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## Comparative Effect of Stevia and Aspartame Sweeteners: A Biochemical, Sero-Biochemical, Histochemical and Immunohistochemical Study

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### Abstract

Aspartame is a common artificial sweetener that is thought to be dangerous. On the other hand, stevioside (stevia) a natural sweetener of plant origin was assumed to be a natural alternative to artificial sweeteners. This study aimed to determine whether aspartame or stevioside affected biochemical, sero-biochemical, and immunohistochemical changes in adult rats following six weeks exposure. Forty-eight rats were used and divided into three groups: Group I: the control group, Group II: rats received aspartame (75 mg/kg/day), Group III: rats received stevioside (45 mg/kg/day). All medications were given orally for 6 weeks. Lipid Peroxidation product (MDA); liver glutathione (GSH); nitric oxide (NO); Liver Superoxidase dismutase activity(SOD); catalase (CAT), Low-density lipoprotein (LDL) ; very low lipoprotein (VLDL) ;the cholesterol in the HDL; Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST); Creatinine, Urea, Total proteins were assessed in blood and liver tissue specimens. In addition, hepatic histopathological and immunohistochemical investigations were done. Aspartame group showed significant increase in most of the investigated biochemical parameters (especially glucose, MDA, liver NO, LDL, VLDL, ALT, AST, creatinine and urea) with significant decrease in serum albumin and the endogenous antioxidants (GSH, SOD, CAT) compared to the control group while stevia-exposed animals showed significant decrease in the same biochemical parameters with significant increase in the same endogenous antioxidants. Moreover, severe histopathological and immunohistochemical changed were observed in liver of Aspartam-

treated animals. However, Stevia- treated rats showed the least changes. Therefore, it can be concluded that Stevia was less harmful than aspartame.

**Keywords:** Aspartame, Stevia, Sweeteners, Immunohistochemical, Biochemical

### Introduction

Sugars are a vital part of a modern diet that contributes to the quantities of fruit, vegetables, and nuts. Sugar is natural and safe to consume in small amounts as part of a healthy diet (*White, 2014*). Sugar can hinder your health when taken in excess. People are turning to sugar substitutes or sweeteners fastly on the guidance of nutritionists and dietitians. Sugar substitutes are sweet food additives that contain fewer calories than sugar. These sugar substitutes can originate from natural or artificial sources. Stevia and sugar alcohols are natural sugar substitutes (*White, 2014*). Artificial sweeteners are usually calorie-free and at least 30 times sweeter than sucrose. Food additives are ingredients added to food to enhance its shelf life, texture, and taste (*Simon and Ishiwata, 2003*).

Aspartame is an artificial sweetener that is commonly used with few calories. It is a methyl ester of a dipeptide (L-aspartyl- L-phenylalanine methyl ester) (*Mourad, 2011*). It has 200 times the sweetness of sucrose. In the intestine, ASP is broken down into its parts: phenylalanine, aspartate, and methanol. (*Frühbeck, 2012*) Each of these parts is toxic to various organisms.

After that, methanol is changed into formaldehyde, and formic acid is also changed into formaldehyde. Formic acid is the main metabolite that evokes the acute toxic effects of methanol in human and animals (*Portela et al., 2007*). In addition, prolonged administration of aspartame in experimental animals caused headaches, dizziness, nervousness, sadness, and liver disorder (*Ashok et al., 2013*). Also reported that many consumers had headaches, dizziness, rashes, bloating, nausea, diarrhea, and digestive and health problems after using artificial sweeteners. These adverse effects on the body could develop over time and cause serious long-term diseases with the consumption of these artificial sweeteners (*Spencer, et al., 2016*). Stevia, the common name for the naturally derived non-nutritive sweeteners, stevia glycosides, extracts from the *Stevia rebaudiana Bertonii* leaves. Stevia is a natural sweetener of medical and commercial value worldwide (*Ahmed et al., 2007*). Stevia is a herb that tastes sweet but has no calories. It can be used despite sugar or as a substitute to artificial sweeteners. (*Curi et al. 1986*) found widely dispersed evidence that stevia helps diabetics control their

blood sugar and raises insulin levels, which suggests that stevia controls how much food they eat. Experimental studies have shown that stevia controls insulin levels (*Chang et al., 2005*). Stevia is very popular and can be consumed by a lot of people. It could also help people control their weight (*Stamataki et al., 2020*).

The current study was performed to compare the effects of aspartame (artificial sweetener) and stevia (natural sweetener) on some biochemical, sero-biochemical and Immunohistochemical parameters in liver of albino rats.

## MATERIAL AND METHODS

### Experimental Chemicals and Drugs:

**1-Aspartame:** The tablets of aspartame purchased from (Amrya pharmaceutical companies in Alexandria - Egypt). Each tablet contains a dose of aspartame of 20 mg (one tablet equals a teaspoonful of sugar and 0.4 calories). Then dissolved in distilled water and given orally to rats for 45 consecutive days at a dose of 75 mg/kg body weight/per day (*Ashok et al., 2014*).

**2-Stevia:** Were purchased from Alpha Nexa Nutritionals Ltd. in Kent (U.S.A.) as a powder. Then dissolved in distilled water and given orally at a dose of 40 mg/kg body weight/day for 45 consecutive days as the predicted human therapeutic dose according to (*Paget and Barnes, 1964*).

Stevioside has the chemical formula C<sub>38</sub>H<sub>60</sub>O<sub>18</sub> (*Lemus-Mondaca et al., 2012*).

### 3. Experimental Design

In the present study, we used 48 male albino adult rats that were almost the same size and weight (100 ± 10 gm). Three groups of rats (n=16) were formed using a random allocation method. Laboratory settings (25 ± 0.5 °C room temperature, 50-60% relative humidity, 12 h dark-light alternation with 12-14 air changes/h) with access to drink and food act as antioxidant protector. There were three groups: one received no treatment (the "control group"), one administered aspartame orally at a dose level of 75 mg/kg body weight/day (the "aspartame group"), and one received stevia orally at a dose level of 40 mg/kg body weight/day (the "stevia group"). Both short-term (21 days) and long-term (45 days) experiments were conducted. After 21 days, eight animals from each group were dissected. All the other animals were kept in same experimental conditions for 45 days before being dissected.

### 4 Sampling:-

#### 4.1 Blood sampling:

After each study length (21 and 45) blood samples were taken from each group's inner canthus of the eye (*halperin et al., 1951*) in empty, dry, and clean tubes, for sero-biochemical analysis, blood samples were maintained in a water bath set to 37 °C for 15 minutes and

then centrifuged at 3000 r.p.m. for 10 minutes and the clear serum was separated carefully.

#### **4.2 Tissue Sampling:**

After 45 days of experiment, liver samples were collected from all groups, sliced into pieces, frozen at -20°C, and then homogenised in phosphate buffer solution (PH 7.4) using an MPW-302 Poland homogenizer.

Liver homogenates are used for the determination of several biochemical parameters. This included Lipid Peroxidation product (MDA); liver glutathione (GSH); Liver Superoxide dismutase activity (SOD); catalase (CAT).

For the histological and histochemical investigations, liver tissues were extracted and kept in 10% buffered formalin. Following fixation, the specimen was washed, dehydrated, embedded, sectioned, and stained for microscopic examination.

### **5 Biochemical Parameters:**

#### **5.1 Carbohydrate profile tests**

Serum glucose was evaluated by the method of (*Trinder, 1969*). The commercial kit was purchased from Randox, U.K.

#### **5.2 Determination of serum Albumin:**

Serum albumin was valued by the method of (*Young 1995 ; Burtis et al., 2012*).

#### **5.3 Sero-biochemical parameters:**

Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were valued in

serum According to (*Murray et al, 1984 ; Young 1995*).

#### **5.4. Determination of serum total protein concentration:**

Serum glucose was valued by the method of (*Koller 1984 ; Burtis et al. 2012*).

#### **5.5. Determination of kidney function**

Creatinine, Urea, spectrophotometrically using commercially available diamond diagnostics kits (Germany) (*Murray and Kaplan et al, 1984*).

#### **5.6 Determination of serum lipid profile:**

##### **5.6.1 Determination of serum cholesterol concentration:**

Serum cholesterol was valued by the method of (*Meiattini et al., 1978; Naito and David 1984*).

##### **5.6.2 Evaluation of Serum Triglycerides concentration:**

The amount of triglycerides in the sample determines the intensity of the resulting colour. (*Young 2002; Burtis et al., 2012*).

##### **5.6.3 Determination of serum high-density lipoprotein (HDL) concentration:**

The sample's low-density lipoprotein (LDL) and very low lipoprotein (VLDL) precipitate with phosphotungstate and magnesium ions. The cholesterol in the HDL fraction that remained in the supernatant after centrifugation is determined (*Friedewald et al., 1972; Lopes - Virella et al., 1977*)

##### **5.6.4 Determination of serum Low-density lipoprotein (LDL) concentration:**

LDL Cholesterol (mg/dl) = {total cholesterol – (triglycerides/5) – HDL Cholesterol}

### **5.7 Oxidative stress markers tests**

**5.7.1 Determination of Malondialdehyde (MDA) concentration in liver tissue:** Serum glucose was valued by the method of (*Draper and Hadley, 1990*).

**5.7.2 Determination of Glutathione (GSH) level in liver tissue:**

according to the method of (*Prins and Loose 1969*).

**5.7.3 Determination of liver Superoxide dismutase (SOD) activity:** SOD was valued by a colorimetric method (*Minami and Yoshikawa 1979*).

**5.7.4 Determination of Catalase (CAT) Activity in liver tissue:** Catalase activity in liver tissue was valued by (*Bock et al. 1980*) method.

### **5.8 Histopathological Methods**

Livers from animals were carefully dissected, removed and washed then subsequently cut into small pieces for histological examination, and placed in 10% buffered formalin. Dehydration of fixed tissues was done using ethyl alcohol and then cleared with xylene. Infiltration with paraffin wax at 60°C was followed by embedding. Paraffin blocks were cut at 6 microns from all specimens. For collagen fiber examination; sections were stained by Mallory's trichrome stain (*Gomori, 1950; modified by Sweat et al., 1968*).

### **5.9 Histochemical Methods:**

Liver samples were sectioned and stored in 10% buffered formalin for subsequent histochemical analysis (*Lillie, 1954*). Furthermore, the following staining methods were used to illustrate specific histochemical characteristics (Carmine Method by Best) (*Best, 1906*)

### **5.10 Immunohistochemical staining:**

Sections were cut from the chosen paraffin blocks at a thickness of 4 micrometers. Primary anti-TNF and anti-caspase-3 antibodies were used for incubation (Sigma,USA). Next, we incubated the samples with the specific secondary antibody (Sigma,USA). Each slide is briefly counterstained with hematoxylin for 30 seconds before being dehydrated and mounted (*Ilić et al., 2019*)

### **Statistical Analysis:**

Representation of data as means  $\pm$  standard error of means (SE) using (SPSS) version 10 program to evaluate all of the acquired data. The significance of variations in mean values between control and treatment rats was determined using (ANOVA) test. Statistical significance was defined as a value of  $p < 0.05$  (*Tello and Crewson, 2003*).

### **Results**

The effects of daily oral administration of stevia and aspartame at a dose levels (**40mg/kg/day**) and (**75/kg/day**), respectively in adult male albino

rats for 21 and 45 successive days on serum enzymatic activity (AST and ALT), liver total protein, urea, and creatinine were studied.

Pieces from the liver of each rat in each group were taken to estimate the oxidative stress biomarkers, including (GSH, SOD, and MDA) at Day 45 of the experiment.

### **Glucose and Albumin**

**Serum glucose:** glucose level showed a significant elevation ( $p < 0.05$ ) at (21 and 45 days) in rats received aspartame compared to control and stevia treated albino rats, meanwhile there was non significant change in stevia treated rats compared to control group all over the experimental period. Table (1)

**Serum albumin** Table (1) recorded that aspartame albumin level showed a significant decrease ( $p < 0.05$ ) at (21 days) and recorded a highly significant reduction at ( $p < 0.01$ ) at (45 days) than stevia and control-treated albino rat, meanwhile stevia treated rat non significantly changed than control group all over the experimental period.

### **Liver function tests**

#### **Liver Aspartate transaminase (AST) activity**

**Table (2)** recorded that Aspartame treated group recorded a highly significant increase at ( $p < 0.01$ ) than the control and stevia groups at 21 days, meanwhile it recorded a very highly significant increase at ( $p < 0.001$ ) than the control group at 45 days. Also, the AST levels of the

Aspartame treated group showed highly significant elevation at ( $p < 0.01$ ) than the stevia-treated group.

#### **Liver Alanine transaminase (ALT) activity**

**Table (2)** recorded that Aspartame treated group recorded highly significant increase at ( $p < 0.01$ ) than the control and stevia groups at 21 days, meanwhile it recorded a very highly significant elevation at ( $p < 0.001$ ) than control group at 45 days. Also, the ALT levels of the Aspartame treated group were raised very highly significant at ( $p < 0.001$ ) than the stevia-treated group.

#### **Liver total Protein (TP) level**

**Table (2)** recorded that aspartame total protein level exhibited a significant decrease ( $p < 0.05$ ) at (21 and 45 days) than stevia and control-treated albino rat, meanwhile stevia treated rat non significantly changed than control group all over the experimental period.

#### **Effect on Urea and Creatinine:**

##### **a- Serum creatinine**

From the data recorded in a **table (4)**,

Aspartame treated group recorded a highly significant elevation at ( $p < 0.01$ ) than the control group and at ( $p < 0.05$ ) than stevia group at 21 and 45 days. Meanwhile, stevia revealed non-significant change than control all over the experiment.

##### **b- Serum urea**

Aspartame treated group recorded a significant increase at ( $p < 0.05$ )

than the control group at 21 days, meanwhile it recorded very highly significant increase at ( $p < 0.001$ ) than the control group at 45 days. meanwhile, the serum urea levels of the stevia-treated group were non significantly changed than the aspartame and control-treated group at 21day and recorded a significant increase ( $p < 0.05$ ) in respect to the aspartame and control group at 45 day .

#### **Lipid profile:**

##### **Total cholesterol(TC) activity**

**Table (3)** recorded that aspartame cholesterol level displayed a significant elevation( $p < 0.05$ ) at (21 days)and recorded a highly significant elevation at( $p < 0.01$ )at(45 days) than stevia and control-treated albino rat , meanwhile stevia-treated rat significantly decreased than the control group at ( $p < 0.05$ )all over the experimental period.

##### **Total triglyceride (TT) activity**

**Table (3)** recorded that aspartame triglyceride level exposed a highly significant elevation( $p < 0.01$ ) at (21and 45 days) than stevia and control-treated albino rat , meanwhile, stevia treated rat showed a highly significant decrease than the control group at ( $p < 0.01$ )all over the experimental period .

##### **- (HDL) cholesterol level**

**Table (3)** recorded that aspartame HDL levels revealed a highly significant decrease ( $p < 0.01$ ) at (21and 45 days) than stevia and control-treated albino rat ,

meanwhile stevia treated rat showed a significant elevation than control group at ( $p < 0.05$ )all over the experimental period.

##### **LDL -chlostreol (LDL) level**

**Table (3)** recorded that aspartame LDL levels exhibited significant elevation( $p < 0.05$ ) at (21and 45 days) than stevia and control-treated albino rat , meanwhile stevia treated rat showed a significant decrease than the control group at ( $p < 0.05$ )all over the experimental period .

##### **Oxidative stress markers**

##### **Liver malondialdehyde activity (MDA)**

From the data recorded in **Table (5)**, aspartame recorded a significant elevation at ( $p < 0.05$ )than the control group and a very highly significant increase than the stevia ( $p < 0.001$ ) .and the stevia recorded a significant decrease at( $p < 0.05$ )than the control group and very highly significant decrease than the aspartame.

##### **Liver glutathione activity (GSH)**

From the data recorded in **Table (5)**, aspartame recorded a significant decrease at( $p < 0.01$ )than the control group and designated a highly significant reduction ( $p < 0.01$ ) in their mean values recording compared to stevia ones. Stevia treated animals designated significant increase ( $p < 0.05$ ) in their mean values recording compared to control ones.

##### **Liver superoxide dismutase (SOD)**

From the data recorded in **Table (5)**, Aspartame treated animals

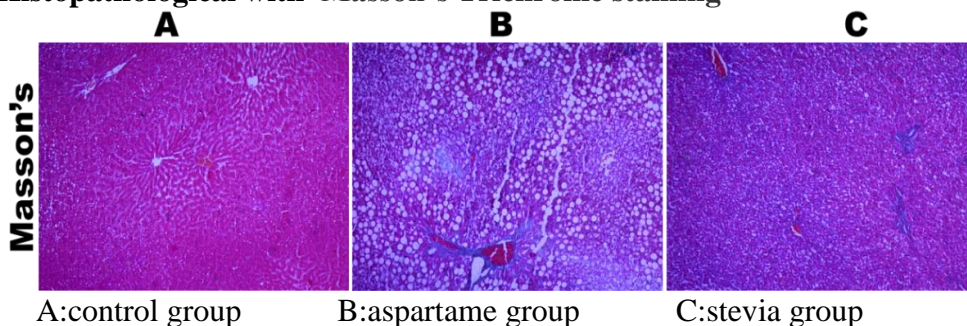
designated a significant decrease at ( $p < 0.05$ ) than the control group and stevia group.

#### Catalase

Aspartame treated animals designated a highly significant

reduction at ( $p < 0.01$ ) than the control group and stevia group., meanwhile, Stevia treated animals designated a significant rise ( $p > 0.05$ ) in their mean values compared to control ones (Table 6).

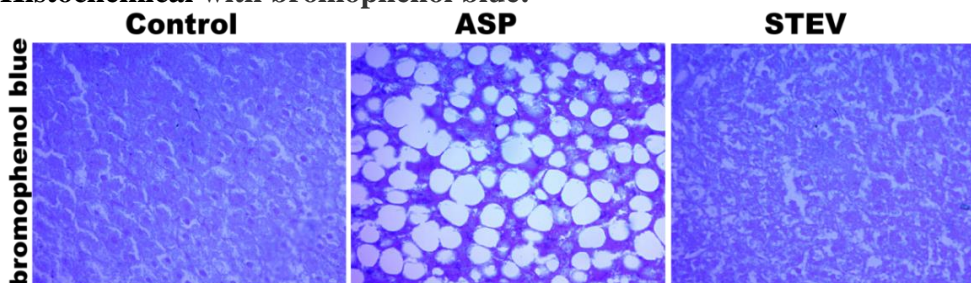
#### Histopathological with Masson's Trichrome staining



Figure(1) :

Masson's Trichrome staining . a) Shows the normal distribution of collagen fibers, stained green, around a portal tract. b) Shows a marked increase in collagen fibers around the blood vessels in the portal tract in the ASP-treated group. c) Liver tissue shows normal collagen fiber deposition stained very light green indicating collagen fiber deposition.

#### Histochemical with bromophenol blue:



Figure(2):

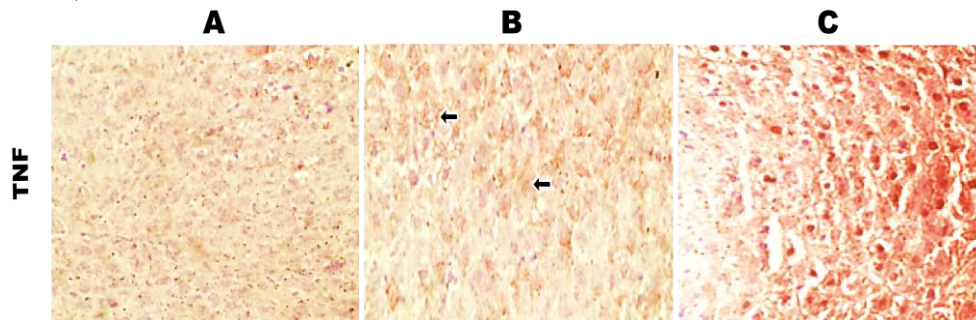
bromophenol blue-stained liver slices revealing a high level of protein content

a) liver of control albino rat showing intensely stained cytoplasm, nucleus, and cell membrane of hepatocytes.

b) ASP treated group showing moderate staining of hepatocytes

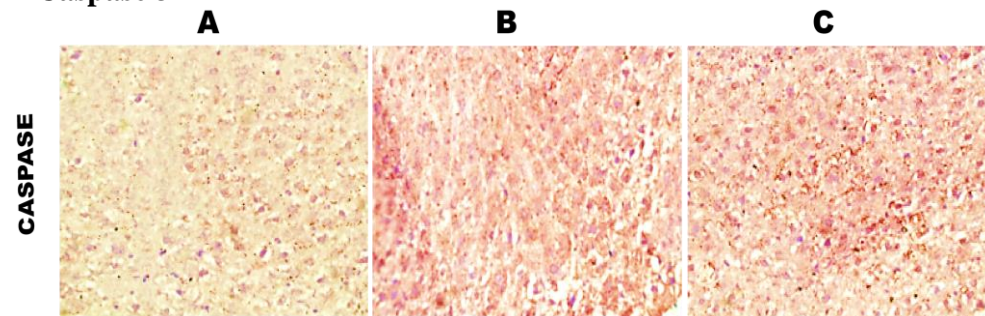
c) STEV group showing strong staining.



**Immunohistochemical staining****1-TNF**

**Figure (3):** TNF-  $\alpha$  staining of liver to detect carcinogenesis

- Control group showed negative expression of TNF-  $\alpha$  in hepatocytes (IHC, 40x).
- ASP group showed strong and diffuse expression of TNF-  $\alpha$  in hepatocytes (IHC, 40x)
- STEV group showed less diffuse expression of TNF-  $\alpha$  in hepatocytes (IHC, 40x) which indicates that the carcinogenic effect is lower in incidence compared to aspartame.

**2-Caspase 3**

**Figure(4):**

- Control group showed negative expression of Caspase 3 in hepatocytes (IHC, 40x).
- ASP group showed moderate and diffuse expression of caspase 3 in most hepatocytes (IHC, 40x).
- STEV group exhibited a significant reduction in the expression of caspase 3 in hepatocytes (IHC, 40x).

**Table 1:** Effect of aspartame and stevia administration on glucose and albumin levels in rats.

Parameters		Serum glucose (mg/dL)	Serum albumin (g/dL)
Time 21 days	Control	117.0 ±8.504 <sup>b</sup>	3.88 ±0.159 <sup>b</sup>
	Aspartame	136.333 ±16.56 <sup>a</sup>	3.80 ±0.100 <sup>*a</sup>
	Stevia	121.0±16.623 <sup>b</sup>	4.26±0.136 <sup>*b</sup>
45 days	Control	102.0±2.489 <sup>b</sup>	3.93 ±0.666
	Aspartame	112.20 ±3.679 <sup>*a</sup>	3.0 ±0.577 <sup>**a</sup>
	Stevia	105.4±1.60 <sup>*b</sup>	4.30±0.20

Values are presented as means ± SEM

\*p < 0.05(Significant)

\*\*p < 0.01(highly Significant)

\*\*\*p < 0.001(Very highly Significant)

a: significant values compared to control.

b: significant values compared to Aspartame treated group.

c: significant values compared to Stevia treated group.

**Table 2:** The effect of aspartame and stevia on liver function

Parameters		AST (IU/L)	ALT(IU/L)	TP(g/dL)
Time 21 days	Control	43 ±6.027 <sup>b</sup>	28.33 ±1.33 <sup>b</sup>	7.13 ±0.13 <sup>b</sup>
	Aspartame	72 ±3.055 <sup>***a</sup>	40.43 ±1.946 <sup>***a</sup>	6.53 ±0.2 <sup>*a</sup>
	Stevia	50.66 ±5.206 <sup>**b</sup>	31.66 ±0.881 <sup>**b</sup>	7.36 ±0.06 <sup>*b</sup>
45 days	Control	45 ±3.701 <sup>b</sup>	33.4 ±0.87 <sup>b</sup>	7.54 ±0.02 <sup>b</sup>
	Aspartame	73.20±3.20 <sup>***a</sup>	51.68 ±3.05 <sup>***a</sup>	6.9 ±0.18 <sup>*a</sup>
	Stevia	54.40±3.385 <sup>**b</sup>	39.02 ±0.54 <sup>***b</sup>	8.22±0.11 <sup>*b</sup>

**Table 3:** The effect of aspartame and stevia on Urea and Creatinine.

Parameters		Serum urea (mg/L)	Serum creatinine (mg/L)
Time 21 days	Control	25.333 ±0.0	0.66 ±0.67
	Aspartame	27.666 ±0.666 <sup>*a</sup>	1.40 ±0.577 <sup>**a</sup>
	Stevia	26.333 ±0.333	0.76 ±0.33 <sup>*b</sup>
45 days	Control	26.260±0.340	0.72 ±0.58
	Aspartame	28.360±0.193 <sup>***a</sup>	1.68 ±0.73 <sup>**a</sup>
	Stevia	27.320 ±0.193 <sup>*a,*b</sup>	0.86±0.814 <sup>*b</sup>

**Table 4:** The effect of aspartame and stevia on lipid profile

Parameters		Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-Cholesterol (mg/dL)	LDL-Cholesterol (mg/dL)
Time 21 days	Control	70. ±2.02	110.66 ±7.31	38.0 ±1.15	38.00 ±5.77
	Aspartame	93.66 ±2.33 <sup>**a</sup>	144.0 ±4.041 <sup>***a</sup>	35.33 ±1.20 <sup>**a</sup>	42.00 ±1.15 <sup>*a</sup>
	Stevia	69.71 ± 2.03 <sup>**b</sup>	91.67 ±5.17 <sup>**b</sup>	47.0 ±1.76 <sup>*a</sup>	33.33 ±.881 <sup>*a</sup>
45 days	Control	75.40 ±1.20	140.0 ±10.59	39.20 ±1.49	41.40 ±1.02
	Aspartame	97.20 ±4.72 <sup>**a</sup>	177.60 ±5.97 <sup>**a</sup>	37.8 ±1.30 <sup>**a</sup>	48.2 0±1.46 <sup>*a</sup>
	Stevia	65.40 ±1.88 <sup>**b</sup>	94.44 ±1.53 <sup>**b</sup>	51.00±4.0 9 <sup>*b</sup>	38.0 ±0.58 <sup>*b</sup>

**Table 5:** The effect of aspartame and stevia on Oxidative Biomarkers

Parameters		MDA (µmol/gtissue)	GSH(mgGSH/g tissue)	SOD(µmol/g tissue)
Time 45 days	Control	4.33 ±1.7346	23.0 ±0.7071	3.78 ±0.598
	Aspartame	5.2 ±2.2408 <sup>*a</sup>	19.80 ±0.969 <sup>**a</sup>	3.04 ±0.448 <sup>*a</sup>
	Stevia	3.33±1.3524 <sup>*b</sup>	24.80±1.09 <sup>**b</sup>	4.34 ±0.403 <sup>*b</sup>

**Table 6 :**The effect of aspartame and stevia on catalase.

Parameters		Catalase (µmol/gtissue)
Time 45 days	Control	14.66 ±1.7346
	Aspartame	11.72±0.832 <sup>**a</sup>
	Stevia	14.82 ±1.98 <sup>*b</sup>

**Discussion**

In the current research, rats given aspartame had significantly higher glucose levels compared to both controls and rats given stevia. On the other hand, the glucose levels of the rats that were given stevia showed no difference between the two groups. Consistent with previous findings (Helal et al., 2019) hyperglycemia was also observed in the aspartame-treated rats, which is not surprising given that long-term consumption of the sweetener has been incriminated in the development of type II diabetes in rodents. Persistent hyperglycemia in diabetes results in an increase in free radical production in all tissues (Proki et al., 2015). Contrary to that, stevia has higher solubility in water and a positive taste profile that is safely metabolized by the body without any side effects (Megeji et al., 2005 ; Geuns et al., 2007).

In the current study, there was an obvious decrease in albumin serum

levels in the aspartame-treated group at 21 and 45 days as compared to the control and stevia-treated group, no change was recorded in albumin level in stevia-treated rats compared to the control. In accordance to aspartame results (Iman et al., 2019) male albino rats were given aspartame at a dose of 50mg/kg/day for 30 day . Regarding liver function our results showed that aspartame at 21 days induced a highly significant increase in the activities of AST and ALT and a higher significant increase at 45 days. Aspartame is thought to cause biochemical alterations in the liver. These changes in enzyme levels could be affected by dose and exposure period. After consuming aspartame for a long time (45 days), the quantity of its metabolites, particularly methanol, rises in the blood, altering the oxidant/antioxidant balance and the surface charge density, resulting in ALT and AST leakage (Humphries

*et al., 2008*). Our result agreed with those reported by (*Oluwatosin and Olubukalo et al .,2016*) in rats given aspartame at three doses 15,35,70 mg/kg/day, all parameters increased from dose 35 g/kg in albino rats dosed aspartame for 90 days.

The result of the total protein significantly decreased at 21 and 45 days in rats received aspartame compared to the control and stevia treated groups, Meanwhile, stevia group showed no changes from the control all over the experimental period following results, those of (*Helal et al .,2019*).

For aspartame (*Mohan and Robert et al .,2009*) in rats given an aqueous extract of stevia leaves powder the changes in total protein. According to (*Mohan and Robert 2009*), blood protein levels were found to be near normal in rats given an water extract of stevia leaves powder, with no significant differences, implying that Stevia rebaudiana leaf extract has an anti-hepatotoxic effect.

The current findings demonstrated that administration of aspartame affects lipid profiles producing a highly significant elevation in serum total cholesterol,(TG), low-density cholesterol (LDL) associated with a significant depletion of HDL at 21 and 45 days of experimental in respective with control and stevia groups these result agreed with serval studies recorded by (*Oluwatosin and Olubukola et al .,2016;Helal et*

*al.,2019*)in rat treated with different doses of aspartame. Our study indicated that stevia sweeteners significantly decreased triglycerides (TG), total cholesterol(TC) and low-density cholesterol( LDL) whereas (HDL) high-density cholesterol was significantly increased compared to control group. These findings are consistent with those of (*Curry and Roberts 2008;Rajesh et al 2009*) who discovered that stevioside dramatically decreases total cholesterol, triglycerides, LDL-C, and VLDL while increasing HDL-C levels when compared to untreated rats. However, (*Park and Cha 2010*) discovered that rats fed stevioside 1 ml/kg /day with a high-fat diet showed lower total cholesterol concentrations than rats received a high-fat diet with water.

Concerning oxidative biomarkers, the present study demonstrated that aspartame at a dose of (20 mg/kg.b.wt / day) for 45 days implies a significant increase in MDA with significant decrease in GSH, SOD, and CAT biomarkers in comparison with control and stevia treated rats similar result was reported by (*Iman.,2011;Saeed.,2016; Mohammed et al.,2017*)

The possible explanation for decreasing in GSH levels in liver tissue homogenates of aspartame-treated animals is due to the reduction in protein thiols from aspartame intoxication (*Ashok and Sheeladevi, 2015*). Methanol, one

of the aspartame metabolites, contributes to the formation of superoxide anion and hydrogen peroxide, which may play a role in the increase of malondaldehyde level (*Parthasarathy et al., 2006 ;Choudhary and Devi, 2014*). On the other hand, the obtained results showed that stevia at a dose (40mg/kg/day) for 45 days induced a significant increase in the concentration of GSH, SOD and CAT accompanied by a significant decrease in MDA .

Total liver antioxidants and liver MDA were both considerably boosted by ingesting stevia. Total phenols in Stevia extract are high, which is a good sign for the antioxidant, free radical scavenging, and inhibition of lipid peroxidation properties of Stevia and may account for the observed improvement in the antioxidant defence mechanism in stevia-supplemented rats compared to those of aspartame (*Shivanna et al., 2013*). Increased GSH production due to Stevia extract may also lower oxidative stress (*Singh et al., 2013 ; Ibrahim et al., 2015*). Some enlarged hepatocytes with foamy cytoplasm were observed 21 and 45 days after oral treatment of stevia at a dose of (40 mg/kg body weight /day). A typical collagenous fibre architecture and distribution were still seen in the majority of the liver, as reported by (*Abo El-naga et al., 2016*).

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### الملخص العربي

#### التأثير المقارن لمحليات ستيفيا والأسبارتام: دراسة كيميائية حيوية ، وكيميائية حيوية مصلية ، وكيمياء نسيجية ، وكيمياء مناعية

ايمان سامح, كوثر عبد الواحد الهادي, سحر سرور, ريهام الطرابيلي, احمد شاکر, رانيا حلمي عبده

في السنوات الأخيرة ، نما سوق المحليات بشكل كبير. الأسبارتام هو محلي صناعي شائع يُعتقد أنه خطير. من ناحية أخرى ، ستيفيوسيد هو نبات يحتوي على السكر ويفترض أنه بديل طبيعي للمحليات الصناعية. الأهداف: كان الهدف من هذه الدراسة هو تحديد ما إذا كان الأسبارتام أو الستيفيوسيد قد أثر على التغيرات الكيميائية الحيوية ، والتغيرات الكيميائية الحيوية المصلية والكيميائية المناعية في الجرذان البالغة بعد ستة أسابيع. المواد والطرق: تم استخدام ثمانية وأربعين فأراً وقسمت إلى ثلاث مجموعات: المجموعة الأولى: المجموعة الضابطة ، المجموعة الثانية: الفئران التي تلقت الأسبارتام (75 مجم كجم / يوم) ، المجموعة الثالثة: تلقت الفئران ستيفيا (45 مجم كجم / يوم). تم إعطاء جميع الأدوية عن طريق الفم لمدة 6 أسابيع. منتج بيروكسيد الدهون (MDA) ؛ الجلوتاثيون في الكبد (GSH) ؛ أكسيد النيتريك (NO) ؛ نشاط ديسموتاز سوپر أوكسيداز الكبد (SOD) ؛ الكاتلاز (CAT) ، البروتين الدهني منخفض الكثافة (LDL) ؛ البروتين الدهني المنخفض جداً (VLDL) ؛ الكوليسترول في HDL ؛ Alanine Aminotransferase (ALT) ، و Aspartate Aminotransferase (AST) ؛ تم إجراء الكرياتينين واليوريا والبروتينات الكلية على عينات الدم والأنسجة. النتائج: أظهرت مجموعة الأسبارتام زيادة في المستويات مقارنة بالمجموعة الضابطة وأظهرت المعالجة بالستيفيوسيد انخفاضاً في المستويات مقارنة بمجموعة التحكم كان للأسبارتام تأثيرات ضارة أكثر من ستيفيا. الخلاصة: Stevioside كان أقل ضرراً من الأسبارتام.