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Protective Role of Alpha Lipoic Acid against the Deleterious Effects of both Natural and Artificial Sweetener (Sucrose and Aspartame) in Albino Rats

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ABSTRACT

Key words: aspartame, sucrose , lipoic acid

This study was planned to throw light on the adverse effect of natural sweetener (sucrose) and artificial sweetener (aspartame) and the possible way of alpha lipoic acid to ameliorate the deleterious effects which may produce from these sweeteners in rats. There is a significant increase of alkaline phosphatase activity of sucrose treated group in comparison to untreated one at 45and 90- day of the experiment at ($P < 0.01$). Administration of lipoic acid did not reduce the potential effect of sucrose. On the other hand, aspartame had no effect on the level of alkaline phosphatase either at 45-day or at 90-day of the experiment. Sucrose had no effect on the level of total serum protein at 45-day and 90-day of the experiment. Administration of lipoic acid in combination of aspartame was significantly increase the level of serum total protein at 45 day of the experiment and had no effect at 90 day of the experiment .Sucrose was significantly decreased the level of albumin /globulin at 45 days of the experiment Administration of sucrose or aspartame was significantly increased level of urea only at 90-dayof the experiment in comparison to untreated group. Lipoic acid in combination of sucrose or aspartame was significantly decreased level of urea at 90-day of the experiment in comparison to sucrose or aspartame treated group. Administration of sucrose or aspartame was significantly increased level of serum creatinine at 90-day of the experiment in comparison to untreated group. Administration of aspartame was significantly decreased and increased the level of serum uric acid at 45 and 90 days respectively while sucrose decreased uric only at 45 day of the experiment in comparison to untreated group. Administration of sucrose or aspartame was significantly increased level of cholesterol and triglycerides during the experiment in comparison to untreated group. Administration of lipoic acid in combination of sucrose or aspartame was significantly decreased level of cholesterol during the experiment in comparison to sucrose or aspartame treated group. Sucrose was significantly increased level of glucose during the experiment in comparison to untreated group. Lipoic acid in combination of sucrose was significantly decreased level of glucose during the experiment in comparison to sucrose treated group. Lipoic acid in combination of aspartame was significantly decreased level of glucose at 45-day of the experiment but had no effect at 90-day of the experiment in comparison to aspartame treated group.

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1. INTRODUCTION

Sweeteners are food additives that are used to improve the taste of foods. There are two types of sweeteners found in food and beverages. Sugars (natural sweeteners) are sweeteners extracted from natural products without any chemical modifications, and sugar substitutes (artificial sweeteners) which are chemically processed. Natural sweeteners are sweet-tasting compounds with some nutritional value. In humans and other animal's sucrose is broken down into its constituent monosaccharide's glucose and fructose by sucrase or isomaltase glycoside hydrolysis which are located in the membrane of microvilli lining the duodenum (kaneko, 2008). Sucrose has a moderately high glycemic index of 80 and most adverse effects of sucrose are related to its content of fructose (Watson et al., 2003). Both human and animal studies have shown that, fructose is a highly lipogenic nutrient that, contributes to insulin resistance, metabolic defects and the development of a prediabetic or diabetic state (Miller and adeeli, 2008). Moreover, fructose feeding can induce free radical formation by

down regulation of HMP shunt enzymes that generate reduced environment in the form of NADPH and NADH (Giardino et al., 1996). Artificial sweeteners, are used for a number of reasons, one of these reasons is for diabetic persons, as they are not metabolized in the body into glucose. Thus, they do not cause a rise in blood sugar nor they because reactive hypoglycemia associated with high GI foods. This allows diabetics to satisfy their sugar cravings while still managing their blood glucose (Tandel, 2011). Other reason for use is for weight loss through limiting food energy intake by replacing high-energy sugar or corn syrup with other sweeteners having little or no food energy (Harvey, et al., 2012). Also Alternative sweeteners are often low in cost because of their long shelf-life and high sweetening intensity (Coultate, 2009).

The FDA approved seven sugar substitutes including stevia، aspartame, sucrose, neotame, acesulfame potassium, saccharin, and advantame. Aspartame is the last one and our choice in the present study because it considered the most consumed one in Egypt under the brand name diet sweet which was discovered in 1965 by James M. Schlatterat the G.D. Searle Company. It is white crystalline powder that is derived from the two amino acids aspartic acid and phenylalanine. It is about 200 times as sweet as sugar and can be used as a tabletop sweetener or in frozen desserts, gelatins, beverages, and chewing gum .When eaten, aspartame undergoes complete hydrolysis in the gastrointestinal tract to methanol and aspartyl phenylalanine, Subsequent secondary cleavage is mediated by dipeptide hydrolases of alpha AspPhe to aspartic acid and phenylalanine.Absorption of aspartate from the intestinal lumen is thought to be carrier-mediated, and Asp may share a carrier with glutamate. The dipeptide AspPhe could theoretically exist in the portal circulation but is not observed in the systemic circulation. An increases in the levels of thiobarbituric acid reactive substances (TBARS) and hydroperoxides were observed in the liver of fructose-fed rats , Reactive oxygen species can themselves reduce the activity of antioxidant enzymes such as CAT and GPx (Datta et al., 2000).Also high sucrose diet enhance hepatic lipogenesis, probably because of the metabolic effects of the component fructose (Shafrir 1991)cellular and membrane phospholipids are the major targets of damaging free radicals and therefore, depletion of phospholipids in liver of high fructose-fed rats could attributed to oxidative stress (Slatter et al,. 2000).In connection with the adverse effect of aspartame Abdel-Salam, et al (2012) studied the effect of the sweetener aspartame on some blood parameters including Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) TBARS, nitrite and GSH, and the result indicated that The administration of aspartame (22.5 and 45 mg/kg) . It did not alter liver TBARS, nitrite, GSH, AST, ALT, or ALP. On the contrary a study belonging to Iman (2011) represented that, the oral administration of ASP (40 mg/ kg) led to a significant elevation in LPO level in the liver and kidney tissue and also showed an increase in MDA level which was accompanied by a decrease in the activities of antioxidant enzymes, SOD and CAT in the liver leading to degrade H2O2, more H2O2 could be converted to toxic hydroxyl radicals that may contribute to oxidative stress due to methanol metabolite from ASP. In concerning to alpha lipoic acid it has been documented to have positive effects on a wide variety of clinical conditions, which is completely consistent with its effect in decrease of the oxidative stress (Li et al., 2013).

Alpha-lipoic acid is water and lipid soluble antioxidant a property that allows it to concentrate in cellular and extracellular environments (May et al., 2007). It functions as a cofactor in several mitochondrial multienzyme complexes involved in energy production in humans and animals (Shay et al., 2009). Alpha-Lipoic Acid acts as coenzyme of pyruvate and the alpha-ketoglutarate dehydrogenase multienzyme complex of the tricarboxylic acid cycle and has metal chelating, free radical scavenging and antioxidant regenerating abilities (Caylak et al., 2008). It protects against oxidative stress both in peripheral tissues and central nervous system (Winiarska et al., 2008). This study was planned to throw light on the adverse effect of natural and artificial sweetener (sucrose and aspartame) on some serum biochemical constituents and the possible role of alpha lipoic acid to ameliorate these deleterious effects which may produce from these sweeteners in rats.

2. MATERIAL AND METHODS

2.1. Materials:

2.1.1. Experimental animals:

A total number of 60 albino rats (average weight from 150 to 200g) were obtained from Egyptian company for production of vaccine, Sera and Drugs, and kept for a period of 14 days for adaptation with the environment before starting the experiment.

The animals were housing in clean metal cages (6 cages, 10 rats each), with 12 hour day-night cycle at temperature of 22 C and humidity of 45 % to 46 %. The rats were fed a balanced commercial diet and the drinking water provided ad libitum.

2.1.2. Experimental design:

The rats were randomly allotted into 6 groups as presented in table (1).

Table (1): Experimental design of rats treated with sweeteners

Rat groups	Treatment	References
Group 1	plain water (-ve control)	Binesh et al (2013)
Group 2	Alpha lipoic acid 5 % (+ control)	Tory et al (1999)
Group 3	Sucrose (10 %)	Binesh et al (2013)
Group 4	Aspartame (100 mg/kg body weight)	Dr Lesley (2013), Omar et al (2009), Butchko et al .(2002)
Group 5	Sucrose (10 %) + lipoic acid (5%)	Tory et al (1999)
Group 6	Aspartame (100 mg/kg bw.) +lipoic acid (5%)	Binesh et al (2013)

2.1.3. Samples:

The experiment will last for 3 months and two blood samples were collected at 45 and 90 dayafter treatment. The collecting serum samples were kept frozen at -20 c until analyzed. The treated rats were sacrificed after fasting, the liver of each rat will quickly remove, divided into three parts ,one third was homogenized and frozen until analyzed and the others were sock into frozen saline then preserved into 10 % formalin.

2.1.4. Chemicals

2.1.4.1. Kits of liver functions tests, Kidney function tests, lipid profiles, Diabetic profiles obtained from Randox laboratory.

2.1.4.2. Table sugar (sucrose) (10 %).

2.1.4.3. Aspartame tablet (Diet sweet 20 mgtablet): it was obtained from Al-Amreya for pharmaceutical industry, Alexandria, Egypt.

2.1.4.4. Alphalipoic acid capsule (thiotex forte 600 mgcapsule): it was obtained from Marcryrl pharmaceutical industries, El-obour city –Egypt.

All reagents and chemicals were of analytical grade0fhigher purity.

2.2.1. Collection of blood samples:

At the end of experiment (periods45 and 90 day) as well after overnight fasting, blood samples were drawn from the retro-orbital plexus of rats of all groups under diethyl ether anesthesia before sacrificing by decapitation. Blood samples were collected without anticoagulant in clean and dry Wassermann tubes and left in a slope position to clot at room temperature. The tubes were centrifuged at 3000 rpm for 5 minutes and the non hemolysed serum was carefully separated and transferred into clean dry Eppendorf which kept frozen at -20^{0}C until being used for biochemical analysis.

2.2.2. Handling of rat livers and kidneys tissue.

Rats of each group were eviscerated. Liver and kidney tissues were removed from the carcasses and washed by ice -cold saline buffer to remove the blood and then blotted in filter papers and divided into three parts, one of them will be homogenized and the whole three parts were kept frozen at -20^{0}C for biochemical analysis

2.2.3. Biochemical assays

Determination of total serum proteins (Doumas et al., 1975), serum albumin (Doumas et al.,1971), alanine aminotransferase (Young, 1990), aspartate aminotransferase (Young, 1990), alkaline phosphatase (Tietz et al.1983), urea nitrogen (Young, 2001), creatinine (Todd and Henry, 1984), cholesterol (Searcy, 1969), triglycerides (Stein, 1987), and blood glucose (Caraway and Watts,1987).

3. Results and discussion

The results presented in table (2) indicated that, there is a significant increase of the AST level in sucrose treated group in comparison to untreated group or group treated with sucrose and lipoic acid only at

45-day of the experiment. There is a significant increase of the AST level in aspartame treated group in comparison to untreated group or group treated with aspartame and lipoic acid only at 90-days of the experiment. The results presented in table (3) indicated that, there is a significant decrease of the ALT level in sucrose treated group in comparison to untreated group or group treated with sucrose and lipoic acid at 45-day of the experiment. There is a significant increase of the ALT level in aspartame treated group in comparison to untreated group or group treated with aspartame and lipoic acid at45 day and 90-days of the experiment. The results observed in Table (4) indicated that, there is a significant increase of alkaline phosphatase level of sucrose treated group in comparison to untreated group either at 45-day or 90- day of experiment at ($P < 0.01$). Administration of lipoic acid did not reduce the potential effect of sucrose. While administration of aspartame had no effect on the level of alkaline phosphatase either at 45 day or at 90 day of the experiment. Similar results were obtained by Mohamed D. Morsy, et al., (2014) who showed a significant increase in ALT, AST and ALP. Moreover, Marko et al., (2015) concluded that treatment with ASP in rats (40 mg/kg/daily for six weeks) caused an increase in the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). On the other hand, Abdel-Salam, et al (2012) studied the effect of the sweetener aspartame on some blood parameters including Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and the results indicated that the administration of aspartame (22.5 and 45 mg/kg) did not alter AST, ALT, or ALP. Alpha lipoic acid exerts strong oxidative protection in the liver and kidney against free radical induced cellular damage (Morakinyo et al., 2012).

The results observed in Table (5) indicated that sucrose had no effect on the level of total serum protein at 45 day and 90 day of the experiment. While administration of aspartame had no effect on total serum protein at 45 day of the experiment but it significantly decreases its level at 90 day of the experiment. Administration of lipoic acid in combination of aspartame significantly increased the level of total serum at 45 day of the experiment and had no effect at 90 day of the experiment. The results observed in Table (6) indicated that sucrose had no effect on the level of serum albumin at 45 day and 90 day of the experiment. While administration of lipoic acid in combination with sucrose resulted in a significant decreased of level of serum albumin in comparison to untreated group. Administration of aspartame had no effect on total serum protein at 45 day of the experiment but it significantly decreased its level at 90 day of the experiment. Administration of lipoic acid in combination of aspartame was significantly increase the level of total serum at 45 day of the experiment and had no effect at 90 day of the experiment. Administration of aspartame was significantly decreased the level of serum albumin during the experiment. Alphalipoic acid exerts strong oxidative protection in the liver and kidney against free radical induced cellular damage (Morakinyo et al., 2012).

The results observed in Table (7) indicated that sucrose was significantly increased the level of globulin at 45 day and 90 day of the experiment albumin in comparison to untreated group. While administration of lipoic acid in combination of sucrose resulted had no effect on the level of serum globulin in comparison to sucrose treated group. Administration of aspartame was significantly increased the level of serum globulin at 45 day and 90 day of the experiment in comparison to untreated group. Administration of lipoic acid in combination of aspartame was significantly increase the level of total serum at 45 day of the experiment and had no effect at 90 day of the experiment. The results observed in Table (8) indicated that sucrose was significantly decreased the level of albumin /globulin at 45 day but not at 90 day of the experiment in comparison to untreated group. Administration of aspartame was significantly increased the level of serum globulin at 90 day of the experiment and had no effect at 45-day of the experiment in comparison to untreated group. The obtained results were not in agreement with those described by Morsy, et al., (2014) who revealed that Sucrose 10 %, did not show any significant variation in serum albumin. Alpha lipoic acid exerts strong oxidative protection in the liver and kidney against free radical induced cellular damage (Morakinyo et al., 2012).

The results observed in Table (9) indicated that sucrose was significantly decreased the level of serum total albumin at 90-day but not at 45-day of the experiment in comparison to untreated group. Administration of aspartame was significantly increased the level of serum globulin at 90 day of the experiment and had no effect at 45-day of the experiment in comparison to untreated group. Administration of lipoic acid in combination with

sucrose or aspartame was significantly decreased the serum total bilirubin at 90-day of the experiment. Our results are not in agreement with those obtained by Morsy, et al., (2014) who revealed that Sucrose 10 %, did not show any significant variation in serum albumin. Alpha lipoic acid exerts strong oxidative protection in the liver and kidney against free radical induced cellular damage (Morakinyo et al., 2012)

The results observed in Table (10) indicated that administration of sucrose or aspartame was significantly increased level of urea at 90-day but not at 45-day of the experiment in comparison to untreated group. Administration of lipoic acid in combination of sucrose or aspartame was significantly decreased level of urea at 90-day of the experiment but not at 45-day in comparison to sucrose or aspartame treated group. The results observed in Table (11) indicated that administration of sucrose or aspartame was significantly increased level of serum creatinine 90-day but not at 45-day of the experiment in comparison to untreated group. The results observed in Table (11) indicated that administration of sucrose or aspartame was significantly decreased level of serum uric acid at 45-day but sucrose had no effect at 90-day and aspartame significantly increased uric acid at 90-day of the experiment in comparison to untreated group. Similar results were described by Kamal et al., (2011) concluded that 65% high sucrose induced obesity, represent the best available rat model to study nephropathy, which riskily affect the kidney via elevating urea and creatinine. On the contrary, Peter et al.,(1992)concluded that initial studies using 10% ,11% and 50% sucrose intake in Wistar rats during months showed no evidence of renal perturbations as serum urea nitrogen and creatinine and urinary findings did not suggest renal damage. Alpha lipoic acid exerts strong oxidative protection in the liver and kidney against free radical induced cellular damage (Morakinyo et al., 2012).

The results observed in Table (13 and 14) indicated that sucrose or aspartame was significantly increased level of cholesterol and triglycerides during the experiment in comparison to untreated group. Lipoic acid in combination of sucrose or aspartame was significantly decreased level of cholesterol during the experiment in comparison to sucrose or aspartame treated group. Similar results were also described by Singleton et al. (1999) who mentioned that Serum triglycerides increased after consumption of the drink sweetened with glucose and fructose, but not aspartame, indicating that unlike glucose and fructose, aspartame does not enhance postprandial lipemia following lipid loading. On the other hand Marko et al.,(2015) study revealed that treatment with ASP caused an increase in the concentrations of, cholesterol, LDL-cholesterol, as well as a decrease in the levels of HDL-cholesterol in the serum. On the other hand, Morsy, et al., (2014) revealed that Sucrose 10 %, did not show any significant variation in serum cholesterol, triglycerides. Okunoet al.,(1986) administered a single dose of 500 mg aspartame and the results showed no significant change after 2 weeks' administration in blood cholesterol and triglyceride. Supplementation with alpha lipoic acid caused a decrease in lipid peroxidation, plasma cholesterol, triglycerides, and low density lipoprotein cholesterol, and an increase in high-density lipoprotein cholesterol. (Yang et al.,2008). Alpha lipoic acid exerts strong oxidative protection in the liver and kidney against free radical induced cellular damage (Morakinyo et al., 2012).

The results observed in Table (15) indicated that sucrose was significantly increased level of glucose during the experiment in comparison to untreated group. Administration of lipoic acid in combination with sucrose was significantly decreased level of glucose during the experiment in comparison to sucrose treated group. Administration of aspartame was significantly increased level of glucose at 45-day and decreased its level at 90-day of the experiment in comparison to untreated group. Administration of lipoic acid in combination of aspartame was significantly decreased level of glucose at 45-day of the experiment but had no effect at 90-day of the experiment in comparison to aspartame treated group. The results obtained by Morsy, et al., (2014) revealed that Sucrose 10 %, did not show any significant variation in glucose. While both Sucrose 30 %, and Sucrose 60 %, showed a significant increase in glucose.

Table (2): Effect of aspartame or sucrose on serum AST activities in rats.

Groups	N	45-Day	90-Day	t-value
Control	4	Ba 120.00±5.18	Da 121.25±9.73	**0.11 NS**
Lipoic acid	4	Ca 113.75±2.53	Eb 101.50±5.06	**2.16 ***
Sucrose	4	Aa 135.00±3.03	Ca 126.25±10.11	**0.82 NS**
Sucrose + lipoic	4	Ca 113.00±17.01	Ca 125.75±12.85	**0.59 NS**
Aspartame	4	Db 106.50±8.17	Aa 134.50±7.60	**2.50 ***
Aspartame + lipoic acid	**4**	**Eb** **98.50±10.52**	**Ba** **131.25±14.97**	**3.79***

-Means within the same column of different capital litters are significantly different at (P < 0.05).
-Means within the same raw of different small litters are significantly different at (P < 0.05)
NS = Non significant * = Significant at (P < 0.05) ** = Significant at (P < 0.01)

Table (3): Effect of administration of aspartame or sucrose on the ALT levels in rats.

Groups	N	45-Day	90-Day	t-value
Control	4	Ba 38.50±5.14	Ca 38.00±4.64	**0.07 NS**
Lipoic acid	4	Cb 34.00±2.94	Ba 40.50±1.04	**2.08***
Sucrose	4	Ca 35.75±2.06	Ca 39.00±3.42	**0.81 NS**
Sucrose + lipoic	4	Ca 35.75±0.63	Ba 41.50±3.97	**1.43 NS**
Aspartame	4	Aa 45.75±5.27	Aa 43.00±2.97	**0.45 NS**
Aspartame + lipoic acid	**4**	**Da** **30.25±3.73**	**Da** **30.00±3.81**	**0.47 NS**

-Means within the same column of different capital litters are significantly different at (P < 0.05).
-Means within the same raw of different small litters are significantly different at (P < 0.05)
NS = Non significant * = Significant at (P < 0.05) ** = Significant at (P < 0.01)

Table (4): Effect of aspartame or sucrose on the activity of alkaline phosphatase in rats.

Groups	N	45-Day	90-Day	t-value
Control	4	Bb 31.25±1.70	Ba 34.50±3.12	**0.91 NS**
Lipoic acid	4	Ba 33.25±2.59	Ba 33.25±2.56	**0.01 NS**
Sucrose	4	Aa 38.50±2.18	Aa 39.75±3.88	**0.28 NS**
Sucrose + Lipoic	4	Aa 37.25±4.44	Aa 37.25±4.77	**0.01 NS**
Aspartame	4	Ba 33.50±3.75	Ba 34.50±3.48	**0.19 NS**
Aspartame + Lipoic acid	**4**	**Aa** **38.50±0.87**	**Ca** **30.00±3.19**	**2.57***

-Means within the same column of different capital litters are significantly different at (P < 0.05).
-Means within the same raw of different small litters are significantly different at (P < 0.05)
NS = Non significant * = Significant at (P < 0.05) ** = Significant at (P < 0.01)

Table (5): Effect of aspartame or sucrose on the levels of serum total protein in rats.

Groups	N	45-Day	90-Day	t-value
Control	4	Ba 6.10±0.25	Ba 6.50±0.47	**0.74 NS**
Lipoic acid	4	Aa 6.73±0.28	Aa 6.68±0.21	**0.14 NS**
Sucrose	4	Aa 6.83±0.35	Ba 6.43±0.35	**0.80 NS**
Sucrose + lipoic	4	Aa 6.78±0.15	Ba 6.50±0.27	**0.88 NS**
Aspartame	4	Ba 6.15±0.24	Aa 6.70±0.32	**1.37 NS**
Aspartame + lipoic acid	**4**	**Aa** **6.70±0.60**	**Aa** **6.65±0.31**	**0.07NS**

-Means within the same column of different capital litters are significantly different at (P < 0.05).
-Means within the same raw of different small litters are significantly different at (P < 0.05)
NS = Non significant * = Significant at (P < 0.05) ** = Significant at (P < 0.01)

Table (6): Effect of aspartame or sucrose on the values of serum albumin in rats.

Groups	N	45-Day	90-Day	t-value
Control	4	Aa 4.23±0.15	Aa 4.23±0.19	**0.60 NS**
Lipoic acid	4	Aa 4.23±0.22	Ba 3.98±0.30	**0.66 NS**
Sucrose	4	Aa 4.25±0.21	Aa 4.15±0.10	**0.43 NS**
Sucrose + lipoic	4	Ca 3.93±0.06	Ca 3.65±0.26	**1.02 NS**
Aspartame	4	Ba 4.10±0.21	Ca 3.73±0.35	**0.91 NS**
Aspartame + lipoic acid	**4**	**Ba** **4.00±0.26**	**Aa** **4.13±0.49**	**0.22 NS**

-Means within the same column of different capital litters are significantly different at (P < 0.05).
-Means within the same raw of different small litters are significantly different at (P < 0.05)
NS = Non significant * = Significant at (P < 0.05) ** = Significant at (P < 0.01)

Table (7): Effect of aspartame or sucrose on serum globulin levels in rats.

Groups	N	45-Day	90-Day	t-value
Control	4	Da 1.88±0.39	Ba 2.28±0.48	**0.64 NS**
Lipoic acid	4	Ba 2.50±0.23	Aa 2.70±0.48	**0.37 NS**
Sucrose	4	Ba 2.58±0.26	Aa 2.28±0.42	**0.61 NS**
Sucrose + Lipoic	4	Ba 2.85±0.10	Aa 2.85±0.42	**0.01 NS**
Aspartame	4	Ca 2.05±0.45	Aa 2.98±0.56	**1.28 NS**
Aspartame + Lipoic acid	**4**	**Aa** **2.70±0.71**	**Ba** **2.53±0.54**	**0.19 NS**

-Means within the same column of different capital litters are significantly different at (P < 0.05).
-Means within the same raw of different small litters are significantly different at (P < 0.05)
NS = Non significant * = Significant at (P < 0.05) ** = Significant at (P < 0.01)

Table (8): Effect of aspartame or sucrose on the albumin/globulin ratios in rats.

Groups	N	45-Day	90-Day	t-value
Control	4	Aa	Aa	**0.29 NS**
		2.68±0.71	2.36±0.81	
Lipoic acid	4	Ca	Ba	**0.28 NS**
		1.73±0.17	1.63±0.31	
Sucrose	4	Ca	Aa	**0.81 NS**
		1.70±0.18	2.01±0.34	
Sucrose + lipoic	4	Ca	Ba	**0.06 NS**
		1.38±0.03	1.40±0.26	
Aspartame	4	Aa	Ba	**1.26 NS**
		2.40±0.62	1.48±0.40	
Aspartame + lipoic acid	**4**	**Ba**	**Aa**	**0.83 NS**
		2.03±0.76	**2.12±0.78**	

Table (9): Effect of aspartame or sucrose on serum total bilirubin in rats.

Groups	N	45-Day	90-Day	t-value
Control	4	Ba	Ca	**0.31 NS**
		0.28±0.05	0.25±0.06	
Lipoic acid	4	Ba	ABa	**0.31 NS**
		0.33±0.05	0.35±0.06	
Sucrose	4	Ba	Ba	**0.26 NS**
		0.33±0.09	0.30±0.04	
Sucrose + Lipoic	4	Ba	Ca	**0.77 NS**
		0.30±0.04	0.25±0.05	
Aspartame	4	Ba	Aa	**0.87 NS**
		0.30±0.04	0.38±0.08	
Aspartame + Lipoic acid	**4**	**Aa**	**Ba**	**0.94 NS**
		0.43±0.08	**0.33±0.08**	

-Means within the same column of different capital litters are significantly different at (P < 0.05).
-Means within the same raw of different small litters are significantly different at (P < 0.05)
NS = Non significant * = Significant at (P < 0.05) ** = Significant at (P < 0.01

Table (10): Effect of aspartame or sucrose on values of serum urea in rats.

Groups	N	45-Day	90-Day	t-value
Control	4	Ba	Ba	**0.39 NS**
		28.25±2.02	27.25±1.49	
Lipoic acid	4	Ba	Ba	**0.78 NS**
		29.00±0.91	30.25±1.32	
Sucrose	4	Ba	Aa	**0.55 NS**
		29.50±2.63	31.25±1.70	
Sucrose + lipoic	4	ABa	Ba	**0.77 NS**
		30.25±2.29	27.75±2.29	
Aspartame	4	ABa	Aa	**0.68 NS**
		30.00±1.58	31.50±1.50	
Aspartame + lipoic acid	**4**	**Aa**	**Ba**	**0.23 NS**
		31.00±2.04	**30.25±2.39**	

Table (11): Effect of aspartame or sucrose on values of serum creatinine in rats.

Groups	N	45-Day	90-Day	t-value
Control	4	Aa	Da	**0.92 NS**
		0.48±0.03	0.43±0.05	
Lipoic acid	4	Cb	Ba	**2.04 ***
		0.40±0.07	0.58±0.05	
Sucrose	4	BCb	Aa	**2.95 ***
		0.43±0.05	0.63±0.05	
Sucrose + lipoic	4	Bb	Aa	**3.13 ***
		0.45±0.03	0.63±0.05	
Aspartame	4	BCb	ABa	**3.65 ****
		0.43±0.03	0.60±0.04	
Aspartame + lipoic acid	**4**	**Da**	**Ca**	**1.48 NS**
		0.30±0.04	**0.48±0.11**	

Table (12): Effect of aspartame or sucrose on values of serum uric acid in rats.

Groups	N	45-Day	90-Day	t-value
Control	4	Aa	Bb	**0.26 NS**
		4.03±0.50	3.88±0.25	
Lipoic acid	4	Ba	Ba	**0.46 NS**
		3.93±0.14	3.83±0.17	
Sucrose	4	Ca	Ba	**0.13 NS**
		3.50±0.36	3.80±0.57	
Sucrose + lipoic	4	Aa	Ca	**0.91 NS**
		4.20±0.58	3.55±0.41	
Aspartame	4	Ba	Aa	**1.31NS**
		3.75±0.66	4.68±0.25	
Aspartame + lipoic acid	**4**	**Ca**	**Aa**	**1.49 NS**
		3.50±0.37	**4.23±0.32**	

-Means within the same column of different capital litters are significantly different at (P < 0.05).
-Means within the same raw of different small litters are significantly different at (P < 0.05)
NS = Non significant * = Significant at (P < 0.05) ** = Significant at (P < 0.01)

Table (13): Effect of aspartame or sucrose on values of serum cholesterol in rats.

Groups	N	45-Day	90-Day	t-value
Control	4	Ea	Da	**0.68 NS**
		78.50±5.24	85.25±8.43	
Lipoic acid	4	Ca	Da	**0.51 NS**
		85.00±4.08	88.75±5.98	
Sucrose	4	Ab	Aa	**3.57****
		106.75±3.92	124.00±2.80	
Sucrose + lipoic	4	Ba	Ca	**0.91 NS**
		86.50±2.87	90.50±9.58	
Aspartame	4	Db	Ba	**2.08***
		83.25±4.96	97.25±4.52	
Aspartame + lipoic acid	**4**	**Fa**	**Ea**	**0.32 NS**
		69.25±2.25	**67.75±4.09**	

Table (14): Effect of aspartame or sucrose on the values of serum triglycerides in rats.

Groups	N	45-Day	90-Day	t-value
Control	4	Db	Ca	**2.98 ***
		54.50±2.96	66.50±2.72	
Lipoic acid	4	Ca	Da	**0.46 NS**
		57.00±3.34	55.25±1.65	
Sucrose	4	Ca	Ca	**1.17 NS**
		59.25±5.57	66.75±3.15	
Sucrose + Lipoic	4	Ab	Aa	**2.85 ***
		74.25±3.35	92.25±5.34	
Aspartame	4	Cb	Ca	**1.57NS**
		58.00±3.19	67.00±4.74	
Aspartame + Lipoic acid	**4**	**Bb**	**Ba**	**0.45NS**
		71.00±4.95	**74.00±4.30**	

Table (15): Effect of aspartame or sucrose on the levels of serum glucose in rats.

Groups	N	45-Day	90-Day	t-value
Control	4	Ca	Cb	**1.78 NS**
		85.25±9.53	67.00±3.70	
Lipoic acid	4	Ea	Ba	**0.50 NS**
		80.00±7.82	84.75±5.28	
Sucrose	4	Aa	Aa	**1.10 NS**
		96.50±3.12	87.50±7.51	
Sucrose + lipoic	4	Fa	Cb	**2.64 ***
		78.75±3.09	67.00±3.19	
Aspartame	4	Ba	Da	**1.72 NS**
		87.50±12.20	65.50±3.62	
Aspartame + lipoic acid	**4**	**Da**	**Ca**	**1.52 NS**
		82.00±5.97	**67.50±7.41**	

-Means within the same column of different capital litters are significantly different at (P < 0.05).
-Means within the same raw of different small litters are significantly different at (P < 0.05)
NS = Non significant * = Significant at (P < 0.05) ** = Significant at (P < 0.01)

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