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Aspartame-Induced Oxidative Stress on Liver and Kidney in Normal and Diabetic Adult Male Rats

KEYWORDS: Aspartame, diabetes, LPO, AST, ALT, urea, creatinine

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ABSTRACT *The present study was designed to evaluate whether the daily administration of aspartame (ASP) by 200 mg/kg bwt for 4 weeks induces oxidative stress and biochemical changes in the liver and kidney of rats. Forty adult male albino rats were divided into four equal groups {control, ASP (200 mg/kg bwt), streptozotocin-induced diabetes (D) (70 mg/kg bwt), and D+ASP). Levels of lipid peroxidation (LPO) products a long with liver and kidney functions were investigated. The results revealed increase of LPO in ASP group compared to control and decrease in D+ASP groups compared to ASP. AST and ALT activities were increased in ASP and D groups compared to control. Creatinine level and urea concentration showed an elevation in all treated groups compared to control. This study improved that ASP administration may be responsible for oxidative stress that induce disturbance of liver and kidney function.*

Introduction

Aspartame (ASP) (L-aspartyl-L-phenylalanine methylester) is one of the most widely artificial sweeteners consumed in so many products worldwide in various countries (1). ASP is widely used (62%) as a non-nutritive sweetener in food, drinks and pharmaceuticals (2). It is an artificial sweetener possessing 180-200 times the sweetness potency of sucrose and has a calories value of 4/Kcal/g. It was approved by the Food and Drug Administration (FDA) in 1981 (3).

The European Union Scientific Committee on Food maintained the established acceptable daily intake (ADI) of ASP in humans at 40 mg/kg bwt (4). After administration to humans and experimental animals, ASP is rapidly and completely metabolized to 40% aspartic acid, 50% phenylalamine and 10% methanol (5).

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or receptor insensitivity to endogenous insulin. While exogenous insulin and other medications can control many aspects of diabetes assorted complications affecting the vascular system, kidney, and peripheral nerves are common and extremely costly in terms of longevity and quality of life (6).

Diabetes is associated with the generation of reactive oxygen species (ROS) causing oxidative damage (7) particularly to pancreas, liver, kidney etc. (8).

Material and Methods

Animals:

Forty adult male Sprague-Dawely albino rats, weighting 225±5g were used in this work obtained from Assiut University Joint Animal Breeding.

Experimental design and procedures:

Rats were divided into four equal groups, ten rats for each. The experimental period was 4 weeks. At the end of the experiment, rats were killed and specimens of liver and kidney were removed and stored at -80°C.

Induction of diabetes Mellitus:

The overnight fasted rats received a single intraperitoneal injection of streptozotocin (STZ) (70 mg/kg bwt) (9). After 48 h animals were considered diabetic. The rats with fasting diabetes having blood glucose level of 250 mg/dl or above were considered diabetic. Rats with 400 mg/dl blood glucose level and above during the experiment treated with 100-200µ/l of insulin to avoid death.

Measurement of LPO and Biochemical investigations:

LPO was based on that of Ohkawa et al. (10). Blood samples were collected from rat orbital sinus. Alanine aspartate amino transferase (AST), Alanine aminotransferase (ALT), urea and creatinine were measured using enzymatic colorimetric assay kits (Diamond Diagnostics).

Statistical analysis:

Results were expressed as means ± SEM. Differences between means were tested by one way ANOVA followed by the student- Newman- Keul t-test.

Results

The quantitative data of the liver are summarized in Table (1). In ASP administered and diabetic groups, the level of LPO (as indicated by higher MDA) significantly increased ($P<0.001$) versus those of control rats. When ASP was given to diabetic animals, it inhibited ($P<0.05$) the LPO level.

The activity of AST was nonsignificant increased while ALT significantly ($P<0.001$) stimulated in rats after ASP administration compared to controls. In diabetic rats, the activity of AST and ALT were stimulated ($P<0.001$) versus that of controls. Administration of ASP to diabetic animals nonsignificant inhibited both the activity of AST and ALT.

As shown in Table (2), the level of LPO in kidney of ASP administered and diabetic groups stimulated significantly ($P<0.001$) versus controls. The administration of ASP to diabetic animals showed nonsignificant inhibition of LPO level.

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The levels of urea and creatinine were nonsignificant increased after administration of ASP versus control. In diabetic group, the levels of urea and creatinine significantly stimulated by ($P<0.05$) and ($P<0.001$), respectively versus those of controls. When diabetic rats administered with ASP, the level of urea was significant stimulated ($P<0.01$) while the creatinine level was nonsignificant inhibited versus diabetic animals.

Discussion

In the present study administration of aspartame enhance the elevation of LPO in the homogenates of the liver and kidney tissues. The increase of LPO level is taken as direct evidence for oxidative stress (11). This is well supported by other reports Parthasarathy et al. (12) who observed an increase LPO level after methanol administration in the lymphoid organs. Similarly, a significant increase of LPO level in the kidney of rats after treatment with formaldehyde (13) and in both liver and kidney after ASP administration (14) was recorded.

The alternation after aspartame administration is related to its metabolite methanol. Methanol is primary metabolized by oxidation to formaldehyde and then to format. These processes are accompanied by the formation of superoxide anion and hydrogen peroxide (12) .The prime target for free radical reactions during aspartame metabolism is the unsaturated bonds in membrane lipid. Consequent peroxidation results in a loss in membrane fluidity and receptors alignment, suggesting oxidative damage to macromolecules such as lipids (15).

An increase in the activities of AST and ALT in hepatic tissue after ASP administration was reported in rats (16). Also, ASP caused significant increase in both levels of the liver and kidney function markers (17, 14). In addition, methanol administration significantly increased serum urea and creatinine levels (12). These observations were consistent with the finding of our study. When liver and kidney cell membrane is damaged due to involvement of oxidative stress during ASP metabolism, a variety of enzymes normally located in the cytosol are released into the blood stream (18).

It was reported that hypoglycemia provoked over production of ROS and impairment of antioxidant enzymes leading to oxidative stress and organ dysfunction (19). In the present study, diabetic rats showed increased LPO level substantially in the liver and kidney. These results agree with many authors who observed that LPO level are reported to be increased in the liver (20) and in the kidney and liver of diabetic rats (21).

Oxidative stress has been shown to produce glycation of protein, inactivation of enzymes, and alternations in structural functions of collagen basement membrane (22). Oxidative stress may have significant effect in the glucose transport protein (GLUT) or at insulin receptor (23). Scavengers of oxidative stress may have an effect in reducing the increased serum glucose level in diabetes and may alleviate the diabetes as well as reduce its secondary complications.

In the present investigation, the significant elevation in serum creatinine and urea levels indicating impaired renal function of diabetic animals. These observations are consistent with Swaminathan, (24) who explained that the deterioration that characterizes kidney disease of diabetes takes place in and around the glomeruli. Early in the disease, the filtering efficiency diminishes, and important blood proteins are lost in the urine. Later the kidney lost their ability to remove waste products, such as creatinine and urea that when measured in the blood gives an indication of how far a kidney disease has progressed.

In the present study, the D+ASP showed depletion of LPO level, AST and ALT activities compared to diabetic. These results agree with Adaramoye and Akanni, (14) who observed that consumption of ASP by diabetic patients well further aggravate the health condition of these individuals. Other study reported that treating diabetic rats with aspartame improved the kidney function and plasma glucose level (25). These results may be explained as reported previously about hyperglycemia induced oxidative stress play a key role in the development and diabetes its complication**.**

The results of the present study give further data to support the idea that aspartame administration may induce redox imbalance, altered biochemical indices and lipid profile in rats. Morphological investigation on the studied organs in doing.

Table (1): Means ± standard error of lipid peroxidation product (MDA), AST and ALT as well as inhibition and/ or stimulation percentage in the liver of control and different treated rats.

Measurements Groups	LPO (nmol/MDA)	AST (U/L)	ALT (U/L
Control	0.093 ± 0.083[a]	0.096 ± 0.0076[a]	0.071 ± 0.0036[a]
Aspartame (ASP) I% or S% VS control	0.289 ± 0.0348[b] S= 210%	0.101 ± 0.010[a] S= 4.88%	0.142 ± 0.0103[b] S= 100%
Diabetic (D) I% or S% VS control	0.248 ± 0.0398[b] S= 94.2%	0.188 ± 0.011[b] S= 95.2%	0.262 ± 0.0273[c] S= 269.0%
Diabetes + Aspartame (D+ASP) I% or S% VS ASP I% or S% VS dib	0.181 ± 0.024[a] I= 37.4% I= 27.0%	0.125 ± 0.0104[a] S= 14.9% I= 33.6%	0.242 ± 0.0093[c] S= 70.4% I= 7.6%

Values in the same column with unlike superscript letters are significantly differing.

Table (2): Means ± standard error of lipid peroxidation product (MDA), urea and creatinine as well as inhibition and/or stimulation percentage in the kidney of control and different treated rats.

Measurements Groups	LPO (nmol/MDA)	Urea (mg/dl)	Creatinine (mg/dl)
Control	0.061 ± 0.0852[a]	34.088 ± 1.449[a]	0.191 ± 0.0871[a]
Aspartame (ASP) I% or S% VS control	0.260 ± 0.0135[c] S= 76.7%	41.36 ± 4.758[a] S= 5.66%	0.369 ± 0.0495[a] S= 48.2%
Diabetic (D) I% or S% VS control	0.257 ± 0.0118[c] S= 75.5%	57.042 ± 4.520[b] S= 38.85%	1.071 ± 0.0751[b] S= 82.2%
Diabetes + Aspartame (D+ASP) I% or S% VS ASP I% or S% VS dib	0.163 ± 0.0077[b] I= 37.3% I= 36.6%	81.37 ± 7.131[c] S= 94.18% S= 29.9%	0.904 ± 0.126[bc] S= 59.2% I= 18.5%

Values in the same column with unlike superscript letters are significantly differing.

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