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Spirulina platensis and alpha lipoic acid are protective against deleterious effects of aspartame on the liver and kidneys of rabbits

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A B S T R A C T

Spirulina and alpha lipoic acid are the most important antioxidants. The purpose of this study was to explore the adverse effect of (Aspartame) and the protective role of alpha lipoic acid and spirulina in alleviating the deleterious effects of aspartame on the liver and rabbit kidneys. Forty two Rabbits were classified into seven equal groups. Group I: (Control group) received no drugs. Group II: rabbits administered with alpha-lipoic acid (100 mg/kg b. wt/day). Group III: rabbits received spirulina platensis (1500 mg/kg b. wt/day). Group IV: rabbits received aspartame (250 mg/kg. b. wt/day). Group V: rabbits received aspartame (250 mg mg/kg b. wt) and treated with alpha- lipoic acid (100 mg/kg b. wt). Group VI: rabbits received aspartame (250 mg mg/kg b.wt) and treated with spirulina (1500 mg/kg b. wt). Group VII: rabbits received aspartame (250 mg mg/kg b.wt) and treated daily with alpha- lipoic acid (100 mg/kg b. wt) and spirulina (1500 mg/kg b. wt) for 8 weeks. Blood samples for serum separation were collected once from all animal groups after eight weeks of experiment. The results revealed a significant increase in serum liver marker enzymes (ALT), (AST), (ALP), kidny function tests (urea, creatinine, uric acid) and lipid profile (total cholesterol and triacylglycerols) and total protein concentration with a significant reduction in serum albumin in aspartame- administrated group. Coadministration of spirulina and alpha lipoic acid with aspartame treated rabbits ameliorate aspartame dangerous effects as revealed by a significant enhancement in all previous biochemical parameters to its normal ranges. These results suggested that, the antioxidant and anti-inflammatory activity of spirulina and alpha lipoic acid could curtail liver impairment and enhanced the harmful impact of aspartame on rabbits liver and kidney.

Key words: Aspartame, Spirulina platensis, alpha-lipoic acid, Hepatorenal function, Rabbits.
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1. INTRODUCTION

Aspartame (ASP) is a dietary low-calorie artificial sweetener and is widespread used over the last 30 years. ASP is found in more than 6000 products, including yoghurt, soft drinks, candy, chewing gum, tabletop sweeteners fruit juices, jellies, gelatins and drugs such as vitamins and sugar-free cough drops (Choudhary and Devi, 2014). ASP represents a higher source of oxidative stress since it is rapidly metabolized into methanol, phenyl-alanine and aspartic acid. Methanol is further metabolized by oxidation to formaldehyde and then to formate. These processes are accompanied by formation of superoxide anion and hydrogen peroxide which cause harmful effects of acute intoxication in many organs especially on liver functions. ASP consumption, the concentration of its metabolites increases in the blood (Humphries et al., 2008). After tissue damage, some of the enzymes find their way

into the serum leaking through membranes with altered permeability. Activity determinations of serum enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and γ-glutamyl transpeptidase (γGT)) are a valuable tool in clinical diagnosis. The measurement of these enzymes' activities in tissues and body fluids can be used to estimate the degree of toxic-ity of a chemical compound on an organ/tissues (Vijan, 2010; Abdhilash et al., 2011). This is in accordance with (Amin et al., 2016) who notice marked elevation in creatinine and urea due to the aspartame toxicity**.** As the kidney has an important role in excretion of various waste metabolites from the body, studies on nephrotoxic effect of artificial sweeteners, especially aspartame, have increased (Martins and Azoubel, 2007). Additionally, sugar-sweetened beverages (SSBs) are any beverage with added sugar, which includes soft drinks (soda), fruit drinks, iced tea, and energy and vitamin water drinks. The primary cause of gout is hyperuricemia due to excess urate production or impaired renal excretion of uric acid. (Terkeltaub, 2003).

Spurulina has stimulatory effect on the physical stamina and acts as liver detoxifier, bowel cleanser and as catalyst for the absorption of essential elements. (Hyo-Jin et al., 2006). Spirulina strongly induces antioxidant enzyme activity, helps to prevent lipid peroxidation and DNA damage and scavenges free radicals.
Alpha lipoic acid acts as coenzyme of pyruvate and It protects against oxidative stress both in peripheral tissues and central nervous system (Winiarska et al., 2008). Also, alpha lipoic acid exerts strong oxidative protection in the liver and kidney against free radical induced cellular damage (Morakinyo et al., 2012). This study was designed to investigated the adverse effect of artificial sweetener (aspartame) on some serum biochemical constituents of liver and kidney function and the possible protective role of spirulina and alpha lipoic acid to ameliorate these deleterious effects which may produce from aspartame administration in rabbits.

2. MATERIAL AND METHODS

2.1 Experimental Animals.

Forty two white male New-Zealand Rabbits of 4-6 weeks old age and average body weight 800-1200 g were used in the experimental investigation of this study. Rabbits were obtained from Laboratory Animals Research Center Faculty of Veterinary Medicine, Benha University. Rabbits were housed in separated metal cages (6 per cage) and they were kept on a well balanced ration and fresh clean drinking water ad-libitum. Rabbits were kept at a constant environmental and nutritional condition throughout the whole period of experiment. All rabbits were left for 15 days for acclimatization before the start of the experiment.

2.2. Chemicals and antioxidants

The chemicals and antioxidants used in the present study were:
2.2.a. Aspartame:
Aspartame was purchased from Al-Ameriya pharma company, Egypt. Aspartame was manufacture in the form of tablets each one tablet contains 20 mg of aspartame.
2.2.b. Spirulina platensis
Spirulina microalgae blue green powder was obtained from Cairo National Research Center - Dokki-Egypt.
Preparation and dosage of Spirulina: Spirulina was freshly prepared by dissolved in distilled water and administered orally using stomach tube in a daily dose of 1500 mg/kg body weight (Colla, et al.,2008).
2.2.c. Alpha lipoic acid (thioctic acid).
Synonyms: thioctic acid, 6, 8-dithiooctanoic acid, 1, 2dithiolane-3-pantanoic acid. Alpha lipoic acid (Thiotacid)® tablets was manufactured by Eva pharma Egypt.
Note: The content of each tablet 600 mg of α-Lipoic acid was dissolved in propylene glycol which manufactured by El-Nasr Pharmaceutical Chemicals Co. Abu Zaabal, Egypt.
Dosage: DL-α-Lipoic acid was given orally in a daily dose of 100 mg/kg body weight (Şehirli et al., 2008).

2.3 Experimental design

After acclimatization to the laboratory conditions, the rabbits were randomly divided into seven equal groups, each one consisting of sixanimals placed in individual cages and classified as follows:
Group I: (Control group) received no drugs.
Group II: rabbits administered with alpha-lipoic acid (100 mg/kg b.wt/day)

Group III: rabbits received spirulina platensis (1500 mg/kg b. wt/day).
Group IV: rabbits received aspartame (250 mg/kg. b. wt/day). Group V: rabbits received aspartame (250 mg mg/kg b. wt) and treated with alpha- lipoic acid (100 mg/kg b. wt).
Group VI: rabbits received aspartame (250 mg mg/kg b.wt) and treated with spirulina platensis (1500 mg/kg b. wt).
Group VII: rabbits received aspartame (250 mg mg/kg b.wt) and treated daily with alpha- lipoic acid (100 mg/kg b. wt) and spirulina platensis (1500 mg/kg b. wt) for 8 weeks.

2.4. Sampling

2.4.1. Blood samples

Twenty-four hours after the last dose of alpha-lipoic acid and spirulina platensis administration, blood samples were collected by vein puncture of the marginal ear vein from all animal groups in dry, clean tubes and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 r.p.m for 15 minutes. The clean, clear serum was processed directly for determination of AST, ALT and ALP activities, then kept in a deep freeze at -20°C until used for subsequent biochemical analysis. All sera were used for determination of the following parameters: Total protein, albumin, urea, creatinine, uric acid, total cholesterol and triacylglycerols.

2.5. Biochemical analysis.

Serum (ALT and AST), ALP, total protein, albumin ,urea, creatinine, uric acid, total cholesterol, triacylglycerol were determined according to the method described by Reitman, and. Frankel, (1957). Tietz et al., (1983). Gomal et al.,(1949); Doumas et al.,(1971); Young, (1990); Tietz,.,(1986)., Trivedi,.,(1978)., Caraway and Watts (1987), Todd and Henry (1984); respectively.

2.6. Statistical analysis

The results were expressed as mean ± SE using SPSS software program version 16 (SPSS© Inc., USA). The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparison among the groups for testing the inter-grouping homogeneity. Values were considered statistically significant when $p<0.05$.

3. RESULTS

The obtained results in table (1) revealed that oral aspartame administration to rabbits for 8 weeks exhibit a significant increase in serum ALT, AST, ALP activities and total protein concentration with marked reduction in serum albumin level when compared with control group. On the other hand, coadministration of spirulina and alpha lipoic acid with aspartame treated rabbits for 8 weeks caused significant decrease in serum ALT, AST, ALP activities and total protein concentration and markedly increased serum albumin level when compared to aspartame treated group. Additionally, no significant changes were noticed in all pervious parameters among the three groups (control normal(G1), alpha- lipoic acid (GII) and spirulina (GIII) administered rabbits.

The obtained data table (2) demonstrated that, oral aspartame administration to rabbits for 8 weeks induced a significant increase in serum urea, creatinine, uric acid in addition to total cholesterol and triacylglycerol concentrations when compared with control group. Meanwhile, spirulina and alpha-lipoic acid administration with aspartame treated rabbits for 8 weeks exhibit a significant decrease in serum kidney function tests (urea ,creatinine, uric acid) as well as total cholesterol and triacylglycerol concentrations when compared to aspartame administered group. Furthermore, no significant changes were noticed in all pervious parameters among the three groups (control normal (G1), alpha-lipoic acid (GII) and spirulina (GIII) administered rabbits

Table (1): Effect of Spirulina or/and alpha-Lipoic acid on serum liver marker enzymes activities, total protein and albumin concentrations in aspartame administered male rabbits.

Animal groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total protein (g/dl)	Albumin (g/dl)
G I:Control	14 ± 0.7 ^{d}	16.4 ± 1.106^{e}	50.6 ±1.28^{d}	7.06 ±0.06^{c}	3.86 ± 0.13^{d}
G II:L.A	12.8± 0.37^{d}	17 ± 0.54^{e}	46.6 ±1.07^{d}	7.26 ±0.07^{bc}	3.92 ± 0.37^{ab}
G III:Spirulina	13.6± 0.5^{d}	18 ± 0.58^{e}	49.75 ± 2.01^{d}	5.8 ± 1.27^{c}	4.04 ± 0.13^{a}
G IV:Aspartame	48.8 ± 3.1^{a}	76.8 ± 1.52^{a}	124 ± 3.68^{a}	8.9 ± 0.12^{a}	2.76 ± 0.16^{c}
GV:Asp+L.A	34 ± 1.14^{b}	34 ± 1.14^{b}	65.4 ± 2.48^{c}	7.42 ± 0.03^{b}	3.64 ± 0.09^{b}
GVI:Asp+Spir	29.6 ± 0.24^{c}	29.6 ± 0.24^{c}	88.2 ± 2.49^{b}	7.1 ± 0.04^{bc}	3.62 ± 0.03^{c}
G II:Asp+L.A+Spir	21 ± 0.63^{d}	21 ± 0.63^{d}	47.6 ±1.28^{cd}	6.98 ± 0.26^{c}	4.52 ± 0.59^{a}

Data are presented as (Mean ± SE). SE = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table (2): Effect of Spirulina or/and alpha-Lipoic acid on serum lipid profile (total cholesterol, triacylglycerol) and kidney function tests (urea, creatinine, uric acid) concentrations in aspartame administered male rabbits.

Animal groups	Total Cholesterol (mg/dl)	Triacylglycerols (mg/dl)	Urea (mg/dl)	creatinine (mg/dl)	Uric acid (mg/dl)
G I: Control	103± 4.91^{d}	50 ±1.58^{de}	18 ± 1.14^{cd}	0.66 ± 0.08^{bcd}	2.87 ± 0.13^{c}
G II: L.A	92 ±2.07^{e}	45 ±1.58^{e}	16.6 ±0.50^{cd}	0.64 ±0.01^{cd}	3.24 ±0.08^{bc}
G III: Spirulina	95.6 ± 2.50^{de}	47 ± 2.00^{e}	15.2 ± 0 .80^{d}	0.56 ± 0 .04^{d}	2.94 ± 0 .04^{c}
G IV: Aspartame	180 ± 1.37^{a}	86 ± 1.87^{a}	48.4 ± 2.97^{a}	1.81 ± 0.13^{a}	7.02 ± 0.32^{a}
GV: Asp+L.A	121.2 ± 1.74^{c}	58 ± 1.22^{c}	24.2 ± 0.05^{c}	0.85 ± 0.2^{b}	3.26 ± 0.10^{b}
GVI: Asp+Spir	139.4 ± 4.14^{b}	66 ± 1.87^{b}	22.2 ± 0.66^{c}	0.82 ± 0.02^{bc}	3.44 ± 0.11^{b}
GII: Asp+L.A+Spir	113.4 ± 1.72^{c}	52 ± 2^{cd}	20.6 ± 0.6^{bc}	0.8 ± 0.03^{bc}	3.08 ± 0.03^{bc}

Data are presented as (Mean ± SE). SE = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

4-DISCUSSION

Aspartame (E 951) is the most commonly used non-nutritive artificial sweeteners in over 100 countries in more than 6000 products harmacidical product &feed, drugs and including soft drinks, fruit juice, baked goods, chewing gum, candy, puddings, canned foods, ice cream, yogurt, table sweeteners and plenty of other foods and beverages (Magnuson, et al., 2007). The present results showed that, treatment with aspartame caused significant elevation in the activities of serum liver marker enzymes (ALT and AST and ALP) as compare to control group. This results agree with Morakinyo et al.,(2012) who showed that administration of aspartame (100 mg/kg b. wt) with lipoic acid significantly decreased the value of serum AST, ALT and ALP activities as compared to the control group. However, spurulina and alpha- lipoic acid treatment led to limited decrease in ALT and AST and ALP activities and increase in total protein and albumin relating to aspartame group probably because of the remarkable antioxidant property of spurulina and lipoic acid that drastically attenuated liver tissue damage. The increase in serum ALT, AST activities provides another confirmation for liver injury caused by oxidative stress which induced by aspartame. This results agree with Iyaswamy et al., (2017) who reported that ALT and AST located in the cytosol but escape out from the cell into extracellular fluids and blood flowing due to changes in the permeability of hepatocyte membranes due to increased lipid peroxidation induced by oxidative stress. Also, who exhibits that long-term consumption of aspartame may induce changes on the redox status of liver functions. However, aspartame and its

metabolite methanol could induce liver damage via the mechanism of apoptosis and bring out hepatotoxicity. The marked regulation of liver enzymes, total protein and albumin after spurulina and lipoic acid treatment may prove that spurulina and lipoic acid conserves the structural integrity of liver against ASP-induced injury through the capacity to ROS scavenger as well as through stimulation of endogenous antioxidant defense system. Aspartame caused elevation total protein. This is indication for liver dysfunctions and treated by spirulina and alpha-lipoic acid resulted in enhancement of protein level nearly to its normal ranges of control group, this indicate the ability of spiurulina and lipoic acid to modulate the liver function. As well as, the results of the present study are in conformid with those reported by Iyaswamy et al., (2017) who demonstrates that long-term consumption of aspartame may induce changes on the redox status of liver functions; however, aspartame and its metabolite methanol could induce liver damage via the mechanism of apoptosis and bring out hepatotoxicity. The marked regulation of liver enzymes, total protein and albumin after rosemary treatment may prove that rosemary conserves the structural integrity of liver against ASP-induced injury through the capacity to ROS scavenger as well as through stimulation of endogenous antioxidant defense system. A significant increase in serum triacylglycerol and total cholesterol concentrations were observed in aspartame treated rabbits group. Similarly, Marko et al.,(2015) display that treatment with ASP caused an increase in the concentrations of serum total cholesterol, LDL-cholesterol and triacylglycerol. Also**,** Subramanian et al., (2015) shown that, serum total cholesterol, LDL-cholesterol and triacylglycerol were significantly increased. Meanwhile, spirulina and alpha-lipoic acid administration with aspartame treated rabbits for 8 weeks exhibit a significant decrease in serum total cholesterol and triacylglycerol concentrations when compared to aspartame administered group. Likewise, Kurushima et al., (1995) reported that, spurulina platensis caused a marked decrease in the hypercholesterolemia -induced in rabbits. Who added that, spirulina decrease the serum levels of total cholesterol and increase HDL-cholesterol, consequently spirulina act as a protective factor against the development of atherosclerosis. As confirmed with Yang et al., (2008) who reported that supplementation with alpha lipoic acid caused apparent decrease in lipid peroxidation, plasma cholesterol, triglycerides and low density lipoprotein cholesterol concentrations with marked increase in high-density lipoprotein cholesterol level.

In the current study aspartame administration caused significant elevation in serum urea, uric acid and creatinine concentrations.

Correspondingly**,** Adaramoye and Akanni (2016) show that aspartame administration at three different concentrations 15 mg, 35 mg and 70 mg/ kg b.wt./day for 9 weeks significantly increased the levels of total cholesterol, triglycerides and low-density lipoprotein, alanine aminotransferase, urea , creatinine and uric acid. This is another indication for liver dysfunctions as high urea levels mean that activities of the urea cycle enzymes increased which increase the capacity of the liver to synthesize urea and this may relate to the increased catabolism than anabolism of proteins. This suggestion was supported by previous studies of Alsoufi, (2017) and Meyburg et al., (2018). Also, the elevated serum levels of urea and creatinine indicate reduced ability of the kidney to eliminate the toxic metabolic substances. Parthasarathy et al., (2006) showed that methanol administration significantly increased serum urea and creatinine levels. Additionally, Maiuolo, et al., (2016) reported that the liver is one of the main organs for endogenous production of uric acid, and it is eliminated by kidney. The balance of uric acid formation and excretion is driven by several enzymatic pathways which regulated by pathophysiological factors as metabolic products and free radical species. Furthermore, hyper-uricemia is independently associated with the severity of liver damage and elevation of liver enzymes, AST and ALT which recorded in the present study. In addition, the elevations of urea and uric acid also reflect the severity of kidney dysfunction. Furthermore, Adaramoye and Akanni (2016) reported that impairment of kidney association with a justly sudden fall in glomerular filtration rate because of methanol. Methanol the metabolite of aspartame that enters the proximal tubular cells, binds to anionic phospholipids and inducing abnormal function and metabolism of intracellular membranes and other organelles, and developed injury in the tubular epithelial cells in kidney. Meanwhile, spirulina and alpha-lipoic acid administration with aspartame treated rabbits

for 8 weeks exhibit a significant decrease in serum kidney function tests (urea ,creatinine and uric acid) concentrations when compared to aspartame administered group. Spirulina is rich in β-carotene and the bioavailability is as good as the pure β-carotene, vitamin E and vitamin C and selenium, and spirulina extracts could be effective against free radical induced lipid peroxidation which in turn may lead to cellular transformation (Pal *et al*., 2010) and also reduced serum urea, creatinine and uric acid concentrations. Alpha lipoic acid exerts strong oxidative protection in the liver and kidney against free radical induced cellular damage (Morakinyo et al., 2012). So, in the current studies usage of natural antioxidants like spirulina as a protective strategy against toxicity induced by aspartame remain more effective than treated with lipoic acid because the antioxidant potential of *Spirulina* species and protective effects are mediated by phycocyanins, β-carotene, and other vitamins and minerals contained within *Spirulina*.

5. CONCLUSION

It could be concluded that, the potential ameliorating effect of spirulina and alpha-lipoic acid as powerful antioxidant and anti-inflammatory agents in combating free radical-induced oxidative stress and improved the damaging impact of aspartame on liver and kidney tissues. Therefore, these results confirm the strong antioxidant, anti-inflammatory and cytoprotective effects of spirulina and alpha-lipoic acid that may alleviate the undesirable and dangerous effects during aspartame exposure.

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