



ISSN NO. 2320-5407

Journal homepage: <http://www.journalijar.com>

INTERNATIONAL JOURNAL
OF ADVANCED RESEARCH

RESEARCH ARTICLE

Synergistic effect of N-acetyl cysteine and folic acid against aspartame- induced nephrotoxicity in rats

Afaf Abbass Sayed Saleh

Department of Zoology, Women's College, Ain Shams University, Egypt

Manuscript Info

Manuscript History:

Received: 12 March 2014
Final Accepted: 23 April 2014
Published Online: May 2014

Key words:

Aspartame; Nephrotoxicity;
Oxidative stress; Antioxidants; Rat

*Corresponding Author

Afaf Abbass Sayed Saleh

Abstract

The objective of the present study is to explore the synergic and the presumable protective effects of the combination of N-acetyl cysteine (NAC) and folic acid (FA) against aspartame induced nephrotoxicity. Eighteen adult male Wistar rats, weighing 150-170 g, were randomly divided into three groups as follows: first group was given distilled water and served as control group; the second group was given aspartame dissolved in distilled water in a dose of 500 mg /kg b.wt.; the third group was given both NAC and folic acid dissolved in distilled water in a dose of 600 mg /kg b.wt. and 12 mg/kg b.wt. respectively. Serum urea, creatinine, and potassium were significantly increased whereas sodium and calcium were significantly decreased in rats that had received aspartame. Blood hemoglobin, serum iron and ferritin were significantly decreased whereas serum total iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC) were significantly increased. The concentration of reduced glutathione (GSH), and the activity of glutathione peroxidase (Gpx), and catalase (CAT) were significantly decreased whereas the concentration of LPO (TBARS) in kidney tissues was significantly increased. Combined Treatment with NAC and FA significantly restored kidney functions, modulate hematological parameters, reduced lipid peroxidation and enhanced reduced glutathione level.

Copy Right, IJAR, 2014. All rights reserved.

1. Introduction

Aspartame N-(L- α -Aspartyl)-L-phenylalanine, 1-methyl ester is a low-calorie, intense artificial sweetener. It is a white, odourless powder, approximately 200 times sweeter than sugar. In Europe, it is authorised to be used as a food additive in foodstuffs such as drinks, desserts, sweets, dairy, chewing gums, energy-reducing and weight control products and as a table-top sweetener (Lim et al., 2006).

As has been demonstrated by Trocho et al. (1998), aspartame ingestion leads to a significant exposure and accumulation of formaldehyde adducts in the organs and tissues. Formaldehyde is much more toxic to cells when free radical levels are increased. In addition, 40% of aspartame breaks down into an excitotoxic amino acid. There is a worldwide preoccupation with the counteraction of the negative effects of excitotoxic amino acids, which are utilized as food additives or pharmacologic factors, by attenuating or blocking oxidative stress, the essential cause of many renal injury in animals and men (Saito et al., 2005). On the other hand, several studies suggest an association between aspartame consumption and the risk of cancer, diabetes type 2, or pre-term delivery (Soffritti et al., 2010; Englund-Ögge, 2012; Fagherazzi et al., 2013).

N-acetylcysteine is a synthetic precursor of GSH, which stimulates the intracellular synthesis of GSH, acts as a nucleophile to conjugate with reactive metabolites and enhances glutathione S-transferase (GST) activity (Tylickiet

al.,2003). A recent clinical trial demonstrated that NAC can reduce the incidence of contrast-induced nephropathy, including dialysis requirement and mortality, in patients undergoing angiographic procedures (Al-Ghonaim and Pannu, 2006). In experimental animals, several studies have confirmed nephroprotective properties of NAC on gentamicin-mediated nephropathy (Mazzon et al.,2001 ; Patel, 2011), in ifosfamide-induced nephrotoxicity (Mishima et al., 2006; Chen et al.,2008), on AmB-induced renal tubular cell apoptosis (Briguori et al.,2004; Odabasiet al.,2009); chronic kidney disease (CKD) (Agarwal et al.,2004) as well as in ischaemia/reperfusion injury (Tariq et al., 1999; Dhalla et al., 2000; Nitescu et al., 2006). Additional protective effects of NAC may lie in the ability of this compound to decrease NF- κ B activation, reduce kidney inflammation and improve renal function (Ranganet al.,2000; Nitescu et al.,2006) and to improve microcirculation (Polatet al.,2006).

Folic acid is a form of the water-soluble vitamin B9. Folate is a naturally occurring form of the vitamin, found in food, while folic acid is synthetically produced, and used in fortified foods and supplements. Folic acid is essential for growth, development and normal cellular function, and its uptake is mediated by regulated transport system (Wani and Kaur, 2012). Several studies have shown that folic acid supplementation can reduce the risk of, neurological and neuropsychiatric disorders (Manzoor and Runcie, 1976), neural tube defects (Daly et al., 1995), cardiovascular and hematological diseases (Verhaar et al., 2002) and several types of cancer, including cervical, lung, brain, pancreatic, colorectal and breast cancer (Duthie et al., 2002). The antioxidant activity of folic acid is thought to be involved in these effects of folic acid on health, helping with the formation and regeneration of red blood cells and preventing anemia; and increasing energy and endurance (Nakano et al., 2001; Paradisi et al., 2004). Moreover, the anti-inflammatory effect of folic acid is manifested by a decrease in the levels of interleukin and C-reactive proteins (Solini et al., 2006). In fact, folic acid has been reported to have an antioxidant effect against ROS and an alleviating role in hyperhomocysteinemia and its associated endothelial dysfunction (Moens et al., 2008).

The use of an antioxidant in combination is superior to its isolate use in several different animal models of oxidative stress and renal dysfunction (Petronilho et al.,2008; Puica ,2008;Manaliet al.,2011). So the objective of our present study was to explore the interactions and possible synergism and curative potential of NAC and folic acid on aspartame -induced nephrotoxicity in vivo model.

Materials and Methods

2.1. Chemicals

All chemicals were purchased from Sigma Chem. Co., St. Louis, MO, USA.

2.2. Animals

Eighteen male adult Wistar rats weighing 150-175 g were purchased from Helwan Laboratory Farms of Egyptian Organization for Vaccine and Biological preparations. All the animals were maintained under standard laboratory conditions of temperature (25 °C) and 12 h light and dark cycles throughout the experimental period. The rats were provided with a standard rat chow and tap water ad libitum. All animal procedures and experimental protocols were approved by the Department of Zoology Council, Women's College, Ain Shams University, Egypt, which has an ethical authority.

2.3. Experimental protocol

Three groups were formed (n= 6) for oral administration; first group was given distilled water and served as control group; the second group was given aspartame dissolved in distilled water in a dose of 500 mg /kg b.wt./day; the third group was given both NAC and folic acid dissolved in distilled water in a dose of 600 mg /kg b.wt./day and 12 mg/kg b.wt./day respectively. After 42 days of treatment, all the animals were euthanized and decapitated, blood was collected and centrifuged at 3000 rpm for 20 min; the clear serum obtained was used for the evaluation of kidney function. The kidneys were removed immediately, washed in ice cooled 0.15 M NaCl and blotted on a filter paper. Then the tissue was weighed and one hundred milligrams of kidney tissue was homogenized in ice-cold 0.25 M sucrose containing 1mM diethylenetriaminepenta-acetic acid (1:1 w/v). The homogenate was centrifuged at 20,000 g for 20 min at 4°C and the supernatant was used for biochemical assays.

2.4. Determination of serum urea and creatinine

Serum urea and creatinine were carried out using respective diagnostic kits purchased from Randox Ltd., Co. (UK) according to the methods of Fawcett and Scott (1960), Seeling and Wust (1969) respectively.

2.5. Determination of serum electrolytes

Sodium (Na) and Potassium (K) analysis were accomplished by emission flame photometry after suitable dilution as described by Dean (1960). Serum calcium was determined colorimetrically using commercial kits (Human, Germany) according to the method of Goldberg (1973).

2.6. Determination of serum hematology

At the end of the experiment, part of blood samples were collected in EDTA tubes for blood hemoglobin (Hb) estimation and another part were collected in clean dry test tubes for biochemical assays. The serum parameters were analyzed spectrophotometrically by using double beam UV-Visible spectrophotometer (UV-Visible spectrophotometer, VIS-JR, model 1601). Hb was estimated by the cyanmethemoglobin method. Serum iron (Fe), total iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC) were analyzed by commercial kits (Gesan, Italia). Serum ferritin was determined by ELISA technique using commercial kit (Cayman Chem. Co., USA).

2.7. Determination of glutathione, antioxidant enzymes and lipid peroxidation

Glutathione (GSH) content and glutathione peroxidase (Gp_x) activity were assayed using the method of Baker et al. (1990) and Rotruck et al. (1973) respectively. Catalase (CAT) activity in the sample was measured according to the method described by Aebi (1974) by measuring the decrease in absorbance of H₂O₂ at 240 nm. Reactive oxygen metabolites in terms of lipid peroxidation (LPO) as thiobarbituric acid reactive substances (TBARS) concentration was assayed by the method of Hogber et al. (1974). The commercial ELISA kits of GSH, Gp_x, CAT, and TBARS were purchased from Cayman Chem. Co., USA.

2.8. Statistical Analysis

Data were presented as mean \pm standard error (SE) and were statistically analyzed using Student's "t" test. The data were statistically analyzed using analysis of variance (ANOVA) followed by Duncan's multiple range test according to Snedecor and Cochran (1982) by the aid of SPSS program, Version 10, USA.

3. Results

Oral administration of aspartame (500 mg/kg b.wt.) for 42 days significantly increased urea and creatinine in serum. Sodium and calcium were significantly decreased whereas potassium was significantly increased in the experimental groups as compared to the control group (Table 1). These alterations were reverted to near normal levels upon treatment with NAC and folic acid.

Table 2 shows the changes in various hematological parameters in response to oral administration of aspartame after six weeks. Significant decrease in blood hemoglobin (Hb) was observed. Serum iron and ferritin were significantly decreased whereas the changes in serum total iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC) were significantly increased as compared to the control group. The changes in blood Hb and serum iron, TIBC, UIBC and ferritin were modulated in rats that had received both antioxidants.

Remarkable decrease in glutathione (GSH) content was observed. Glutathione peroxidase (Gpx) and catalase (CAT) activities were also significantly decreased in intoxicated rats whereas the concentration of thiobarbituric acid reactive substances (TBARS) in kidney tissues was significantly increased as compared to the control rats group (Table 3). Oral administration of NAC and folic acid were significantly increased kidney GSH, GPX, and CAT. On the other hand, TBARS was significantly decreased.

Table 1
Efficacy of the combination of N-acetylcysteine and folic acid treatment on serum biochemical parameters of nephrotoxic rats .

Parameters	Control	Aspartame group	Aspartame+NAC+FA
Urea (U/L)	14.629 ± 0.427 ^A	22.398 ± 0.731 ^B	17.592 ± 0.559 ^C
Creatinine (U/L)	0.366 ± 0.007 ^A	0.612 ± 0.015 ^B	0.471 ± 0.011 ^C
Na (U/L)	140.89 ± 3.891 ^A	129.08 ± 3.223 ^B	135.66 ± 3.574 ^C
K (U/L)	4.101 ± 0.059 ^A	4.747 ± 0.077 ^B	4.352 ± 0.068 ^C
Ca (mg/dL)	8.946 ± 0.124 ^A	7.883 ± 0.093 ^B	8.365 ± 0.107 ^C

Values are expressed as means ± S.E, n= 6.

A, B, C = means bearing different superscripts within the same row that differ significantly (P< 0.05).

Table 2
Efficacy of the combination of N-acetylcysteine and folic acid treatment on hematological parameters of nephrotoxic rats.

Parameters	Control	Aspartame group	Aspartame+NAC+FA
Hb (g/dl)	14.694 ± 0.227 ^A	12.569 ± 0.151 ^B	13.932 ± 0.197 ^C
Fe (µg /dL)	17.421 ± 0.309 ^A	14.557 ± 0.252 ^B	16.035 ± 0.287 ^C
TIBC (µg /dL)	93.452 ± 1.773 ^A	110.758 ± 2.493 ^B	105.322 ± 1.974 ^C
UIBC (µg /dL)	76.031 ± 1.329 ^A	96.201 ± 1.887 ^B	89.287 ± 1.569 ^C
Ferritin (ng/dL)	88.746 ± 1.438 ^A	69.193 ± 1.127 ^B	76.492 ± 1.298 ^C

Values are expressed as means ± S.E, n= 6.

A, B, C = means bearing different superscripts within the same row that differ significantly (P< 0.05).

Table 3
Efficacy of the combination of N-acetylcysteine and folic acid treatment on kidney biomarker parameters of nephrotoxic rats.

Parameters	Control	Aspartame group	Aspartame+NAC+FA
GSH (nmol/g tissue)	9.269 ± 0.196 ^A	6.956 ± 0.171 ^B	8.092 ± 0.183 ^C
GPx (nmol/mg protein/min)	3.783 ± 0.127 ^A	3.007 ± 0.096 ^B	3.435 ± 0.111 ^C
CAT (nmol/mgprotein/min)	7.452 ± 0.161 ^A	6.138 ± 0.133 ^B	6.882 ± 0.149 ^C
TBARS (ng/g tissue)	2.797 ± 0.029 ^A	4.332 ± 0.057 ^B	3.538 ± 0.044 ^C

Values are expressed as means ± S.E, n= 6.

A, B, C = means bearing different superscripts within the same row that differ significantly ($P < 0.05$).

4. Discussion

This study investigated the capability of the combination of NAC and folic acid compounds to protect against aspartame-induced nephrotoxicity. Our findings confirmed the previous observations that NAC

(Briguori et al., 2004; Chen et al., 2008) and folic acid (Hwang et al., 2010; Ebaid et al., 2013) effectively reduce oxidative stress, restore the normal concentrations of anti-oxidant enzymes, and exhibit anti-inflammatory activity. In addition, the present study proves the synergic effect of both antioxidants upon some biochemical parameters in white rat exposed to aspartame action for six weeks, which has not been established before.

Aspartame is best known by the brand names NutraSweet, Equal, Sweet One and Spoonful. Aspartame is a synthetic chemical combination which is comprised of approximately 50% phenylalanine, 40% aspartic acid, and 10% methanol. Aspartame is found in thousands of foods, drinks, candy, gum, vitamins, health supplements and even pharmaceuticals.

One of the early signs of glomerular dysfunction is the elevation in serum creatinine. Serum creatinine concentration is the most widely used marker in estimating GFR (Nitescu et al., 2006). We hypothesized that, oral administration of aspartame at a dose of 500 mg/kg.b.wt. for six weeks may cause kidney injury that was represented by a significant increase in urea and creatinine concentration in serum. These results confirmed that nephrotoxicity is the major dose-limiting side effect of aspartame as previously reported by Marielza et al. 2007 and Odabasi et al. 2009. These changes reflected the severity of renal insufficiency which occurred in association with a fairly sudden fall in glomerular filtration rate because of the majority of methanol, the by product of administered aspartame metabolism that enters specifically the proximal tubular epithelial cells, binds to anionic phospholipids in the target cells inducing abnormalities in the function and metabolism of multiple intracellular membranes and organelles then developed injury in the proximal tubular epithelial cells of kidney that caused renal failure (Parthasarathy et al., 2006).

It has been found that the combination of NAC and folic acid is superior in preventing aspartame-induced kidney damage. Furthermore, the association use of antioxidants consistently attenuated kidney oxidative damage and improve urea and creatinine levels, suggesting that iron ions are important in the regeneration of the damaged kidney, as previously demonstrated in vitro (Zager et al., 1994; Petronilho et al., 2008).

Serum electrolytes were disturbed significantly in aspartame treated rats as compared to the control animals. Lower value of serum sodium indicates inability of kidney to conserve sodium and chloride. Hemodilution too may be involved in the fall of sodium value via excess of water intake and or increased production of endogenous water. In turn, the reversed increases of potassium appeared to be due to reduced excretion of K aggravated by leakage of intracellular potassium into blood stream as a result of aspartame induced lesions in renal tubular epithelium. These results are in harmony with the data obtained by Heibashy et al. (2009).

Serum sodium and calcium were significantly decreased. Conversely, serum potassium was significantly increased in aspartame-treated rats. These disturbances probably due to the elevated methanol level which produced after aspartame administration. Furthermore, increased methanol levels enhance deposition of calcium as calcium phosphate and carbonate in injured skeletal muscle (Ibrahim and Saleh, 2012).

Also, the toxicity of aspartame may cause an increase in the urinary excretion of calcium and Serum electrolytes were disturbed significantly in aspartame treated rats as compared to the control animals. These results confirmed that there was a link between the food additive aspartame and kidney stones revealing that aspartame increases calcium in urine, a condition called calciuria. Calcium accumulation is the most common reason for the formation of kidney stones (Nguyen et al., 1998; Xu et al., 2013). Folic Acid the B vitamin will stop an enzyme responsible for producing the uric acid. Folic acid (5 mg per day) helps break down uric acid stones. The present study found that both NAC and folic acid significantly suppressed the toxic effect of aspartame by alleviating the cellular toxicity activating the anti-oxidant defence system (Table 1).

A small amount of aspartame significantly increases the plasma methanol levels (Davoli, 1986). Methanol is primarily metabolized by oxidation to formaldehyde and then to formate, these processes are accompanied by the formation of superoxide anion and hydrogen peroxide (Parthasarathy et al., 2006). As demonstrated in the present study, aspartame toxicity was associated with a significant decrease in blood Hb. This was paralleled by a greater depletion of serum iron and Ferritin as well as more elevated levels of serum TIBC and UIBC compared to the control group. These alterations may contribute to methanol intoxication that increased levels of free radicals production (Abhilash et al., 2011). A high TIBC and UIBC, usually indicates iron deficiency. Iron deficiency anemia is the most common form of anemia. Anaemia is a common manifestation of chronic kidney disease (CKD) and a significant determinant of overall prognosis and quality of life in patients with renal insufficiency. As inadequate production of erythropoietin is the main mechanism of renal anaemia, in most renal patient provision of sufficient human recombinant erythropoietin (rHuEPO) and iron replacement therapy corrects renal anaemia effectively (Schaefer et al., 2002).

Oxidative stress plays an important role in hemolytic anemias, although it is not the primary etiology of these diseases but it participates in causing damage in RBC, and its effect can be neutralized by antioxidants. NAC significantly prevent RBC alteration and prevent the loss of Hb content (Cuzzocrea et al., 2001). Additionally, NAC proved its antioxidant and protective effects on oxidative stressed human RBCs (Grinberg et al., 2005). The antioxidant activity of folic acid may enhance the modulation of blood Hb and most likely play the most important role in the amelioration of the damage to the target organ. Critically, folic acid has an essential role in the integrity and function of DNA (Joshi et al., 2001). Theoretically, folic acid could interfere with the metabolism, cellular transport, and regulatory functions of the natural folates that occur in the body by competing with the reduced forms for binding with enzymes, carrier proteins, and binding proteins (Smith et al., 2008). Due to antioxidant powerful of NAC and folic acid a remarkable correction was observed in hematological parameters of nephrotoxic rats orally treated with 500 mg of aspartame for six weeks (Table 2).

Antioxidant depletion or deficiency in antioxidants may contribute to oxidative stress. oxidative stress results from an oxidant/antioxidant imbalance, an excess of oxidants, and/or a depletion of antioxidants. Oxidative stress is thought to play an important role in the pathogenesis of kidney disease not only through direct injurious effects, but also by involvement in the molecular mechanisms that control kidney inflammation (Chen et al., 2008; Ebaid et al., 2013).

As demonstrated in the present study, aspartame induced a remarkable depression in glutathione (GSH) content and a significant decrease in the activities of glutathione peroxidase (Gpx) and catalase (CAT) associated with a considerable elevation in the concentration of thiobarbituric acid reactive substances (TBARS) which is a lipid peroxidation product. These results may be due to the damaging effect of free radicals produced following methanol exposure or alternatively could be a direct effect of formaldehyde formed oxidation of methanol on these parameters (Abhilash et al., 2011).

Since glutathione is an endogenous substance that protect cell suffering from oxidative stress, GSH acts as antioxidant by catalyzing the reduction of H_2O_2 to water (Abuja and Albertini, 2001); it can react chemically with singlet oxygen, superoxides and hydroxyl radicals (Singh et al., 2003; Hashimoto et al., 2008). It also be able to attack electrophilic centers and thus protect proteins, lipids and nucleic acids from the attack of electrophilic compounds which are capable of reacting with their SH- groups (Hayes et al., 1991; Ahluwalia et al., 1996). So the depletion of cellular GSH increase cell vulnerability to oxidative stress (Oyama et al., 2002). The decrease in GSH may be due to the rapid reaction of GSH with formaldehyde to form nucleophilic

adducts (Sogut et al.,2004).In addition,the depletion in GSH content seems to have been caused by methanol metabolism depends on GSH (Abhilash et al., 2011).

Furthermore,The decrease in activities of Gpx and CAT may be due to the action of methanol metabolites such as formaldehyde and free radicals.Formaldehyde readily reacts with the amino acids of soluble proteins leading to hydroxymethyl derivatives and intermolecular bridges in proteins (Lu et al.,2012). Free radicals formed during methanol oxidation can also cause formation of protein peroxides. These alterations may result in denaturation, aggregation and fragmentation of proteins, altering physicochemical properties and potentially losing of enzymatic activities (Skrzydłowska et al., 2000).NAC has antioxidant properties and, as a sulfhydryl donor, contributes to the regeneration of endothelium-derived relaxing factor and glutathione (GSH). Additional protective effects of NAC result from its ability to reduce oxygen radicals produced following oxidative processes. This effect can be shown directly by interfering with the oxidants, up-regulating antioxidant systems such as superoxide dismutase (SOD), or enhancing the catalytic activity of GSH peroxidase (GSH-Px) (Grinberg et al., 2005;Zachwieja et al., 2005;Hosseinjani et al.,2013).Additionally, folic acid exerts antioxidant protection in different cell organelles, both in vitro and in vivo(Joshi et al., 2001).

NAC and folic acid were found to significantly restore the normal concentrations of GSH and catalase activity. Catalase enzyme is a hemoprotein that metabolizes the decomposition of H₂O₂ to form oxygen and water. GSH acts as a non-enzymatic anti-oxidant that reduces the amount of H₂O₂, hydroperoxides and xenobiotic toxicity (Kadiska et al.,2000).

LPO in cellular membranes damages polyunsaturated fatty acids tending to reduce membrane fluidity which is essential for proper functioning of the cell. Thus, the increase in TBARS level observed in this study, which is an index of LPO, indicated kidney cell membrane damage after ASP administration. Moreover, elevation in the concentration of lipid peroxidation in the renal cortex explains the nephrotoxicity of aspartame that probably related with the tissue damage by free radicals resulted from aspartame administration. As, Parthasarathy et al. 2006 and Mourad 2011 reported that aspartame is able to generate free radicals as hydrogen peroxide, hydroxyl radical and superoxide anions in rat renal mitochondria.

The primary metabolic pathway of methanol is oxidation to formaldehyde and formate. This process is accompanied by an elevation of in nicotinamide adenine dinucleotide (NADH) level and the formation of superoxide anion and H₂O. Methanol toxicity is associated with the increased production of oxygen radicals due to mitochondrial damage and increased microsomal proliferation. All of these factors have an essential role in lipid peroxidation. The production of formaldehyde and free radicals during methanol intoxication (3 g/kg) in rats has been found to cause modification of proteins and lipid molecules and the inhibition of GSH-Px and GSH reductase activities (Skrzydłowska and Farbiszewski, 1999). Hemolysis and myoglobinuria are other contributing factors in renal injury following methanol intoxication (Verhelst et al.,2004). NAC with free sulfhydryl groups have been found to directly react with electrophilic compounds such as formaldehyde and free radicals. NAC also diminished lipid peroxidation, elevated the GSH level in the liver and erythrocytes, and increased the activity of GSH-related enzymes in the serum, erythrocytes, and liver after methanol ingestion (Skrzydłowska and Farbiszewski, 1999). Folic acid acts as a methyl donor in a range of metabolic and nervous system, biochemical processes, as well as being necessary for DNA synthesis. Additionally, folic acid can not only scavenge thyl radicals but can also repair these thiols at physiological pH. Moreover, a significant inhibition property in microsomal lipid peroxidation was observed (Gliszczyńska-Świąło, 2007).

A considerable amelioration effect was observed in all estimated parameters after treatment with both antioxidants. So the combination of antioxidants NAC and folic acid determined moderate synergistic effect of kidney damages induced by Aspartame administration.

5. Conclusion

It has been found that the combination of NAC and folic acid was more effective against aspartame-induced kidney dysfunction, hematological changes and oxidative stress.

6. ACKNOWLEDGEMENT

The author is thankful to the authorities of Ain Shams University and Head of Zoology Department, for providing support to the study.

References

- AbdElfatah, A.M., Ghaly, I.S., Hanafy, S.M., 2012. Cytotoxic effect of aspartame (diet sweet) on the histological and genetic structures of female albino rats and their offspring. *Pakistan J. Biol. Sci.* 15 (19), 904-918.
- Abhilash, M., Paul, M.V.S., Arghese, M.V. V., Nair, R.H., 2011. Effect of long term intake of aspartame on antioxidant defense status in liver. *Food Chem. Toxicol.* 49, 1203-1207.
- Abuja, P.M., Albertini, R., 2001. Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. *Clin. Chim. Acta.* 306, 1-17.
- Aebi, H., 1974. Assay of catalase, In "Methods in Enzymology" ed. by L. Packer, Academic Press, New York, 105, 121-27.
- Agarwal, R., Vasavada, N., Saches, N.G., Chase, S., 2004. Oxidative stress and renal injury with intravenous iron in patients with chronic kidney disease. *Kidney Int.* 65, 2279-2289.
- Ahluwalia, P., Tewari, K., Choudhary, P., 1996. Studies on the effects of monosodium glutamate (MSG) on oxidative stress in erythrocytes of adult male mice. *Toxicol. Lett.*, 84, 161-165.
- Al-Ghonaim, M., Pannu, N., 2006. Prevention and treatment of contrast-induced nephropathy. *Tech. Vasc. Interv. Radiol.* 9, 42-49.
- Alleva, R.B., Borghi, L., Santarelli, E., 2011. In vitro effect of aspartame in angiogenesis induction. *Toxicol. In vitro*, 25, 286-293.
- Alsuhailani, E.S., 2010. In vivo cytogenetic studies on aspartame. Comparative and functional genomics. 1- 4.
- Andreatta, M.M., Muñoz, S.E., Lantieri, M.J., Eynard, A.R., Navarro, A., 2008. Artificial sweetener consumption and urinary tract tumors in Cordoba, Argentina. *Preventive Medicine.* 47, 136-139.
- Aruoma, O.I., Halliwell, B., Hoey, B.M., Butler, J., 1989. The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Radic. Biol. Med.* 6, 593-597.
- Baker, M.A., Cermiglia, G.J., Zaman, A., 1990. Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large number of biological samples. *Anal. Biochem.* 190, 360-365.
- Briguori, C., Colombo, A., Violante, P., Balestrieri, F., Manganelli, P., Elia, B., Golia, S., Lepore, G, Riviezzo, P., Scarpato, A., Focaccio, M., Librera, E., Bonizzoni, B., Ricciardelli, B., 2004. Standard vs double dose of N-acetylcysteine to prevent contrast agent associated nephrotoxicity. *Eur. Heart J.* 25, 206-211.
- Chen, N., Aleksa, K., Woodland, C., Rieder, M., Koren, G., 2008. N-Acetylcysteine prevents ifosfamide-induced nephrotoxicity in rats. *Br. J. Pharmacol.* 153(7), 1364-1372.
- Ciappuccini, R., Ansemant, T., Maillefert, J.F., Tavernier, C., Ornetti, P., 2010. Aspartame-induced fibromyalgia, an unusual but curable cause of chronic pain. *Clinical and experimental rheumatology.* 28 (6), 131-133.
- Cuzzocrea, S., Mazzon, E., Dugo, L., Serraino, I., Ciccolo, A., Centorrino, T., De Sarro, A., Caputi, A. P., 2001. Protective effects of n-acetylcysteine on lung injury and red blood cell modification induced by carrageenan in the rat. *The FASEB Journal.* 15 (7), 1187-1200.
- Daly, L.E., Kirke, P.N., Molloy, A., Weir, D.G., Scott, J.M., 1995. Folate levels and neural tube defects. Implications for prevention. *J. Am. Med. Assoc.* 274, 1698-1702.
- Davoli, E., 1986. "Serum Methanol Concentrations in Rats and in Men After a Single Dose of Aspartame," *Food and Chemical Toxicology.* 24(3), 187-189.
- De Caro, L., Ghizzi, A., Costa, R., 1989. Pharmacokinetics and bioavailability of oral acetylcysteine in healthy volunteers. *Arzneim. Forsch.* 39, 382-385.
- Dean, J.A., 1960. In "Flame photometry" 1st ed. Mc-Graw-Hill Book Co. New York.
- Dhalla, N.S., Elmoselhi, A.B., Hata, T., Makino, N., 2000. Status of myocardial antioxidants in ischemia-reperfusion injury. *Cardiovasc. Res.* 47, 446-456.
- Draper, H.H., Hadley, M., 1990. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol.* 186:421-431.

- Duthie, S.J, Narayanan, S., Brand, G.M., Pirie, L., Grant, G., 2002. Impact of folate deficiency on DNA stability. *J. Nutr.* 132,2444S-2449S.
- Ebaid , H., Samir, A.E., Bashandy,I., Alhazza, M.,Rady, A., El-Shehry, S., 2013. Folic acid and melatonin ameliorate carbon tetrachloride-induced hepatic injury, oxidative stress and inflammation in rats. *Nutrition & Metabolism.*10, 20.
- Englund-Ögge, L., Brantsæter, A.L., Haugen, M., Sengpiel, V., Khatibi, A., Myhre, R., Myking, S., Meltzer, H.M., Kacerovsky, M., Nilsen., RM., Jacobsson, B., 2012. Association between intake of artificially sweetened and sugar-sweetened beverages and preterm delivery: a large prospective cohort study. *Am. J. Clin. Nutr.* 96, 552-559.
- Fagherazzi, G., Vilier, A., SaesSartorelli D, Lajous, M., Balkau, B., Clavel-Chapelon, F., 2013. Consumption of artificially and sugar-sweetened beverages and incident type 2 diabetes .*Am.J. clin. nut.* 97(3), 517-23.
- Fawcett, J.K., Scott, J., 1960. Determination of blood urea using the berthelot reaction. *J. Clin. Path.* 13, 156-162.
- Fishbane, S., Durham, J.H., Marzo, K., Rudnick, M., 2004. N-acetylcysteine in the prevention of radiocontrast-induced nephropathy. *J. Am. Soc. Nephrol.* 15, 251–260.
- Gliszczynska-Świgło , A., 2007. Folates as antioxidants.*Food Chemistry .* 101 (4), 1480–1483.
- Goldberg, M.H., 1973. Performance of kits used for clinical chemistry analysis of calcium in serum. *Am. J. Clin. Path.*, 59, 836-845.
- Grinberg, L., Fibach, E., Amer, J., Atlas, D., 2005. N-acetylcysteine amide, a novel cell-permeating thiol, restores cellular glutathione and protects human red blood cells from oxidative stress. *Free RadicBiol Med* 38, 136– 145.
- Hashimoto, K., Takasaki, W., Yamoto, T., Manabe, SI., Tsuda, S., 2008. Effect of glutathione (GSH) depletion on DNA damage and blood chemistry in aged and young rats. *J. Toxicol. Sci.* 33 (4), 421 – 429.
- Hayes, P.C., Bouchier, I.A.D., Beckett, G.J., 1991. Glutathione – S – transferase in humans in health and disease. *Gut*, 32, 813- 818.
- Heibashy, M.I.A., El-Nahla, A.M., Ibrahim, A.I. , Saleh, S.Y.A., 2009. Comparative studybetween dimethyl sulfoxide (DMSO), allopurinol and urate oxidase administration in nephrotoxic rats induced with gentamicin. 43rd Annual Veterinary Medical Symposium, College of Veterinary Medicine Nursing and Allied Health, Tuskegee University, Alabama, USA.
- Hogberg, J., Larson, R. E., Kristoferson, A., Orrenius, S., 1974. NADPH-dependent reductasesolubilised from microsomes by peroxidation and its activity. *Biochem.Biophys. Res. Commun.* 65, 836-842.
- Hosseinjani, H., Moghaddas, A., Khalili, H., 2013. N-acetylcysteine for the prevention of non-contrast media agent-induced kidney injury: from preclinical data to clinical evidence. *Eur. J. Clin. Pharmacol.*13 ,1494-8
- Hwang, S.Y., Siow, Y.L., Au-Yeung, K.K., JOK, H., 2010. Folic acid supplementation inhibits NADPH oxidase-mediated superoxide anion production in the kidney.*Am. J. Physiol. Renal. Physiol.* 300,189-198.
- Ibrahim, M.A., Saleh, A.A., 2012. Comparative study of Quercetin or/and Urate Oxidase against Gentamicin - induced Nephrotoxicity and Oxidative Stress in Rat kidneys .*J.of American Science.* 8, 1-9.
- Joshi, R. , Adhikari, S., Patro, B.S., Chattopadhyay, S., Mukherjee, T., 2001. Free radical scavenging behavior of folic acid: evidence for possible antioxidant activity. *Free Radical Biology and Medicine.*30 (12) , 1390– 1399.
- Kadiska, M.B., Gladen, B.C., Baird, D.D., Dikalov, A.E., Sohal, R.S., Hatch, G.B., Jones, D.P., Mason, R.P., Barret, J.C., 2000. Biomarkers of oxidative stress study: are plasma antioxidants markers of CCl4 poisoning? *J. Free. Rad.Biol. Med.* 28, 838-845.
- Lim, U., Subar, A.F., Mouw, T., Hartge, P. , Morton, L.M., Stolzenberg-Solomon , R., Campbell, D., Hollenbeck, A.R., Schatzkin, A., 2006. Consumption of aspartame-containing beverages and incidence of hematopoietic and brain malignancies.*Cancer Epidemiology, Biomarkers and Prevention.* 15, 1654-1659.
- Lu, K., Gul, H., Upton, P.B., Moeller, B.C., Swenberg, J.A., 2012. Formation of hydroxymethyl DNA adducts in rats orally exposed to stable isotope labeled methanol. *Toxicological Sciences.* 126, 28- 38.
- Manzoor, M., Runcie, J., 1976. Folate-responsive neuropathy: report of 10 cases. *Br. Med. J.* 1, 1176-1178.

- Marielza, R., Martins, I., Azoubel, R., 2007. Effects of Aspartame on Fetal Kidney: A Morphometric and Stereological Study. *Int. J. Morphol.* 25(4), 689-694,
- Mazzon, E., Britti, D., De Sarro, A., 2001. Effect of N-acetylcysteine on gentamicin-mediated nephropathy in rats. *Eur. J. Pharmacol.* 424, 75-83.
- Mishima, K., Baba, A., Matsuo, M., Itoh, Y., Oishi, R., 2006. Protective effect of cyclic AMP against cisplatin-induced nephrotoxicity. *Free Radic. Biol. Med.* 40, 1564-1577.
- Moens, A.L., Champion, H.C., Claeys, M.J., Tavazzi, B., Kaminski, P.M., Wolin, M.S., Borgonjon, D.J., Nassauw, V. L., Haile, A., Zviman, M., Bedja, D., Wuyts, F.L., Elsaesser, R.S., Cos, P., Gabrielson, K.L., Lazzarino, G., Paolocci, N., Timmermans, J.P., Vrints, C.J., Kass, D.A., 2008. High-dose folic acid pretreatment blunts cardiac dysfunction during ischemia coupled to maintenance of high-energy phosphates and reduces postreperfusion injury. *Circulation.* 117, 1810-1819.
- Mourad, I.M., 2011. Effect of aspartame on some oxidative stress parameters in liver and kidney of rats. *African Journal of Pharmacy and Pharmacology.* 5(6), 678-682.
- Nakano, E., Higgins, J.A., Powers, H.J., 2001. Folate protects against oxidative modification of human LDL. *Br. J. Nutr.* 86, 637-639.
- Nguyen, U.N., Dumoulin, G., Henriot, M.T., Regnard, J., 1998. Aspartame ingestion increases urinary calcium, but not oxalate excretion, in healthy subjects. *J. Clin. Endocrinol. Metab.* 83(1), 165-8.
- Nitescu, N., S. E., Ricksten, N., Marcussen, B., Haraldsson, U., Nilsson, S.B., Guron, G., 2006. N-Acetylcysteine attenuates kidney injury in rats subjected to renal ischaemia-reperfusion. *Nephrol. Dial. Transplant.* 21, 1240-1247.
- Odabasi, Z., Karaalp, A., Cermik, H., 2009. Reduction of Amphotericin B-Induced Renal Tubular Apoptosis by N-Acetylcysteine. *Antimicrob. Agents Chemother.* 53 (7), 3100-3102.
- Oyama, Y., Sakai, H., Arata, T., Okano, Y., Akaike, N., Sakai, K., Noda, K., 2002. Cytotoxic effects of methanol, formaldehyde and formate on dissociated rat thymocytes: a possibility of aspartame toxicity. *Cell Biol. Toxicol.* 18(1), 43-50.
- Paradisi, G., Cucinelli, F., Mele, M.C., Barini, A., Lanzone, A., Caruso, A., 2004. Endothelial function in postmenopausal women: effect of folic acid supplementation. *Human Reproduction.* 19(4), 1031-1035.
- Parthasarathy, J.N., Ramasundaram, S.K., Sundaramahalingam, M., Pathinasamy, S.D., 2006. Methanol induced oxidative stress in rat lymphoid organs. *J. Occup. Health.* 48, 20-27.
- Patel, M.B., Deshpande, S., Shah, G., 2011. Evaluation of efficacy of vitamin E and N-acetyl cysteine in gentamicin-induced nephrotoxicity in rats. *Ren. Fail.* 33(3), 341-7.
- Petronilho, F. L., Constantino, L., Souza, B., 2008. Efficacy of the combination of N-acetylcysteine and desferrioxamine in the prevention and treatment of gentamicin-induced acute renal failure in male Wistar rats. *Oxford Journals Medicine Nephrology Dialysis Transplantation.* 24(7), 2077-2082.
- Polat, C., Tokyol, Ç., Kahraman, A., 2006. The effects of desferrioxamine and quercetin on hepatic ischemia-reperfusion induced renal disturbance. *Prostaglandins Leukot Essent Fatty Acids* 74, 379-383.
- Puica, C., Craciun, C., Borsa, M., Rusui, M., Roman, I., Cristescu, M., 2008. Protective effects of bioactive antioxidants complex against aspartame exposure during gestation: biochemical, morphological and ultrastructural studies on new-born rat brain. *Life Sciences Series.* 18, 195-208.
- Rangan, G.K., Wang, Y., Tay, Y.C., 2000. Cytokine gene expression in adriamycin nephropathy: effects of antioxidant nuclear factor kappa B inhibitors in established disease. *Nephron.* 86, 482-490.
- Reiter, R.J., 2000. Melatonin: lowering the high price of free radicals. *News Physiol. Sci.* 15 (5), 246-250.
- Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hapeman, D.G., Hoekstra, W., 1973. Selenium Biochemical roles as a component of glutathione peroxidase. *Science.* 179, 588-590.
- Saito, Y., Nishio, K., Yoshida, Y., Niki, E., 2005. Cytotoxic effect of formaldehyde with free radicals via increment of cellular reactive oxygen species. *Toxicology.* 1 (2-3), 235-45.

- Schaefer, R.M., Teschner, M., Kosch., M., 2002. Folate metabolism in renal failure. *Nephrol Dial Transplant.* 17 (5), 24–27.
- Schernhammer, E.S., Bertrand, K.A., Birmann, B.M., Sampson, L., Willett, W.W., Feskanich, D., 2012. Consumption of artificial sweetener and sugar-containing soda and risk of lymphoma and leukemia in men and women. *Am. J. Clin. Nutr.* 96, 1419-1428.
- Seeling, F., Wust, T., 1969. Modified methodology determination of creatinine. *Z. Ernahrungswiss.* 2(4), 169-176.
- Singh, P., Mann, K.A., Mangat, H.K., Kaur, G., 2003. Prolonged glutamate excitotoxicity: Effects of mitochondrial antioxidants and antioxidant enzymes. *Mol. Cell Biochem.* 243 (1-2), 139-145.
- Skrzydłowska, E., Elas, M., Farbiszewski, R., Roszkowska, A., 2000. Effect of methanol intoxication on free-radical induced protein oxidation. *J. Appl. Toxicol.* 20 (3), 239-243.
- Skrzydłowska, E., Farbiszewski, R., 1999. Protective effect of N-acetylcysteine on reduced glutathione, reduced glutathione-related enzymes and lipid peroxidation in methanol intoxication. *Drug Alcohol Depend* 57, 61–67.
- Smith, J.D., Terpening, C.M., Schmidt, S.O.F., Gums, J.G., 2001. Relief of fibromyalgia symptoms following discontinuation of dietary excitotoxins. *Annals of pharmacotherapy*, 35, 702-706.
- Smith, A.D., Kim, Y.I., Helga Refsum, H., 2008. Is folic acid good for everyone? *Am. J. Clin Nutr.* 87, 517-533.
- Snedecor, G.W., Cochran, W. C. 1982. "Statistical method" 7th Ed. The Low. Univ. press, Ames Iowa, U.S.A.
- Soffritti, M., Belpoggi, F., Manservigi, M., Tibaldi, E., Lauriola, M., Falcioni, L., Bua, L., 2010. Aspartame administered in feed, beginning pre-natally through life span, induces cancers of the liver and lung in male Swiss mice. *Am. J. Ind Med* 53 (12), 1197-206.
- Soffritti, M., Belpoggi, F., Padovani, M., Lauricia, M., DagliSpositi, D., Minardi, F., 2004. Life-time carcinogenicity bioassay of toluene given by stomach tube to Sprague-Dawley rats. *Eur. J. Oncol.* 9, 91:102.
- Sogut, S., Songur, A., Ozen, O.A., Ozyurt, H., Sarsilmaz, M., 2004. Does the sub acute (4 week) exposure to formaldehyde inhalation lead to oxidant /antioxidant imbalance in rat liver. *J. Gen. Med.* 1 (3), 26-32.
- Solini, A., Santini, E., Ferrannini, E., 2006. Effect of short-term folic acid supplementation on insulin sensitivity and inflammatory markers in overweight subjects. *Int. J. Obes. (Lond)* 30, 1197-1202.
- Stanley, L., 2013. Review of data on the food additive aspartame. Supporting Publications 399, 191. European Food Safety Authority.
- Stegink, L.D., Filer, L.J., Bell, E.F., Ziegler, E.E., Tephly, T.R., 1989. Effect of repeated ingestion of aspartame-sweetened beverage on plasma amino acid, blood methanol and blood formate concentrations in normal adults. *Metabolism*, 38, 357-363.
- Tariq, M., Morais, C., Sobki, S., Al Sulaiman, M., Al Khader, A., 1999. N-acetylcysteine attenuates cyclosporin-induced nephrotoxicity in rats. *Nephrol Dial Transplant.* 14, 923–929.
- Trocho, C., Pardo, R., Rafecas, I., Virgili, J., Remesar, X., Fernández-López, J.A., Alemany, M., 1998. "Formaldehyde derived from dietary aspartame binds to tissue components in vivo". *Life sciences* 63 (5), 337–349.
- Tylicki, L., Rutkowski, B., Horl, W.H., 2003. Antioxidants: a possible role in kidney protection. *Kidney Blood Press Res.* 26, 303–314.
- Verhaar, M.C., Stroes, E., Rabelink, T.J., 2002. Foliates and cardiovascular disease. *Arterioscler. Thromb. Vasc. Biol.* 22, 6-13.
- Verhelst, D., Moulin, P., Haufroid, V., Wittebole, X., Jadoul, M., Hantson, P., 2004. Acute renal injury following methanol poisoning: analysis of a case series. *Int. J. Toxicol.* 23:267–273.
- Wani, N.A., Kaur, J., 2012. Adaptive transport of folic acid across renal epithelia in folate-deficient rats. *J. Physiol. Sciences*. 62 (6), 461-468.
- Wani, N.A., Kaur, J., 2012. Adaptive transport of folic acid across renal epithelia in folate-deficient rats. *J. Physiol. Scie.* 62 (6), 461-468
- Xu, H., Zisman, L.A., Coe, L.F., and Elaine M. Worcester, M.L., 2013. Kidney stones: an update on current pharmacological management and future directions. *Expert Opin Pharmacother.* 14(4), 435–447.
- Zachwieja, J., Zaniew, M., Bobkowski, W., Stefaniak, E., Warzywoda, A., Ostalska-Nowicka, D., 2005. Beneficial in vitro effect of N-acetyl-cysteine on oxidative stress and apoptosis. *Pediatr Nephrol* 20, 725–731.
- Zager, R.A., Fuerstenberg, S.M., Baehr, P.H., 1994. An evaluation of antioxidant effects on recovery from ischemic acute renal failure. *J. Am. Soc. Nephrol.* 4, 1588-1597.