on rats.

In biological study on stevioside, *Mori et al., (1981)* found that, no abnormal signs were seen in mating performance or fertility in any of the groups and no external, internal, or skeletal anomalies attributable to stevioside were observed in the fetuses when male and female rats were fed on stevioside at concentration of 0.15, 0.75, or 3%. *Baket and O' Brient, (1986)* reported that stevioside is a stable molecule at 100 °C, when maintained in solution in the pH range 3-9 , although it decomposes quite at alkaline pH levels of greater than 10 under these conditions. *Aze et al., (1991)* carried out subchronic oral toxicity study on stevioside for 13 weeks at dose levels of 0.0, 0.31, 0.62, 1.25 and 5% to determine the appropriate dose levels for a 2 year study. From result a concentration of 5% was concluded to be a suitable maximum tolerable dose of stevioside for a 2 year carcinogen city study in rats. *Xili et al., (1992)* suggested an acceptable daily dose from stevioside for humans should be 7.94 mg/kg body weight. *El-Said Aattia et al, (1993)*, reported that, on the basis of chemical, physical and sensory properties of the experimental combinations, it was demonstrated that adding any sweeteners as replacements for sucrose resulted in a decrease in the quality and acceptability of the resultant cake. A formulation involving fructose and polydextrose gave the product, its acceptability which was similar to that of the control sample. At the same time, cake samples achieved 40% reduction in calories. *Farag (1998)* studied the effect of stevioside of three levels 5mg stevioside /100gm diet on both cold and heated treatment for 2 hours at 160 °C and 40mg stevioside /100gm diet in biological, biochemical and histological examination extended for three months. Regarding to serum glucose values in all groups were in normal range, serum triglycerides and serum total cholesterol, there was insignificant changes between control and tested groups. HDL-C the serum HDL-C male and female rats were around the same values of the control group. On the other hand, concerning LDL-C, there was significant increase in male rats on 5mg stevioside/100gm diet by heat compared with control, other results were around the control group level.

The aim of this investigation was to provide a partial solution of some nutrition problems as diabetic ones through the utilization of stevioside as sweetener for biscuit production. Therefore, this study was carried out to investigate the effect of adding stevioside to wheat flour on rheological properties of dough, evaluation of baking quality and organoleptic of produced biscuits. Biological evaluation and biochemical parameters for experimental rats and histological examination for some organs of rats were carried out.

MATERIALS AND METHODS

1-Materials

- American wheat flour (72% extraction) was obtained from North Cairo Mills company, AL-Salam City, Cairo, with following specifications:

Moisture	Protein*	Fat*	Fiber*	Ash*
12.10	1175	0.80	0.75	0.57

* On dry weight basic

- Stevioside (white crystalline powder having a molecular weight of 804.9, and is approximately 300 times sweeter than sucrose), was obtained from L.M.G. International Trade Corporation LTD, Cairo, Egypt.
- Weaning male albino rats were obtained from Helwan farm.

2-Methods

Stevioside quantity used for biscuits production as shown in table (1):

Table 1. Stevioside quantity used for biscuits production.

Treatment No.	Wheat flour (gm)	Stevioside quantity (gm)	Sugar quantity (gm)
1	100	0.0	30
2	100	0.025	22.5
3	100	0.050	15
4	100	0.075	7.5
5	100	0.100	0.0

Preparation of biscuits

Wheat flour 100 gm, stevioside (0, 0.025, 0.05, 0.075 and 0.1 gm), sugar (30, 22.5, 15, 7.5 and 0.0 gm) respectively, and salt (0.36 gm). Mix vegetable fat (16 gm) with

sugar and salt using electric beater for 3 min. add milk powder (2.6 gm) to the sugar mixture, mix for 1 min. at low speed, mix steviosoide and wheat flour in a dough mixer for 1 min. Scrape down and continue to mix for 3 min. Roll the dough in a cookie sheet, bake at 180 °C for 12 min. according to *Wade (1998).*

Sensory evaluation

Score sheet of produced biscuits are shown in the following table :

Table 2. Sensory evaluation of biscuits

Properties	Score
Taste	20
Odor	20
Texture	20
Color	20
Mouthfeel	10
Appearance	10
Overall acceptability	100

Chemical analysis

Moisture, protein, fat, crude fiber, ash, reducing sugar and non-reducing sugars were determined according to the *A.O.A.C. (1990).*

Rheological properties

-Farinograph and extensograph tests were carried out according to the *A.A.C.C. (1993).*

Biological evaluation

Animal feeding experiments

Thirty-six adult male rats were used in this study. The average weight of them was 120-130g. All rats were fed on basal diet for two weeks. The basal diet consisted of 12% casein, 10% corn, 0.2% cholin chloride, 5% cellulose, 4%salt mixture, 1% vitamin mixture and 67% starch according to Lanepeter and Pearson (1971). Water and food were provided adlibtum. At the end of two weeks, 18 of rats were divided into 3 groups (6 rats each) and fed for continuous 3 weeks: The 1st group [(G1) Normal control] was fed on basal diet and considered as normal control. The 2nd group [(G2) 0.01% stevioside added [before baking]. The 3rd group [(G3) 0.01% stevioside added [after baking]. The other 18 rats were fasted overnight then each rat was injected with active alloxan solution 150mg/kg. To ensure occurrence of diabetes in rats, blood sample was taken after 48 hours of injection to determine blood glucose. Then these diabetic rats (18 rats) were divided into other 3 groups (6 rats each) and

fed also for continuous 3 weeks. The 4th group [(G4) Diabetic control] was fed on basal diet and considered as diabetic control. The 5th group [(G5) 0.01% stevioside added [before baking]. The 6th group [(G6) 0.01% stevioside added [after baking]. During the experiment period, the body weight of rats was recorded. At the end of experiment, rats were fasted overnight and anaesthetized using ethyl ether and the blood collected and the serum was separated by centrifuge and stored at (-20 °C) for biochemical analysis.

Biochemical parameters of blood

Blood glucose was determined using the method of *Trinder (1969)*, serum total cholestrol was determined according to the method described by *Allain et al., (1974)*. serum triglycerides were determined according to the method described by *Fossatip and Preciple (1982)*. Urea in plasma was determined calorimetrically at 525nm according to the method of *Caraway (1975)*. Glutamate pyruvate amino transferase (GOT) and glutamate oxaloacetate amino transferase (GPT) activities were determined colorimetrically at 540nm according to the method of *Reitman and Frankel (1957)*.

Histological examination

Samples of liver, kidney, cerebrum, cerebellum, heart and spleen were taken from each rat justly after slaughtering them, immediately fixed in 10% neutral formalin and processed for staining with hematoxylin and eosin. All these methods outlined by *Druy and Wallington (1980)*.

Statistical analysis

The data of sensory evaluation of produced biscuit, and that of serum analysis were subjected to statistical analysis (T – test) according to *Snedecor and Cochran (1967)*.

RESULTS AND DISCUSSION

Rheological properties

The data in table (3) showed that addition of stevioside at level of (0.025, 0.050, 0.075 and 0.100%) to wheat flour resulted in slightly increased concerning water absorption which recorded (63.1, 63.2, 64 and 64.1%) respectively compared with control (63%). Regarding to dough stability, no effect was observed with [0.025% stevioside] treatment and gradually decreased from 7min. in control to (6, 5.5 and 5.5 min.) after addition (0.050g, 0.075g and 0.100g stevioside) respectively. Proportional number was 2.8 in control and [0.025g stevioside] treatment while slightly decreased to (2, 19 and 1.6) after addition (0.050g, 0.075g and 0.100g stevioside) to treatments

after addition of (0.025g, 0.050g, 0.075g and 0.100g stevioside) treatments) respectively. These results confermed the results obtained from farinograph parameters. The aformentioned results coincide with those obtained by *Asikainen, (1990).*

Sensory evaluation of produced biscuits

From table (4) we observed that the best treatment is No.2[(96.10%) (0.025g stevioside)] then treatment No.3 [(95.10%) (0.050g stevioside)] compared with No.1[(93.10%) (control)] while treatment No.4 [(91.90%) (0.075g stevioside)] is the nearst to treatment No.1 (93.10%) [control], but treatment No.5 [(86.30%) (0.100g stevioside)] had the lowest value conecrning taste, odur, texture, colour, shape, light and total compared with control. The aformentioned results coincide with those obtained by *(Bullock et al, 1992).*

Body weight gains and feed efficiency of rats fed on the produced biscuits

Table (5): showed the effect of feeding on biscuits sweetened with stevioside on body weight gain and feed intake of the experimental rats. The body weight gain (highly) in 1st group (normal control), 2nd group and 3rd group recorded 53, 47.3 and 49.1 g respectively while the body weight gain was the lowest in 4th group (diabetic control), 5th group and 6th group in which they recorded 3.5, 0.5 and 1.3g respectively in this respect, the loss in body weight could be explained by that the insulin deficiency results in an abnormal balance in inter-hormone control. Thus, the insulin-deficient animal is in a state of hormonal imbalance favoring the action of corticosteroide, growth hormone, and glucagon which add to the stimulation of gluconeogensis, lipolysis and decreased intracellular metabolism of glucose. Dehydration occurs because that the water required excess glucose in the urine. The obtained data is in line with the finding of *levy et al., (1991),* and *Blackburn et al., (1997).* On the other hand, food efficiency was almost the same, where it recorded 0.216, 0.19 and 0.253 in 1st group (normal control), 2nd group and 3rd group respectively while recorded 0.014, 0.001 and 0.005 in 4th group (diabetic control), 5th group and 6th group respectively.

Organs weight of rats fed on the produced biscuits

Table (6): showed the effect of feeding on biscuits sweetened with stevioside on the relative ratio of some organs, i.e. liver, heart, kidney, brain and spleen to body weight of the alloxanized rats compared with control.

The relative ratio of liver to body weight showed that the value ranged between (3.21 to 3.35 %).

Heart relative ratio of 4th group (diabetic control) rats showed similar value (0.48%) as 5th group while ranged between (0.49% to 0.51%) for 1st group (normal control), 2nd group, 3rd group and 6th group.

The relative ratio of kideny to body weight showed that the value ranged between (0.71% to 0.91%).

The relative ratio of brain to body weight showed that the value ranged between (1.14% to 1.79%).

Spleen relative ratio of diabetic rats 4th showed similar value (0.021%) as 6th group while ranged between (0.22% to 0.027%) for 1st group (normal control), 2nd group, 3rd group, 5th group. The aformentioned results coincide with those obtained by *Boy, (1973).* It could be noticed that the changes of body weight of rats due to the feeding on stevioside and these results were non-significant.

Biochemical parmeters of food

The data in table (7-11) showed levels of glucose, triglycerides, urea, cholesterol, GOT and GPT in blood for rats were fed on different produced biscuits. From these data declared that diagnostic differences between each ingredient during three weeks.

Data in table (7) showed that blood glucose of rats in G1 (normal control) was (90±2.35mg/dl) and constant during the experiment period. G2 for non-diabetic rats showed non-significant increase (92±3.01mg/dl) after one weak then after three weeks increased to (94±2.01mg/dl). G3 (stevioside added after baking) for non-diabetic rats decreased after one week from (102 ±5.25 mg/dl) to (88±2.36mg/dl) after tree weeks compared with normal control. G5 (stevioside added before baking) and G6 (stevioside after baking) for diabetic rats showed significant decrease after three weeks (90±3.32, 80±2.01, mg/dl) respectively compared with G4 (diabetic control).

In table (8), data showed that, blood triglyceride of rats after three weeks in G2 and G3 changed insignificantly compared with normal control. Triglyceride value for G5 and G6 showd significant decrease (68±2.25 and 85±5.54mg/dl) respectively, compared with diabetic contro G4 (162±4.25mg/dl).

Data illustrated in table (9) showed that blood urea of rats in G2 and G3 for non-diabetic increased to (57±0.24mg/dl) and (56±0.36mg/dl) compared with normal

control (42±1.02 mg/dl). While G5 and G6 for diabetic, increased to (74±0.02 and 75±0.024mg/dl) respectively, compared with diabetic control (64±2.04mg/dl).

Obtained data in table (10) showed that in G2 and G3 there are non-significant changes in blood cholesterol values in rats compared with normal control. Regarding to (diabetic control) G4 it was (112±3.33mg/dl) and decreased to (92±2.36, 75±5.25mg/dl) for G5 and G6 respectively,

The data in table (11) showed that blood GOT and GPT of rats, GOT showd that non-significant differences between G2 and G3 (89.01mg/dl, 90.11mg/dl) and normal control (90.05 mg/dl). While G5 and G6 showded significant decrease (105.4, 109.3mg/dl) compared with diabetic control G4 (127.5 mg/dl). The same trend was observed with GPT.The aforementioned results are in coincide with those obtained by *Farag, (1998).*

Table 3.Effect of adding stevioside on the rheological properties of wheat flour.

Treatments	Farinograph		Extensograph	
	Water absorption (%)	Dough stability (min.)	Proportional number (R/E)	Dough energy (cm^2)
100% W.F. (control)	63.0	7.0	2.8	70.0
W.F.+St.(0.025%)	63.1	7.0	2.8	67.0
W.F+St.(0.050%)	63.2	6.0	2.0	51.0
W.F+St.(0.075%)	64.0	5.5	1.9	54.0
W.F.+St.(0.10%)	64.1	5.5	1.6	40.0

W.F.= Wheat Flour St. = Stevioside

Table 4. Sensory evaluation of produced biscuits.

Treatments	Teste (20)	Oder (20)	Texture (20)	Colour (20)	Shape (10)	Light (10)	Total (100)
G1 100 % WF	18.20 ±0.088	19.00 ±0.995	19.20 ±0.995	18.90 ±0.369	8.800 ±0.025	9.000 ±0.442	93.10 ±0.225
G2 WF + (0.025 St. %)	19.50 ±0.145	19.20 ±0.654	19.60 ±1.258	19.70 ±0.987	9.600 ±0.055	9.500 ±0.665	97.10 ±0.654
G3 WF + (0.05 St. %)	19.00 ±0.440	19.10 ±0.369	19.10 ±0.036	19.20 ±0.369	9.400 ±1.369	9.300 ±0.025	95.10 ±1.002
G4 WF + (0.075 St. %)	17.10 ±0.295	19.00 ±1.254	18.80 ±0.554	18.70 ±0.456	9.100 ±1.362	9.200 ±0.664	91.90 ±0.994
G5 WF + (0.1 St. %)	15.00 ±0.288	18.80 ±0.036	18.10 ±1.025	18.10 ±0.665	8.300 ±0.0664	8.000 ±0.025	86.30 ±0.258

$P<0.05 = 1.6$ E- St. = Stevioside

Table 5. Body weight, body gain, feed intake (in gms) and feed efficiency of rats in different groups at the end of experiment.

Rat group	Initial body weight	Final body weight	body weight gain	Daily body weight	Feed intake day/gm	Feed efficiency
G1 (Normal control)	122.7	175.7	+53	+1.76	8.21	+0.216
G2 St. before baking	123.0	170.3	+47.3	+1.57	8.22	+0.190
G3 St. after baking	127.0	176.3	+49.1	+1.63	6.44	+0.253
G4 (Diabetic control)	129.0	133.6	+3.5	+0.11	7.51	+0.014
G5 St. before baking	121.0	121.9	+0.5	+0.01	6.51	+0.001
G6 St. after baking	125.0	126.3	+1.3	+0.04	6.91	+0.005

Table 6. The averge percentage of the weight of some organs to the total body weight of different groups at the end of experiment.

Rat group	Liver (%)	Heart (%)	Kidney (%)	Brain (%)	Spleen (%)
G1 (Normal control)	3.33	0.50	0.71	1.14	0.24
G2 St. before baking	3.34	0.51	0.72	1.42	0.25
G3 St. after baking	3.35	0.53	0.73	1.45	0.27
G4 (Diabetic control)	2.91	0.48	0.91	1.79	0.21
G5 St. before baking	3.21	0.48	0.81	1.55	0.22
G6 St. after baking	3.42	0.49	0.79	1.51	0.210

Table 7. Blood glucose of rat groups feed on stevioside for three weeks.

Rat group	After (1) week		After (2) weeks		After (3) weeks	
	Mg/dl	%	Mg/dl	%	Mg/dl	%
G1 (Normal control)	90 ±2.35	100	90 ±2.35	100	90 ±2.35	100
G2 St. before baking	92 ±1.29	102.2	108 ±3.25	120	94 ±3.01	104.4
G3 St. after baking	102 ±5.25	113.3	96 ±4.25	106.6	88 ±2.36	97.77
G4 (Diabetic control)	325 ±3.02	361.1	325 ±3.02	361.1	325 ±3.02	361.1
G5 St. before baking	114 ±2.01	126.6	96 ±8.25	106.6	90 ±3.32	100
G6 St. after baking	117 ±4.25	130	100 ±5.25	111.1	80 ±2.01	80.88

Table 8. Blood triglyceride of rats group fed on stevioside for three weeks.

Rat group	After (1) week		After (2) weeks		After (3) weeks	
	Mg/dl	%	Mg/dl	%	Mg/dl	%
G1 (Normal control)	135 ±5.2	100	135 ±5.2	100	135 ±5.2	100
G2 St. before baking	100 ±1.25	70.07	116 ±2.3	85.95	130 ±1.02	114.8
G3 St. after baking	110 ±7.25	81.48	129 ±4.02	95.55	140 ±1.42	103.7
G4 (Diabetic control)	162 ±4.25	170	162 ±4.25	170	162 ±4.25	170
G5 St. before baking	84 ±0.96	62.22	74 ±5.25	54.81	68 ±3.25	50.37
G6 St. after baking	115 ±2.11	85.18	90 ±3.02	66.66	85 ±5.54	62.96

Table 9. Blood urea of rats group fed on stevioside for three weeks.

Rat group	After (1) week		After (2) weeks		After (3) weeks	
	Mg/dl	%	Mg/dl	%	Mg/dl	%
G1 Normal control)	42 ±1.02	100	42 ±1.02	100	42 ±1.02	100
G2 St. before baking	66 ±1.01	157.1	62 ±3.25	147.6	57 ±0.24	135.7
G3 St. after baking	50 ±0.01	119	58 ±1.32	138	56 ±0.36	133.3
G4 (Diabetic control)	64 ±2.04	152.3	64 ±2.04	152.3	64 ±2.04	152.3
G5 St. before baking	60 ±1.25	142.8	72 ±1.36	171.4	74 ±0.02	176.1
G6 St. after baking	52 ±0.65	123.8	48 ±2.01	114.2	75 ±0.02	178.5

Table 10. Blood cholesterol of rats group fed on stevioside for three weeks.

Rat group	After (1) week		After (2) weeks		After (3) weeks	
	Mg/dl	%	Mg/dl	%	Mg/dl	%
G1 Normal control)	100 ±3.1	100	100 ±3.1	100	100 ±3.1	100
G2 St. before baking	78 ±1.03	78	84 ±2.25	84	97 ±3.01	97
G3 St. after baking	90 ±1.51	90	94 ±3.54	94	102 ±1.22	102
G4 (Diabetic control)	112 ±3.33	112	112 ±3.33	112	112 ±3.33	112
G5 St. before baking	80 ±2.25	80	90 ±4.25	90	92 ±2.36	92
G6 St. after baking	78 ±1.03	78	75 ±2.25	75	72 ±5.25	72

Table 11. Blood GOT and G P T of rats group fed on stevioside for three weeks.

Rat group	After (1) week		After (2) weeks		After (3) weeks	
	GOT	G P T	GOT	G P T	GOT	G P T
	Mg/dl	Mg/dl	Mg/dl	Mg/dl	Mg/dl	Mg/dl
G1 Normal control)	90.05 ±3.21	23.11 ±4.25	90.05 ±3.21	23.11 ±4.25	90.05 ±3.21	23.11 ±4.25
G2 St. before baking	90.11 ±2.02	22.15 ±2.66	98.11 ±2.21	25.01 ±1.22	89.01 ±2.25	23.12 ±1.33
G3 St. after baking	91.33 ±2.11	19 ±1.25	100 ±2.01	21.55 ±2.87	90.11 ±1.01	22.11 ±2.01
G4 (Diabetic control)	127.5 ±0.33	49.11 ±1.65	127.5 ±0.33	49.11 ±1.65	127.5 ±0.33	49.11 ±1.65
G5 St. before baking	100.2 ±1.02	29.25 ±3.36	104.5 ±2.25	30.1 ±2.36	105.4 ±2.25	31.16 ±1.25
G6 St. after baking	112 ±2.35	32.01 ±2.00	113.1 ±1.02	36.44 ±2.01	109.3 ±2.25	35.22 ±1.01

Histological examination

a. Liver

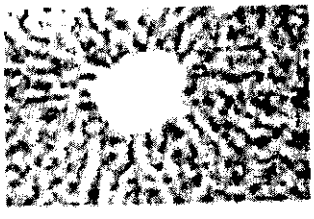


Fig. (15.1)

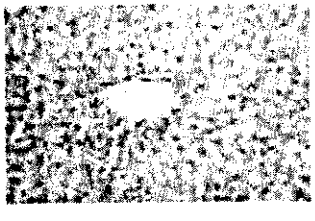


Fig. (15.2)

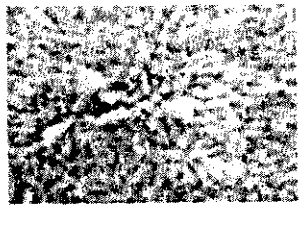


Fig. (15.3)

Photomicrograph of liver (H&E stain X 200)

The Histological examination of liver of normal rats fed on basal diet (1st group) showed that the hepatocytes were arranged in hepatic cards of one cell thick and normal central vein and normal portal area Fig (15.1.). The liver in both 2nd group (rats fed on basal diet contained stevioside added before baking) and 3rd group (rats fed on basal diet contained stevioside added after baking) showed the same normal structure as that of the 1st group. While liver of diabetic rat fed on basal diet (4th group) showed normal liver structure except very few lymphocytic infiltration in portal area Fig (15.2.). The diabetic rats fed on basal diet contained stevioside added before baking (5th group) showed some histological changes of liver. These changes were lymphocytic infiltration in portal area, and some hepatocytes showed vacuolar degeneration with pyknotic nuclei Fig (15.3.). The last group in which the diabetic rats fed on basal diet contained stevioside added after baking showed no changes in the structure of liver.

b. Kidney

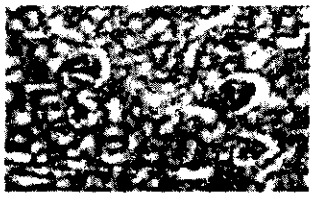


Fig (16.1)

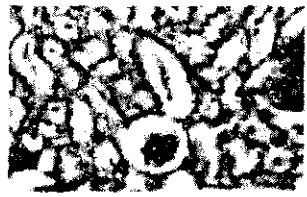


Fig (16.2)

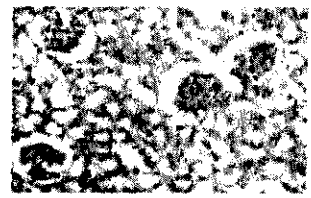


Fig (16.3)

Photomicrograph of kidney (H&E stain X 200)

The kidney of normal rats fed on basal diet (1st group) showed normal structure of both cortex and medulla. Both of renal corpuscles and renal tubules appeared normal (Fig 16.1.). Also, kidney of normal rats fed on basal diet contained stevioside added before baking (2nd group) showed normal structure of the kidney. The examined kidney of normal rats fed on basal diet contained stevioside added after baking (3rd group) showed few changes as coaguletive degeneration in the epithelium of renal tubules, pyknotic nuclei of some tubules, congestion of some glomeruli and lymphocytic infiltration between the renal tubules. Also, hyaline casts were appeared in the lumina of 4th, 5th and 6th group showed normal structure of renal tissue Fig (16.2.).

C. Cerebral cortex

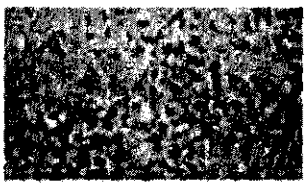
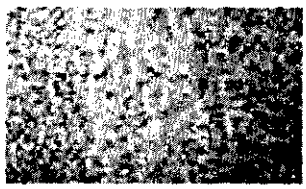
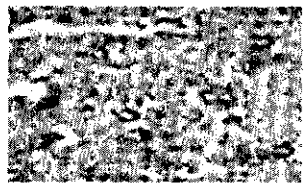




Fig (17.1) Fig (17.2) Fig (17.3)

Photomicrograph of Cerebral cortex (H&E stain X 200)

Layers of Cerebral cortex of normal rats fed on basal diet (1st group) showed normal structure, Fig (17.1.). The hiostological structure of Cerebral cortex of each of 2nd, 3rd and 4th groups showed no changes and appeared similar to that of the 1st group Fig (17.2.). The Cerebral cortex of diabetic rats fed on basal diet contained stevioside added before baking (5th group) showed very few neurotic foci, contained degenerated cells with pyknotic nuclei, Fig (17.3.). The diabetic rats fed on basal diet contained stevioside added after baking (6th group) showed normal histological structure of Cerebral cortex.

d. Cerebellum

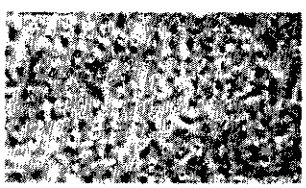
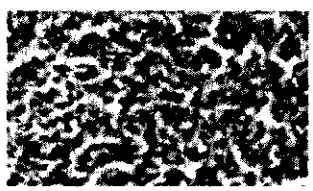
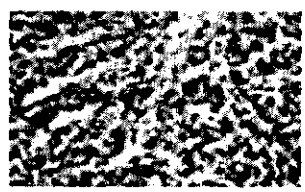
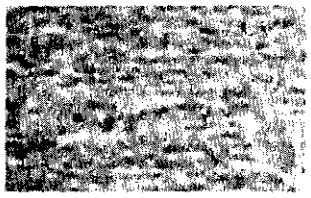
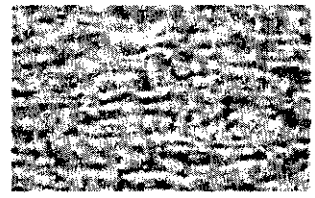
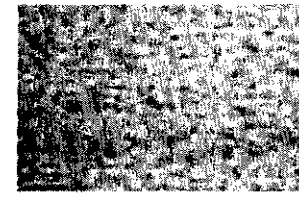




Fig (18.1) Fig (18.2) Fig (18.3)

Photomicrograph of Cerebellum (H&E stain X 200)

The normal rats of 1st, 2nd and 3rd groups showed normal histological structure of cerebellum. It was formed from three layers outward, medecular layer, purkinje cell layer and granular layer, Fig (18.1.). Also, the microscopical pictures of cerebellum of diabetic rats of 4th, 5th and 6th group are normal and similar to that of the 1st group, Fig (18.2.). Also, the 5th group showd very few areas of neurotic foci the tissue. Note the presence of pyknotic nuclei, Fig (18.3.).

e. Heart

Fig(19.1) Fig(19.2) Fig(19.3)

Photomicrograph of Heart (H&E stain X 200)

The heart of normal rats 1st and 2nd groups showed normal structure. The cardiac muscle appeared as uninucleated cardiac muscle fibers forming network, Fig (19.1.). Also, the same picture appeared in the heart of rats in 3rd group except little vascular congestion, Fig (19.3.). The histological structure of heart of diabetic rats in of 4th, 5th and 6th group are normal as similar as that of the 1st group, Fig (19.2.).

f. Spleen:

The histological structure of spleen in all examined rats of different groups showed normal structure of white pulp and red pulp. The aforementioned results coincide with those obtained by *Abd El-Baky (1999)* and *Boy (1973).*

We can conclude from the aforementioned results that all the histological changes due to added stevioside are considered non-significant changes.

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استخدام مستخلص نبات الإستيفيا فى إنتاج البسكويت

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- الاستفيوسـيد يعتـبر مـن المحليات الطبيعية الذى يستخرج من نبات الإستيفيا وهذا المركب درجة حلاوته تعادل ٢٥٠-٣٠٠ مرة قدر السكروز.
- أضـيف هذا المركب الى البسكويت بنسب [٠,٠٢٥ – ٠,٠٥ – ٠,٠٧٥ – ٠,١ %] لكل ١٠٠ جرام دقـيق مـرة قبل الخبز ومرة بعده – وتم إجراء التحاليل الكيميائية والريولوجية والحسية . أجريت تجـربة بيولوجـية ودراسة تشريحية للأعضاء الداخلية لحيوانات التجارب . ويمكن تلخيص النتائج فيما يلى :

- أفضل المعاملات حسيا كانت النسبة ٠,٠٢٥ % تليها النسبة ٠,٥ %.
- انخفاض مستوى الجلوكوز فى الحيوانات المصابة الى ما يقارب من المجموعة الضابطة- ونفس الاتجاه للكوليسترول والدهون الثلاثية .
- لا يوجـد فـرق معـنوى بيـن المجموعـات المخـتلفة بالنسبة لليوريا عند التغذية على الإستيفيوسيد.
- الفحـص التشريحى [والذى قام به طبيب متخصص] يوضح أنه لا توجد تغيرات معنوية عـند إضافة الاستفيوسيد – وذلك للأعضاء تحت الفحص وهى : الكبد – القلب – الكلى – المخ – الطحال.