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# Effect of N-acetylcysteine (NAC) On Hepatotoxicity of Aspartame

**Darwish, A. Sh.; Eman Sh. M. Laz and Faten, G. El-Said**

Biochemistry Department,& Chemistry Department, Zagazig, Animal Health Research Institute

## Abstract

The present study was designed to examine the ability of N-acetylcysteine (NAC) to attenuate Aspartame as a synthetic sweetener (ASP)-induced hepatotoxicity in adult albino rats. Forty adult male rats, weighing 150-170 g, were randomly divided into four groups as follows: first group was given distilled water and served as control group I, Group II: received aspartame (ASP) dissolved in distilled water in a dose of 500 mg /kg. b.wt./day, Group III: received NAC dissolved in distilled water in a dose of 600 mg /kg b.wt./day, and Group IV: Rats received NAC dissolved in distilled water in a dose of 600 mg /kg b.wt./day and aspartame (ASP). Administration of ASP at a dose level of500 mg/kg b.wt. to rats for 42 days significantly elevated the levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gammaglutamyltransferase( GGT), tumor necrosis factor (TNF-α) and hepatic alphafetoprotein (AFP) activity which indicate injury to the liver function. Also, total cholesterol, triglycerides and low-density lipoprotein (LDL) increased significantly. On the other hand ASP decreased serum protein, albumin, high-density lipoprotein (HDL) and liver glutathione (GSH) and superoxide dismutase (SOD).These results reflects that ASP intoxication induced marked alterations in liver functions and caused liver atrophy. NAC (600 mg/kg b.wt.) which administered 1h before ASP ameliorated the hepatotoxicity induced by ASP. This was evidenced by a significant reduction in serum ALT, AST, GGT, TNF-α and hepatic AFP activity and a significant restoration in serum protein, albumin, HDL, GSH and SOD. These results indicate that administration of N-acetylcysteine has a strong potential effect against Aspartame-induced damage to liver. This reflects the beneficial role of N-acetylcysteine in treatment of liver injury.

## Introduction

Sweeteners are paid special attention among food additives as their use enables a sharp reduction in sugar consumption and a significant decrease in caloric intake while maintaining the desirable palatability of foods and soft drinks**(Gougeon ,et al., 2004 and**

**Vences-Mejia, et al., 2006)** Aspartame (NLalpha- Aspartyl-L-phenylalanine 1-methyl ester) (ASP) is a synthetic sweetener consumed by a large wide of people **( Grenby, 1991; Lim 2006 and Portela, 2007)**. It can be toxic, principally to the liver and retina, and has a low caloric value formed from the union of two amino acids, aspartic acid and phenylalanine, with sweetening power 180 to 200 times greater than that of sucrose**(Portela 2007).** It is a dipeptide artificial sweetener composed of the amino acids phenylalanine and aspartic acid plus a small quantity of methanol **(Grenby, 1991).**

Since after ingestion ASP, is very efficiently hydrolyzed one of the main concerns derives from the products of hydrolysis, comprising about phenylalanine (50%), aspartic acid (40%) and methanol (10%), and from the presence of degradation products such as 5-benzyl-3,6-dioxo-2-piperazine acetic acid (DPK) and β-aspartame **(Bially et al., 2009)**.

*N*-acetylcysteine (NAC) is nutritional supplement used primarily as a mucolytic agent and sulfate repletion, such as autism, where cysteine and related sulfur amino acids may be depleted. NAC is a derivative of cysteine; an acetyl group that is attached to the nitrogen atom. This compound is sold as a dietary supplement commonly claiming antioxidant and kidney protecting effects. NAC is a precursor of the amino acid L-cysteine and helps glutathione synthesize pathway. The benefit of NAC for the prevention of contrast-induced nephropathy was first reported by (**Tepel, 2000).** NAC acts as an antioxidant by restoring the pool of intracellular reduced glutathione, which is often depleted as a consequence of increased status of oxidative stress and inflammation. Furthermore, NAC also has reducing and antioxidant properties, acting as a direct scavenger of ROS (reactive oxygen species) **(Goncalves, 2010).** It has been used as a chelator of heavy metals to protect against oxidative stress and prevent damage of cells.

The liver's highly specialized tissues regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions**(Athnea, 2005)**.

The metabolism of xenobiotic to a large extent takes place in the liver. The byproducts of such metabolism sometimes are more toxic than the initial substance. This could lead to hepatic damage and the emergence of hepatic disorders **(Weinstein , 2003)**.

The current study was designed to investigate Aspartame-induced hepatotoxicity and to evaluate the potential beneficial effects of Nacetylcysteine supplementation to improve hepatotoxicity in male rats.

## Materials and Methods

### Experimental animals:

Forty male Sprague–Dawely rats, each weighing 150 -175 g, were obtained from the animal house of the National Research Center (Cairo). The animals were housed in stainless steel cages after grouping in batches of four under standard animal house conditions of relative humidity (55 ± 5%), temperature (25 ± 2˚C) and a 12 hr light/12 hr dark cycle. Rats were allowed free access to standard commercial feed and tap water and were acclimatized to laboratory conditions for a period of one week before the onset of experimentation.

### Experimental design:

Animals were allocated to four groups each of ten rats as follows:

**Group I:** Rats received orally distilled water and served as control group.

**Group II:** Rats received aspartame (ASP) dissolved in distilled water in a dose of 500 mg /kg.b.wt./day,for 42 days **(JECFA,1980)**.

**Group III:** Rats received NAC orally, dissolved in distilled water in a dose of 600 mg /kg b.wt./day, for 42 days **(Chen, 2008).**

**Group IV:** Rats received NAC dissolved in distilled water in a dose of 600 mg /kgb.wt./day then after 1 hour rats received ASP (500 mg /kg. b.wt./day)orally, for 42 days.

After 42 days of treatment, the end of experiment, all the animals were sacrificed after 24 hrs of the last dose of different administrations and their blood were collected, by carotid bleeding, in centrifuge tubes and serum was obtained from the blood after centrifugation at 3000 rpm for 15 min.

The liver was immediately excised, cleared of adhering connective tissue and weighed. Serum and liver samples were stored at -20°C until analysis studies.

### Methods of Biochemical studies:

AST and ALT were measured colorimetrically according to **(Reitman and Frankel, 1957)** and GGT by the method of **(Persijn and Slik, 1976)**. Serum total cholesterol was measured according to the method of **(Richmond, 1973)** and triglycerides by using the method of (Bucolo and David, 1973). Determination of total proteins and albumin were estimated depending on the assays depicted by **(Doumas, 1981)** and **(Doumas, 1971)**, respectively. Alphafetoprotein (AFP) was estimated by using the method of **(Wepsic, 1981)**Hepatic glutathione (GSH) was spectrophotometrically assayed in the by the method of **(Sedlak and Lindsay, 1968)**.

**Statistical analysis:**

Statistical analyses were done using InStat version 2.0 (GraphPad, ISI Software, Philadelphia, PA, USA, 1993) computer program. The results were expressed as mean ± SE. Multiple comparisons were done using one-way ANOVA followed by Tukey-Kramer as a post-ANOVA test.

## Results and Discussion

Data listed in Table (1) show that the administration of rats with ASP caused a significant increase ($P$<0.001) in the levels of serum ALT, AST and GGT as compared to the control group I. The results in Table (2&3) revealed that serum total cholesterol, triglycerides, LDL, TNF-α and AFP levels increased significantly ($P$<0.001) while, ASP exerts a significant ($P$<0.001) decrease in serum HDL, total protein and albumin contents in the ASP treated group as compared to the control group. Treated rats with ASP exhibited a significant ($P$<0.001) decrease in hepatic GSH content ($P$<0.001) as compared to the control values group I (Table 4).

The administration of NAC after ASP exhibited significant amelioration in all previous parameters (Table1, 2, 3&4). The results recorded a significant decrease ($P$<0.001) in the levels of serum ALT, AST, GGT, total cholesterol, triglycerides and LDL levels in the treated group IV as compared to the control group I. Also, NAC after ASP treated group showed increase in serum protein, albumin, HDL and hepatic GSH content compared to ASP and the control groups,

Although aspartame (ASP) received food and Drug Administration (FDA) approval in 1981 and has been judged safe by medical groups such as the AMA Council on Scientific Affairs, there has been persistent concern that the use of ASP, an O-methyl ester of the dipeptidel -a-aspartyl-1-phenylalanine, may result in adverse neurologic symptoms or other abnormalities **(Pardridge, 1986; Tsakiris 2006).**

The current study provided evidence that ASP, a widely used sweetening agent in human diet increasing oxidative stress in liver. In this study, ASP (500 mg/kg) was hepatotoxic in rats as evidenced by significant ($P<0.001$) increase in serum AST, ALT and GGT activities as well as a remarkable elevation in the concentration serum total cholesterol, riglycerides and LDL. These data are sensitive indicators of liver injury **(Ozer, 2008; Egbuonu, 2009 and Abhilash et al., 2011)**, due to the increment of oxidation stress associated with a considerable decrease in the autoimmune system and disturbance in the S-H bond. The elevation in levels of serum liver markers, especially AST, ALT, GGT,AFP and TNF-α may be attributed to damage in the liver cells, since these enzymes are located in the cytosol and released into the blood flowing **(Ozer, 2008; Egbuonu, 2009 and Abhilash et al., 2011, Abdel-Salam 2012 and Ashok and Rathinasamy, 2014)**

Also, it showed that the major target organ in ASP poisoning is liver and the primary lesion is acute centrilobular hepatic necrosis **(Ashok and Rathinasamy, 2014, Jaeschke, 2002 and Alipour, 2013).** ASP is metabolized in the gastrointestinal tract into aspartic acid, phenylalanine and methanol. However, the aspartic acid is mostly eliminated through the lungs in the form of carbon dioxide. Also some of the phenylalanine formed in the intestine following ingestion of ASP is excreted in the form of CO2 most of it is incorporated into the pool of amino acids and contributes to protein synthesis. Moreover, methanol is primarily metabolized by oxidation to formaldehyde and then to format. These processes are accompanied by the formation of superoxide anion and hydrogen peroxides, Protein and albumin depletion results in increased toxicity to ASP, which is associated with a significantly decreased rate of hepatic metabolism **(Roberts, 2004 and Abhilash et al., 2011).**

In the present study it was shown that injection of NAC before ASP protected the liver from damage induced by ASP. This protection was clearly reflected by a decrease in serum ALT, AST, GGT, AFP and TNF-α level and by a significant increase in total

proteins and albumin contents. The results also reveal that serum TC, TG, LDL and HDL levels returned approximately to the normal control levels. This is in agreement with **Alturfan (2012)** and **Sadowska (2007)** who reported that NAC is an excellent scavenger of free radicals and chelator of heavy metal **(Sener, 2003 and Ljubisavljevic (2011).** The effect of NAC in ASP treated rats was manifested by the low concentrations of TC, TG, and LDL and the increased HDL concentrations. Similar findings were also reported for other experimental models, which observed a decline in LDL and total cholesterol and an increase in HDL concentrations in treated animals, this effect may be related to the enhancement of the catabolism of cholesterol to form bile acids and the inhibition of cholesterol synthesis and LDL receptor activity. HDL plays an essential role in the transport of cholesterol to the liver for excretion into bile acids **(Dietschy,1997)** which are cytoprotective in hepatocytes because of their ability to activate phosphatidylinositol-3-kinase. The treatment was able to restore the elevated concentrations of TC, TG, and inflammatory indicators, a significant improvement in liver enzymes and lipid metabolism, and a significant increase in HDL cholesterol have also been observed in cases treated with anti-oxidants **(Gomez 2005)**. NAC effectively reduce oxidative stress, restore the normal concentrations of anti-oxidant enzymes, and exhibit anti-inflammatory activity **(Chen 2008).**

**Table (1): The effect of NAC on serum ALT, AST and GGT levels in ASP-induced hepatotoxicity**

| Animal group | ALT(U/ml) | AST(U/ml) | GGT(U/ml) |
|---|---|---|---|
| G1 (control) | 50.76+0.33 | 149.99+1.22 | 34.85+0.35 |
| G2(ASP) | 115.23+0.13a** | 212.95+0.23a** | 63.76+0.87a** |
| G3(NAC) | 53.07+0.09ab** | 146.25+0.80 N.S | 32.91+0.02N.S. |
| G4(NAC+ASP) | 71.37+0.17ab** | 175.29+0.28ab** | 45.30+0.87ab** |

Data are expressed as mean ± S.E. (n = 8 in each group);different symbols means significance between groups.

**Table (2): The effect of NAC on serum cholesterol, triglycerides, LDL and HDL levels in ASP-induced hepatotoxicity.**

| Animal group | Cholesterol(mg/dl) | Triglycerides(mg/dl) | LDL(mg/dl) | HDL(mg/dl) |
|---|---|---|---|---|
| G1 (control) | 69.22 ± 0.508 | 75.29 ± 0.28 | 22.30±0.10 | 31.86±0.10 |
| G2(ASP) | 115.31 ± 0.24 a** | 114.55 ±0.44 a** | 70.33±0.21a** | 22.07±0.21 a** |
| G3(NAC) | 70.61 ± 0.61 b** | 75.35 ± 0.40 b** | 23.47±0.08N.S | 32.07±0.08 b** |
| G4(NAC+ASP) | 92.05 ± 0.22 ab** | 98.61 ±0.14 ab** | 44.40±0.19 ab** | 27.93±0.19 ab** |

Data are expressed as mean ± S.E. (n = 8 in each group); different symbols means significance between groups

**Table (3): The effect of NAC on Serum Total Protein, Albumin, TNF-α and hepatic AFP in ASP-induced hepatotoxicity**

| Animal group | Total Protein(g/dl) | Albumin(g/dl) | TNF-α (Pg/ml) | AFP(U/g) |
|---|---|---|---|---|
| G1 (control) | 7.37±0.05 | 3.64±0.09 | 10.87 ±0.22 | 5.30± 0.32 |
| G2(ASP) | 5.29±0.09 a** | 2.32±0.03 a** | 40.49 ± 0.28 a** | 33.436±0.29 a** |
| G3(NAC) | 7.35±0.05 N.S | 3.60±0.11 b** | 10.89 ± 0.16 N.S | 5.53 ± 0.20 N.S |
| G4(NAC+ASP) | 6.99±0.03 ab** | 3.36±0.07 b** | 21.98 ± 0.17 ab** | 18.62 ±0.30 b** |

Data are expressed as mean ± S.E. (n = 8 in each group); different symbols means significance between groups.

**Table (4): The effect of NAC on hepatic GSH and SOD levels in ASP-induced hepatotoxicity.**

| Animal group | GSH (U/g wet tissue) | SOD (U/g wet tissue) |
|---|---|---|
| G1 (control) | 2.32 ± 0.21 | 18.12 ± 0.30 |
| G2(ASP) | 0.61 ± 0.10 a** | 10.31 ±0.13 a** |
| G3(NAC) | 2.35 ±0.24 b** | 17.87 ±0.32 b** |
| G4(NAC+ASP) | 1.98 ±0.03 b** | 13.645±0.21 ab** |

Data are expressed as mean ± S.E. (n = 8 in each group); different symbols means significance between groups**.**

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