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THE EFFECTS OF STEVIOSIDES, ASPARTAME AND SUCROSE ON SOME BIOCHEMICAL, HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDIES IN RAT LIVER TISSUE

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ABSTRACT

The goal of this study is to compare the effect of Steviosides and aspartame as natural and synthetic low-calorie sweeteners with sucrose as a high calorie widely used sweetener. This was achieved by using 32 male albino rats. Animals were divided into four experimental groups control, sucrose group receiving 10% sucrose solution, aspartame group receiving 75 mg aspartame/kg body weight /day and Steviosides group receiving 40 mg/kg body weight /day. Both Steviosides and aspartame administration to rats for 90 days resulted in significant decreases in final body weights, body weight gain, food consumption and caloric intake compared to the significant increase under sucrose supplementation. Also, sucrose and aspartame administration caused a decline in liver total antioxidant activity, catalase and glutathione with a significant increase in liver malondialdehyde and nitric oxide levels. Quite the opposite, Steviosides supplementation resulted in a significant elevation in liver total antioxidant activity catalase glutathione with a decrease in in liver malondialdehyde and nitric oxide levels. In addition, aspartame supplementation produced a significant elevation in serum glucose, insulin, deterioration in antioxidant markers, liver function tests with related histological liver damage and increased liver PCNA stained nuclei activity. Steviosides has the potential to be used as a non-caloric natural sweetener instead of the synthetic sweetener aspartame due to its antidiabetic and antioxidant potency for controlling free radicals. These results suggest that replacing sucrose by Steviosides in the diet may be an effective strategy to manage food intake without side effects.

KEYWORDS: Aspartame, Proliferating cell nuclear antigen Steviosides, Sucrose.

INTRODUCTION

Sucrose is a natural caloric sweetener extracted from sugar cane. It is extensively used in human nourishment due to its pleasant taste, nutritive value plus its low cost manufacture.[1] Sucrose sweetened beverages induce obesity owing to its adverse effects on body weight.[2] This increases the risk for type II diabetes and cardiovascular diseases.[3]

To counteract this high sucrose demand, non-nutritive sweeteners either synthetic or natural are used to substitute sugars in diverse food products.[4]

So that, individuals struggling with obesity can enjoy foods and beverages without the risk of consuming additional calories contributed by normal sugar-based products.[3]

Aspartame is one of the most controversial and widely used artificial low-calorie sweeteners.

It has been also used as a food additive in soft drinks, desserts, yogurt, chewable multi-vitamins, breakfast cereals, tabletop sweeteners and pharmaceuticals.[5]

There has been much debate regarding the health disadvantages concerning the consumption of aspartame. Aspartame is metabolized in the gastrointestinal tract to aspartic acid, methanol and phenylalanine. Methanol is then oxidized to formaldehyde and latter to formic acid. The toxic effects of formic acid arise from the inhibition of cytochrome oxidase complex at the end of the respiratory chain in mitochondria.[6] Additionally, liver is the main site for the metabolism of aspartame metabolites. The by-products of such metabolism sometimes are more toxic than the initial substance.[7] Besides, aspartame supplementation alters the redox status of the liver with deleterious effects on antioxidant status. In addition long term aspartame supplementation has histological and histopathological alterations.[8] Also, chronic aspartame supplementation can cause damage to

fundamental cellular components leading to subsequent cell death by necrotic or apoptotic mode.[9] These alterations have increased the doubts about the appropriateness of aspartame as a substitute for sucrose in the diet.[10]

Therefore, the idea of using herbal plants is preferred because they are capable to protect the body from damage caused by free radical induced oxidative stress.[11]

Stevia rebaudiana Bertoni is a natural, sweet tasting, calorie free botanical herb. It is 200–350 times sweeter than sucrose. Steviosides are diterpene glycosides obtained from the leaves of *Stevia rebaudiana* Bertoni. This extract can be considered as natural alternative to artificial sweeteners.[12] Thus, it has the potential to assist individuals in regulating their weights as it has a positive effect on caloric substitution.[3]

Furthermore, Steviosides have multipurpose medicinal uses. It is used for the treatment of various conditions such as diabetes, obesity, hypertension, fatigue, depression.[13] However no significant toxicity has been reported with the use of Steviosides.[4]

As well, most liver diseases involve nitrogen and oxygen free radicals that attack macromolecules for the production and perpetuation of hepatic damage. Therefore, the antioxidant properties of Steviosides make this medicinal herb an excellent therapeutic strategy to fight liver disease.[14] This study aims to suggest promising avenues for both manufacturers and individuals to substitute sugar-based products and food additives by Steviosides extracted from *Stevia rebaudiana* Bertoni herb to combat the posed side effects from the long-term use of the artificial sweetener aspartame or the widely used sweetener sucrose.

2. MATERIALS AND METHODS

2.1. Experimental animals

A total number of 32 male albino rats of the strain *Rattus norvegicus* weighing (100-110) grams were bred and maintained under conventional conditions at the experimental animal research unit of Medical Research Center and Bilharzia, Faculty of Medicine, Ain Shams University. They were kept under standard laboratory conditions (25°C, 60–70% relative humidity and a 12-h light/dark cycle), housed in metal cages in a well-ventilated room, and fed a standard commercial chow diet and water. Animals were acclimatized to laboratory conditions for 5 days before the beginning of the study. Animal procedures were made in accordance with the National Institutes of Health (NIH) protocol approved by Ain Shams University.

2.2. Experimental treatments and dosage

Sucrose was administrated orally to rats as 10% sucrose solution dissolved in water (w/v). Sucrose was purchased from ADWIC (El-Nasr pharmaceutical chemicals Company, Egypt). It was orally supplemented to rats for 90 consecutive days according to the method of Kendig et al.[15] This sucrose solution provided a caloric density of (approx. 0.4 kcal/g) similar to most commercially available sugar drinks.

Aspartame (1-methyl N-L-a- aspartyl -L-phenylalanine**)** was purchased as tablet formulation from (Amrya for pharmaceutical industries Alexandria, Egypt). Each tablet contained 20 mg of aspartame (one tablet equal teaspoonful of sugar and 0.4 calorie) Tablets were dissolved in water and given orally to rats at a dose of 75 mg/kg body weight /day for 90 consecutive days according to the method of Ashok and Sheeladevi.[6]

Steviosides extracted from the leaves of *Stevia rebaudiana* Bertoni were purchased as a powder from Alpha Nexa Nutritionals Ltd, Kent (U.S.A.). It was dissolved in water and given orally as the calculated equivalent human therapeutic dose according to Paget and Barnes.[16] at a dose of 40 mg/kg body weight /day for 90 consecutive days.

2.3. Animal grouping

Rats were divided into four groups. The first group served as control group receiving water. The second group represented the sucrose group, the third group represented the aspartame group and the fourth group represented the Steviosides group. Eight animals from each group were dissected after 90 days of experimental study.

2.4. Morphological Investigations

2.4. 1. Growth Rate and Body weight gain

Rats were weighted by means of a Meopta sensitive balance for monitoring initial and final body weights. Average body weight for each rat in all experimental groups was recorded every week from the beginning until the end of the study. Whole weights were recorded to the nearest gram to determine weekly changes. Body weight gain was calculated at the end of the study period by subtracting final body weight from initial body weight for each rat in every group according to the method of Prokić et al.[10]

2.4. 2. Food consumption and Caloric intake

Food consumption was calculated daily in g/rat/day by subtracting the amount of uneaten food at the end of the day from that provided at the beginning of the day.

Caloric intake was calculated according to the method of Prokić et al.[10]

Caloric intake (Kcal/g/day) = Daily food intake (g) × total energy of food (Kcal/kg) /1000

Total energy of food (Kcal/kg) = 3.828 which was calculated according to Santos et al.[17]

2.4. 3. Final and relative liver weight

Dissection was performed after 90 days of experimental study to rats from the four experimental groups. Liver was then removed, weighted and recorded to the nearest

mg. The relative liver weight of each animal was then calculated according to the equation of Aniagu et al.[18]

$$\text{Relative liver Weight} = \frac{\text{Liver weight (g)} \times 100}{\text{Body weight (g)}}$$

2.5. Determination of serum glucose and insulin

After 90 days of experimental study, rats were anesthetized under diethyl ether. Blood was collected by cardiac acupuncture, left to clot then centrifuged. Serum was collected for the determination of serum glucose calorimetrically according to the method of Trinder.[19]

Serum insulin was assayed by ELISA (Sandwich Immunoassay Technique) using commercial kits (Immunospec Corporation, USA) according to the method of Eastham.[20]

2.6. Determination of liver function tests

Liver tissue samples from all experimental groups were washed in saline, cut into pieces, frozen at -20°C. They were then homogenated in phosphate buffer solution (PH 7.4). Liver tissue homogenates were used in the determination of liver function tests. Liver aspartate aminotransferase (AST), alanine aminotransferase (ALT) were determined by the colorimetric method according to the method of Young et al.[21] Liver total Protein and albumin were also determined color metrically according to the method described by Ashwood and Knight,[22] using reagent kits obtained from Diamond Diagnostic, Cairo, Egypt. Liver alkaline phosphatase (ALP) was determined by the colorimetric method according to the method of Belfield and Goldberg,[23] using reagent kits obtained from Spectrum Diagnostic, Hannover, Germany. The kinetic colorimetric method of gamma-glutamyl transferase (GGT) was performed according to the method of Shaw et al.[24] using reagent kits obtained from Spectrum Diagnostic, Hannover, Germany.

2.7. Determination of oxidative stress markers

Liver homogenates were also used for the determination of total antioxidant activity according to the method described by Koracevic et al.[25] Malondialdhyde (MDA) was measured in liver tissue homogenates using thiobarbituric acid (TBA) assay modified according to the method of Draper and Hadley,[26] Catalase (CAT) activity in liver tissue was determined by the method of Bock et al. [27]. Liver Glutathione (GSH) content was assayed according to the method of Prins and Loose.[28] Nitric oxide (NO) level in liver tissue homogenates was measured by the determination of total nitrite concentration in the sample (R&D systems, Minneapolis, USA) adopted by Green et al.[29]

2.8. Histological investigations

For histological examination, other parts of rats' liver were placed in 10% buffered formalin, dehydrated, cleared with xylene, infiltrated at 60°C and embedded with paraffin wax. Paraffin blocks were cut at 5 mm and affixed to slides and then stained with hematoxylin and eosin (H&E) for general histological examination.[30]

2.9. Immunohistochemical staining of proliferating cell nuclear antigen (PCNA)

From 10% formalin-fixed paraffin-embedded samples, 4-mm-thin sections were prepared and stained with PCNA. Immunohistochemistry staining steps were performed following the manufacturer's instructions (Thermo Scientific, UK) according to the method of Woods et al.[31] Positive antigens were stained brown under light microscopy. The count of immunopositively stained PCNA cells was carried out by of a cell Imaging Software Leica microsystem on fine picture (x400) randomly selected.

2.10. Statistical Analysis

Data were expressed as means ± standard error of means (SE). All the recorded data were analyzed using the Statistical Processor System Support (SPSS) version 10-computer program. The significance of differences between means of the control and all treated rats (a) were analyzed using one-way analysis of variance (ANOVA) test. * is considered statistically significant at $P < 0.05$ for all experimental groups in comparison with control group.

3. RESULTS

3.1. Morphological results

3.1. 1. Growth Rate and Body weight gain

Steviosides and aspartame administration to rats for 90 days resulted in significant decreases in final body weights and body weight gain. On the contrary sucrose, administrated animals denoted a significant increase in final body weights and body weight gain when compared to control ones (Table 1).

3.1. 2. Food consumption and Caloric intake

Aspartame and Steviosides treated rats elucidated significant reduction in food consumption /rat/day. The percentages of decrease in food consumption after 90 days were 39.37 and 42.38 in the pre-mentioned groups respectively. It is obvious that the least levels of food consumption were estimated after 90 days of Steviosides treatment to animals to record mean values of (15.92 ±0.338 g/rat/day) against those of control animals of (27.63±0.42 g/rat/day) (Table 1).

Calculated caloric intake signified a pronounced decrease in the mean values of aspartame and Steviosides supplemented animals compared to control group to record a percentage of decrease of (39.04 % and 42.85 %) respectively (Table 1).

3.1. 3. Final and relative liver weight

A significant increase in liver weight and relative liver weight were observed in sucrose administrated rats whereas Steviosides treated animals signified a pronounced decrease in their liver weights when compared to those of control animals (Table 1).

Table 1: The effect of Steviosides, Aspartame and Sucrose on growth rate, body weight gain, food consumption, caloric intake, final and relative liver weight

Parameters	Control		Sucrose	Aspartame	Steviosides
Initial body weight (g)	Mean ±SE	124.6±1.43	126.0±2.16	126.6±1.029	125.2±0.66
Final body weight (g)	Mean ±SE	336.00±16.37	383.80* ±6.23	269.40* ±3.41	250.60* ±3.04
Body weight gain (g)	Mean ±SE	211.4±16.52	257.8* ±5.87	142.8* ± 4.27	125.4* ±2.48
Food consumption (g/rat/day)	Mean ±SE	27.63±0.42	27.92±0.28	16.75* ±0.337	15.92* ±0.338
Caloric intake (Kcal/g/rat/day)	Mean ±SE	0.105±.0016	0.112* ±.00107	0.064* ±.0013	0.060* ±0.00128
Final liver weight (g)	Mean ±SE	8.52±0.34	11.53* ±0.19	8.02±0.29	6.38* ±0.33
Relative liver weight %	Mean ±SE	0.025±.0003	0.030* ±0.0004	0.029* ±0.0013	0.0254±0.0012

**: Significant change at P < 0.05 of all experimental groups in comparison with control group.*

3.2. Serum glucose and insulin levels

The increase in serum glucose level in aspartame treated animals was statistically significant after 90 days compared to control animals. Also, there was a significant increase in the mean values of serum glucose levels to record mean values of (198.8±4.53 mg/dl) compared to (146.60 ±1.32 mg/dl) of controls. On the other hand, Steviosides administration for 90 days caused a 6.54 % decrease in serum glucose level compared to the control group (Table 2).

The elevation in serum insulin levels was recorded at the end of the study period to record (6.4±0.47 µU/mL) and (3.20±0.15 µU/mL) in sucrose and aspartame supplemented animals respectively compared to **(**1.8 ±0.10 µU/mL) of control rats. (Table 2)

3.3. Liver function tests

A significant increase in liver AST, ALT, ALP and GGT activities were recorded in aspartame treated rats compared to control animals. Quite the reverse, Steviosides administration for 90 days to rats did not cause a significant variation in liver AST, ALT, ALP, GGT activities compared to control ones (Table 2).

A slight decrease in the mean values of liver total protein was denoted in rats administrating sucrose, aspartame and Steviosides for 90 days. Similarly, liver albumin levels recorded a percentage of decrease of (38.54%, 43.51% and 10.30%) in sucrose, aspartame and Steviosides groups respectively compared to control group (Table 2).

Table 2: The effect of Steviosides, Aspartame and Sucrose on serum glucose, insulin levels and liver function tests.

Parameters	Control		Sucrose	Aspartame	Steviosides
Glucose (mg/dl)	Mean ±SE	146.60±1.32	198.8* ±4.53	161.4* ±0.74	137.00* ±4.28
Insulin (µU/mL)	Mean ±SE	1.8 ±0.10	6.4* ±0.47	3.20* ±0.15	2.62* ±0.139
AST(U/L)	Mean ±SE	91.08±2.58	115.38* ±1.41	140.40* ±9.43	94.81±0.60
ALT(U/L)	Mean ±SE	46.67±1.18	81.24* ±1.10	118.24* ±0.57	45.83±2.07
ALP (IU/L)	Mean ±SE	11.93±0.99	53.14* ±3.84	77.26* ±1.76	14.01±1.51
GGT (U/mL)	Mean ±SE	1.77±0.011	2.01±0.073	2.52* ±0.061	1.89±0.17
Total protein (g/L)	Mean ±SE	39.36±1.37	35.32±2.77	34.59±2.77	39.19±0.60
Albumin (g/L)	Mean ±SE	2.62±0.27	1.61* ±0.22	1.48 * ±0.18	2.35 ±0.23

*: Significant change at P < 0.05 of all experimental groups in comparison with control group groups in comparison with control group

Aspartate aminotransferase: AST, Alanine aminotransferase: ALT, Alkaline phosphatase: ALP, Gamma-glutamyl transferase: GGT.

3.4. Oxidative stress markers

3.4. 1. Liver total Antioxidant, catalase activity (CAT) and glutathione activity (GSH)

Sucrose administration to rats for 90 days denoted a significant ($p<0.05$) decrease in liver total antioxidant and GSH activities compared to control rats. Statistically, a significant decline in total antioxidant, catalase and GSH activities in liver tissue was recorded in aspartame group. Their percentages of reduction compared to the control group were 35.03%, 26.48% and 58.33% in the pre-mentioned antioxidant parameters respectively. Quite the opposite, Steviosides treated animals denoted a significant ($p<0.05$) elevation in liver total antioxidant catalase and GSH activities. Their percentages of enhancement were recorded to be 36.09 % 35.17% and 16.66% compared to control animals (Table 3).

3.4. 2. Liver malondialdehyde activity (MDA) and nitric oxide activity (NO)

At the end of the study period aspartame treated animals manifested a significant ($p<0.05$) increase in liver MDA and NO levels. The percentages of elevation were recorded to be 137.71% and 228.7% respectively compared to control group. On the contrary, Steviosides administration to rats recorded a percentage of decrease

of 70.17 % 32.54 % in liver MDA and NO respectively when compared to control animals (Table 3).

Table 3: The effect of Steviosides, Aspartame and Sucrose on liver oxidative stress markers.

Parameters	Control		Sucrose	Aspartame	Steviosides
Total antioxidant (mmol/liter)	Mean ±SE	5.68±0.17	4.51* ±0.29	3.69* ±0.38	7.73* ±0.38
CAT (µmol/sec/g tissue)	Mean ±SE	2.53±0.13	2.35±0.19	1.86* ±0.18	3.42* ±0.095
GSH (mgs GSH/g tissue)	Mean ±SE	0.24±.0050	0.13* ±.0060	0.10* ±.0036	0.28* ±.0107
MDA (µmol/g tissue)	Mean ±SE	1.14±0.17	1.10±0.24	2.71* ±0.77	0.34* ±.037
NO (µmol/g)	Mean ±SE	10.11±0.26	16.85* ±1.25	33.24* ±1.19	6.82* ±0.84

*: Significant change at $P < 0.05$ of all experimental groups in comparison with control group
Malondialdehyde activity: MDA, Catalase Activity: CAT, Glutathione activity: GSH, Nitric oxide Activity: NO.

3.4.3. Histological investigations

Photomicrograph of liver sections from rats administrating sucrose for 90 days showed fatty degeneration and necrosis which was widely spread especially at the periphery of hepatic lobules. The cytoplasm of these degenerative cells was vacuolated and faintly stained. A few number of nuclei showed early signs of pyknosis (Fig. 1-b) compared to the normal hepatic pattern of the liver sections from control group (Fig. 1-a). In addition, histological examination of rat liver sections of aspartame group (Fig. 1-c) revealed general loss of hepatic architecture, presence of multi nuclear cells and increased Kupffer cells were observed. Hepatic degeneration advanced in many regions to vascular or coagulative necrosis that was recognized by coagulation of the cytoplasm of neighboring hepatocytes. This brings about considerable distortion of the normal lobular architecture and clear fatty degeneration. Lymphocytic accumulation could also be seen around the engorged blood vessels (Fig. 1-c). On the contrary Steviosides administration for 90 days revealed normal liver architecture pattern. The liver cells were arranged in the form of cords -radiating from central vein to the periphery (Fig. 1-d).

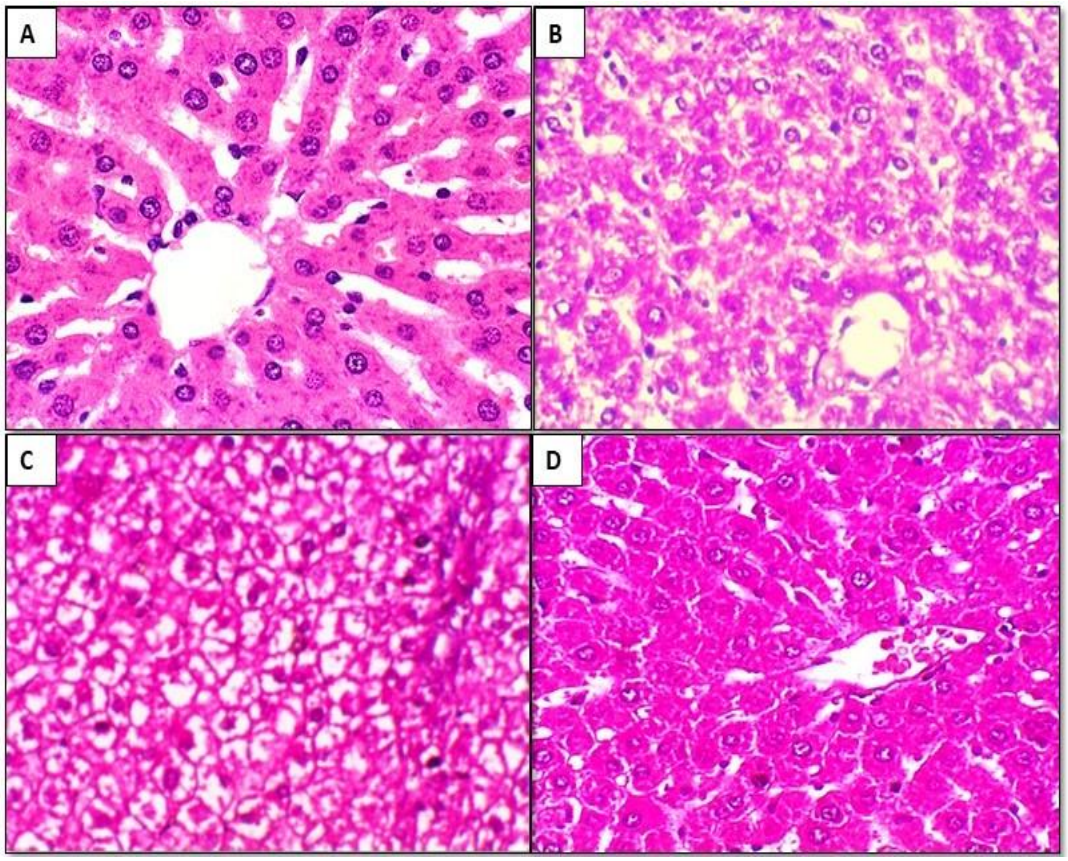

Figure 1: Photomicrographs of rat liver sections stained by H&E after 90 days showing normal liver architecture of control group (Fig. 1-a). Liver sections of sucrose group showed fatty degeneration with some vacuolated degenerative hepatic cells (Fig. 1-b). Hepatic pattern of the liver sections from aspartame group displayed general loss of hepatic architecture, hepatic necrosis with clear fatty degeneration. Lymphocytic accumulation is apparent around the engorged blood vessels (Fig. 1-c). Steviosides group revealed normal liver architecture pattern (Fig. 1-d).

3.4.4. Immunohistochemical investigations of proliferating cell nuclear antigen (PCNA)

Photomicrograph of liver section of control rat stained with PCNA showed homogenously small number of colored stained nuclei (Fig. 2-a) whereas photomicrograph of liver section of sucrose group showed increased PCNA deeply stained positive in hepatocytes (Fig. 2-b). Photomicrograph of liver section of aspartame group manifested highly increased activity of the immune histochemistry of proliferating cell nuclear antigen stainable materials manifested as deeply stained, dispersed intense nuclei scattered all over the liver tissue (Fig. 2-c). Nevertheless, photomicrograph of liver section of Steviosides group showed normal activity of the histochemistry of proliferating cell nuclear antigen (Fig. 2-d). All these photo micrographs were confirmed with the cell Imaging Software count which demonstrated a significant increase in immunopositively PCNA stained cells in aspartame and sucrose groups with mean values of (130.06 ± 0.81 and 110.24 ± 0.84) respectively against (70.04 ± 0.38) of control group (Table 4).

Table 4: The effect of Steviosides, Aspartame and Sucrose on proliferating cell nuclear antigen (PCNA) activity.

Parameter	Control		Sucrose	Aspartame	Steviosides
PCNA stained cells	Mean ±SE	70.04 ± 0.38	110.24* ± 0.84	130.06* ± 0.81	80.50 ± 0.48

*: Significant change at $P < 0.05$ of all experimental groups in comparison with control group

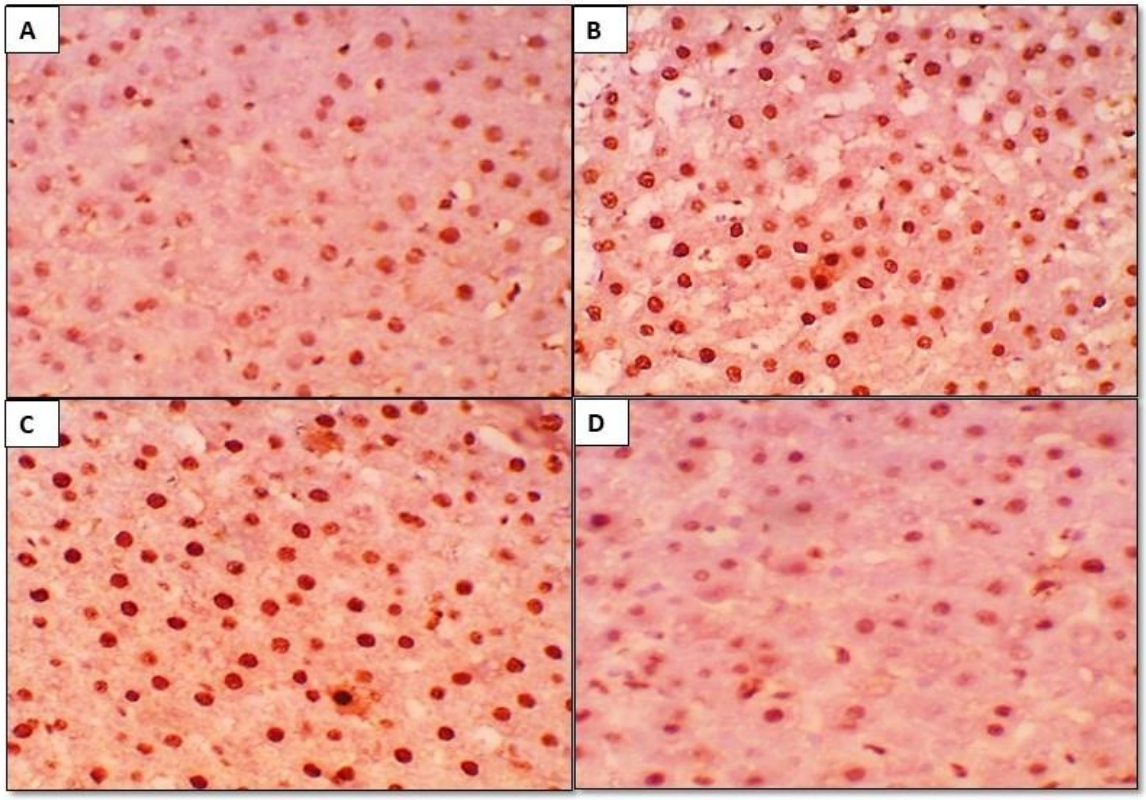

Figure 2: Photomicrograph of liver section of control rat stained with PCNA showed small number of colored stained nuclei (Fig. 2-a). Liver sections of sucrose group showed increased PCNA deeply stained positive in hepatocytes (Fig. 2-b). Liver sections of aspartame group manifested highly increased deeply stained PCNA nuclei (Fig. 2-c). Liver sections of Steviosides group showed normal activity of the proliferating cell nuclear antigen (Fig. 2-d).

4. DISCUSSION

It has long been noticed that the chronic intake of the familiar nutritive sweetener sucrose in children and adults is related to the incidence of obesity and fatty liver disease.[32] Hence, the use of non-caloric sweetener either artificial or natural has become increasingly popular over the past several years.[3]

Here in the present study, oral sucrose administration to rats for 90 consecutive days produced a significant increase in body weight and body weight gain. On the contrary, either aspartame supplementation or oral Steviosides supplementation to animals for the same duration caused a significant decline in body weight and body weight gain. In general, sucrose overload increased liver and relative liver weights. These results were in agreement with[8] who stated that supplementation of high sucrose diet to male rats resulted in significant increase in the total body weight, liver weight and liver index. This may be related to the additional energy that was taken from the extra calories contained in the oral sucrose solution in contrast to the recognized weight loss

in the group of animals receiving aspartame [33].It is worth mentioning that excess sugar is stored in the liver in the form of glycogen. Then the accumulated sucrose load is poured back into the bloodstream in the form of fatty acids, which are transported to storage areas of the body thus increasing body weight.[1]

On the other hand, reports suggest that there is an association between the degree of sweetness, appetite, food intake and energy. During the consumption of aspartame, sweet taste can be experienced in the absence of energy. The consumption of aspartame without energy results in a weakening of the natural sweetness energy association [34]. This lack of response to sweet taste may result in decreased food intake causing weight control.[35]

There was a decrease in total caloric intake in the Steviosides group followed by aspartame group compared to that of sucrose group. This might be related to the reduced caloric and carbohydrate intake in Steviosides which confirms the positive role of Steviosides rich in Stevia herb as a caloric substitute that may help individuals in regulating their weight gain.[3] Also, this may be related to the decrease in feeding efficiency due to the loss of appetite of Steviosides supplemented rats.[4]

Again, the present investigation elucidated that the significant elevation in serum glucose and insulin levels after oral sucrose and aspartame administration against a profound decrease in serum glucose levels under Steviosides supplementation. High sucrose diet can cause abdominal fat accumulation and obesity that are believed to pose a greater risk for the development of insulin resistance and metabolic syndrome.[8]

In addition, consuming sucrose-sweetened beverages increased triglyceride synthesis and deposition which causes fatty liver in rats**.** These alterations associating high sucrose diet is also possibly due to fructose effects on hepatic lipogenesis. The entry of fructose into glycolysis bypasses is the main route controlling step of glycolysis, thus providing unregulated amounts of the lipogenic substrates acetyl-CoA and glycerol-3-phosphate.[36] This was also confirmed by the increased fatty degeneration in H& E hepatic tissue sections of rats administrating sucrose.

Similarly, aspartame treatment produced an increase in serum glucose level. This may be related to the development of the primary stages of type II diabetes.[10] Besides, taste receptors have been found in the gut of rodents and humans as well. Sweet taste receptors in the gut respond to aspartame. Aspartame combines with the gut sweet taste receptors that are expressed in gut enteroendocrine cells causing the release of the glucagon like peptide-1 from enteroendocrine L cells**.** Glucagon like peptide-1 act on the pancreas to enhance insulin and decrease glucagon secretion.[37] In addition, Phenylalanine which represents one of the metabolites of aspartame is suggested to stimulate pancreatic β-cell function and insulin secretion.[35]

However, the significant reduction in serum glucose levels in rats supplementing Steviosides is likely due to the increase in the intracellular: extracellular concentration gradient of glucose,[38]

This may be also related to the enhancing action of Steviosides on insulin utilization by directly acting on β-cells without altering the K+ ATP channel activity, slowing down gluconeogenesis and inhibiting intestinal glucose absorption.[39]

Once more, the results of the current research established a significant decrease in liver total antioxidant level, CAT and GSH due to sucrose and aspartame supplementation for 90 days. In case of sucrose administration increased oxidative glucose metabolism itself increases mitochondrial production of the superoxide anion (O_2^-) which will be converted to the hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2).[38] Based on our results, aspartame treatment for 90 days increased the concentrations of liver MDA, NO levels. The increased levels of NO are taken as direct evidence of oxidative stress. Besides, the increase in MDA level and the decreased GSH level confirms liver and cell membrane damage after aspartame administration.[5] This may be attributed to its metabolite methanol which helps in the formation of superoxide anion and hydrogen peroxide.[40]

Oxidative stress products that are produced from the accumulation of aspartame metabolites in the body also inhibit cell membrane Na^+ K^+ ATPase activity. This in turn causes drastic decrease in GSH which is a contributing factor for the increase in nitric oxide level.[35] The marked decrease in liver GSH levels after aspartame administration may be also related to the increase in formate metabolite which inhibits oxidative phosphorylation, causing decreased intracellular synthesis of ATP.[5]

Contrary to that oral supplementation of Steviosides kept liver antioxidant enzymes near to the control values. Moreover, Steviosides supplementation increased significantly liver total antioxidant, decreased liver MDA. The suggested amelioration of the antioxidant defense mechanism in the Steviosides supplemented rats compared to those of aspartame may be due to the antioxidant, free radicals scavenging and inhibition of lipid peroxidation properties of Steviosides.[41]

As well, Steviosides had the potential to increase the biosynthesis of GSH thereby reduces the oxidative stress. The ability of the Steviosides to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation thus reducing the rate of the chain reaction.[42]

Interestingly, Steviosides increased methionine that is directly involved in the regulation of the glutathione antioxidative system. It also increased tryptophan that is involved in the regulation of the defense system through its action as a precursor of antioxidants and its effect on the inflammatory response.[39] As well, the phenolic compounds present in Stevia extract are capable of decreasing liver MDA. This may be related to the enhancement of the detoxifying enzyme γ-glutamyl transpeptidase.[13] All these results were histologically and immunohistochemically confirmed by the restoration of liver architecture under Steviosides supplementation.

In the concurrent study, sucrose supplementation was associated with a significant increase in liver AST, ALT, ALP accompanied by a mild elevation in γ -GT. This rise in liver enzymes is related to hepatic injury resulting from liver tissue inflammation due to long term sucrose supplementation.[8]

The significant increase in levels of ALP, AST, ALT and γ-GT in rats treated with aspartame can be used to estimate the degree of toxicity of aspartame on tissues.[35] Aspartame toxicity has been inflicted on the plasma membrane of liver cells. This alters the oxidant/antioxidant balance and the surface charge density. Accordingly, liver cell plasma membrane is damaged. This causes leakage of ALT, AST in the cytosol then to the blood stream.[10] The elevation in serum ALP levels is often related to extra hepatic bile obstruction and infiltrative liver disease. Likewise, serum ALP activity is increased due to aspartame supplementation because of de novo synthesis and elution from hepatocyte and biliary epithelial membranes.[35] Besides, gamma glutamyl transferase (γ-GT) is a microsomal enzyme present in hepatocytes and its primary role is to metabolize extracellular GSH to allow precursor amino acids to be assimilated and reused for intracellular GSH synthesis. The increase in serum γ-GT is a defense mechanism reflecting the induction of cellular γ-GT when there is oxidative stress as a result of aspartame administration.[43] Also, the alterations in total protein observed in aspartame treated rats could be due to increased free radical production by methanol metabolite of aspartame.[40]

Furthermore, results of the present study showed also mild insignificant changes in liver function activities in rats administrating Steviosides for 90 days compared to normal control group. This supports the idea that liver enzyme activities do not change significantly under Steviosides supplementation. This suggests that Steviosides had a protective effect against liver damage in addition to its membrane stabilizing activity.[13]

PCNA has important roles in the metabolism of nucleic acid, DNA replication, DNA excision repair, cell cycle control, chromatin assembly, and RNA transcription.[44] From the findings of this study, it appears that liver tissue from rats administrating aspartame expressed PCNA stained nuclei more than those subjected to sucrose when compared to control group. Abnormal cell proliferation is the main feature of carcinogenesis so making inhibition of the excessive proliferation of tumor cells is an effective treatment approach.[45] Taken together that the malignant nature of liver cells is expressed in terms of proliferative activity, so the increased frequency and high PCNA immune-reactivity present in aspartame group may reflect the uncontrolled proliferation of the neoplastic hepatocytes. Also, the high frequency of PCNA expression in aspartame group may probably reflect that in most cases of hepatocyte destruction, there are small numbers of hepatocytes which are undergoing controlled regenerative proliferation.[46]

In conclusion, Steviosides extracted from the leaves of *Stevia rebaudiana* Bertoni has the potential to be used as a non-caloric natural sweetener instead of the synthetic sweetener aspartame due to its antidiabetic and managing food intake action. Similarly, it could be used as antioxidant agent for controlling free radicals in many diseases with resultant health benefits. As well, Steviosides have no serious genotoxic effects making it a perfect sugar non-calorie substitute. These results suggest that replacing sucrose by Steviosides in the diet may be an effective strategy to manage food intake

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest concerning this article.
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