

POTENTIAL PROTECTIVE ROLE OF SELENIUM IN ACRYLAMIDE INTOXICATION. A BIOCHEMICAL STUDY

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ABSTRACT. Acrylamide, a neurotoxic and carcinogenic chemical compound is formed by frying, roasting, grilling or baking carbohydrate-rich foods at temperatures above 120°C. Acrylamide intake is associated with significantly altered levels of total cholesterol, LDL-cholesterol, triglycerides, creatinine, urea and uric acid in the blood. This study investigates the effect of selenium (as sodium selenite and as a dietary supplement - Celnium®) on the evolution of some biochemical parameters, in Wistar rats, which received high doses of acrylamide. The administration of sodium selenite and Celnium® produced an improvement in liver cell integrity (decrease in aspartate aminotransferase (AST) activity and iron blood level), liver activities (decrease in direct bilirubin, total bilirubin, total cholesterol, LDL-cholesterol) and renal function (decrease in creatinine, uric acid and urea levels) in the treated groups as compared to the group which received only acrylamide.

Keywords: acrylamide, biochemical parameters, dietary supplement, selenium

INTRODUCTION

Acrylamide (AA), a small but highly reactive molecule, has been used during the last fifty years for the production of polyacrylamide polymers. The first communication-cord regarding the presence of acrylamide in starch rich foods that undergo thermal processes has been made in 2002 by Swedish researchers, producing great concern mainly because of its well known toxicity (Dybing et al., 2003; Elmore et al., 2005; Eriksson, 2005; Mottram et al., 2002). The underlying mechanism of acrylamide formation is not yet known in detail. However, researches on food model systems concluded that the Maillard reaction is the main pathway of formation (Leufven et al., 2003; Friedman, 2003; Pedreschi et al., 2006; Stadler et al., 2002). Based on the available data, food is estimated to have a significant contribution to the total exposure of the general public to acrylamide. Average intake for the general population was estimated to be in the range of 0.3 - 0.88 µg/kg/day. Therefore, the presence of acrylamide in foodstuffs may be an important risk factor regarding the general population health and also a concern for authorities (Grivas et al., 2002; Stadler et al., 2002).

Acrylamide is easily absorbed by the body through all possible entry points: inhalation - cigarette smoking, ingestion - drinking water from flocculent-treated water, eating (sugar containing polyacrylamides used in food refining processes or potato chips, French fries, processed cereals) and through skin absorption (cosmetics) (Shipp et al., 2006).

Epidemiological studies of human industrial and accidental exposures suggest that the nervous system is

the principal site for toxicity in humans. Consequently, the carcinogenic action has been reconfirmed by the experimental researches on laboratory animals and by epidemiologic studies. Further more, acrylamide was found to be a genotoxicant and a reproductive and developmental toxicant. Since acrylamide has been classified as a Group 2A carcinogen by the International Agency for Research on Cancer and a Category 2 carcinogen and Category 2 mutagen by the European Union, this finding caused worldwide concern (IARC, 1994).

The AA metabolite glycidamide has the ability to form deoxyribonucleic acid (DNA) adducts, which account for a genotoxic and cancer risk increasing agent (Törnqvist, 2005). It has been demonstrated that AA is transformed to its metabolite glycidamide through the hemoglobin (Hb) - adducts in both animals and humans. In animals, chronic acrylamide exposure causes neurotoxicity, morphological and biochemical damage in hepatic, renal, nervous and testicular tissues. Different toxic effects of acrylamide might be also caused by an increase in lipid peroxidation in case of disfunctions of endogenous antioxidant defence system. Components of this system such as enzymes (glutathione peroxidase, thioredoxin reductase) and reduced glutathione are known to protect biological systems against oxidative stress (Shrivastava et al., 1983; Tong et al., 2004).

Selenium is an important biological antioxidant, action in which it is involved as a part of glutathione-SH-peroxidase, the selenoenzyme that catalyses reduction of lipid peroxides and of hydrogen peroxide and thus prevents the nocive effects of lipid peroxidation with

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a high content of unsaturated fatty acids and protects erythrocytes from haemolysis. Selenium protects cells and cell membranes from oxidative processes, facilitating reaction between oxygen and hydrogen and ions transfer at membrane level.

Nowadays, there is an increased interest in dietary supplements with antioxidant properties that can reduce the effects of deleterious food constituents. Selenium plays an important biological role in living organisms, mainly through its presence in selenoproteins (such as glutathione peroxidases, thioredoxin reductases, iodothyronine deiodinases) (Moghadaszadeh et al., 2006).

This paper presents preliminary results regarding the protective potential of Selenium (as sodium selenite) and some selenium dietary supplements (Celnium) in Wistar rats in experimental oral intoxication with increased dose of acrylamide.

MATERIALS AND METHODS

Animal treatments

Adult Wistar male rats (Laboratory Animal Center of Cantacuzino Institute of Research, Bucharest, Romania), weighing 180-220 g, were housed in groups of five in plexiglas cages with the floor covered with sawdust. Animals were maintained in a controlled environment (12 hours light/dark cycle with lights on at 07.00, temperature of $20 \pm 1^\circ\text{C}$) before and throughout the experimental period.

The rats had free access to food (standard lab rat chow), except 18 hours before the experiment but tap water *ad libitum*. All experiments involving animals were approved by the University's Committee for Bioethics and animal experimentation according to international rules of conduct. Studies were carried out using 4 groups of 10 Wistar male rats each which received as follows:

- group 1 (control) – saline 0 control group;
- group 2 – acrylamide (50 mg/kg/day), orally, 10 days = AA group;
- group 3 – acrylamide (50 mg/kg/day), orally and sodium selenite 1 mg/kg/day orally, 10 days = AA + Se group;
- group 4- acrylamide (50 mg/kg/day) and Celnium® (dietary supplement containing selenium from *Saccharomyces cerevisiae*) 0.50mg Se/kg/day orally, 10 days = AA + Ce group.

At the end of treatment, rats were fasted for 12 h before being anesthetized and sacrificed.

Blood analysis

Blood samples were collected in Vacuette® tubes without anticoagulant for performing a series of biochemical analysis, in order to assess certain types of pathological alterations:

- a) In order to assess liver cell integrity, by indirectly evaluating the permeability of the hepatocyte membrane, the enzymatic activities of alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) as well as iron blood levels have been determined;
- b) For assessing activities of the liver, blood levels of urea, total bilirubin, direct bilirubin, total cholesterol, LDL-cholesterol and triglycerides have been determined;
- c) For assessing the renal function, urea, creatinine, and uric acid blood levels have been determined.

All the assays were performed using **ELITech kits**.

Our previous studies also included groups of rats which received only sodium selenite or selenium from Celnium® (in above mentioned doses) but there were no significant differences compared to the control group.

The statistical analysis was performed using analysis of variance (ANOVA) one way. Tukey test was used for multiple comparisons (software Stats Direct version 2.6). Results are presented as means $\pm$ standard deviation. A value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSIONS

The data obtained from the experiments are presented in charts, representing the level of the determined parameter for each study group.

Liver cell integrity

Acrylamide administration produced an 12.76 %, respectively 21.56 % increase in AST and ALT activities, as compared to the control group (Figs. 1 and 2). There was a significant decrease (36.64 %) in AST activity in group 3, compared to group 2. In the case of group 4, the decrease in serum AST activity was lower (11.82 %), the enzymatic activity being almost the same as in the case of the control group (Fig. 1). As opposed to AST, the ALT serum activity increased by 10.48 % in group 3 and by 54.03 % in the group 4, compared to group 2 (Fig. 2). ALT is an enzyme present in hepatic cells; AST is also present in hepatic cells as well so as red blood cells, cardiac and skeletal muscle. The increase in aminotransferase activities suggests that acrylamide is involved in liver damage. Due to the significantly increase in ALT activity in group 4 versus group 3, group 2 and control group, these results suggest that sodium selenite and Celnium® supplementation may favor liver cell damage. The data regarding the effect of acrylamide on serum transaminases activities are in accordance with those obtained from other studies. Awad et al. (1998) showed that AA significantly increased AST leakage at 30 min of incubation with 10 mM of AA. Totani et al. (2007) showed that there was a relatively little increase in serum levels of ALT and AST in rats exposed to acrylamide for 12 weeks. Allam A.A. et al. (2010) also found that acrylamide increased serum ALT and AST activities.

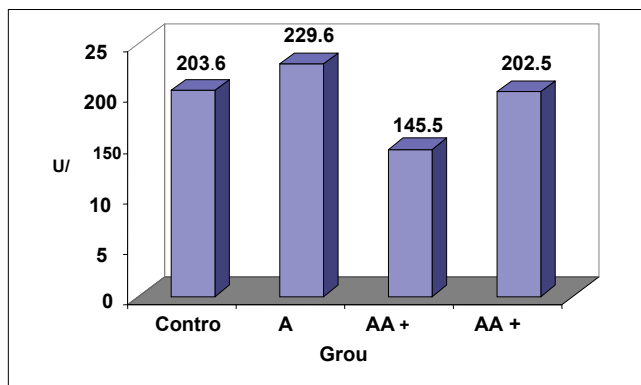


Fig. 1. Blood levels of AST

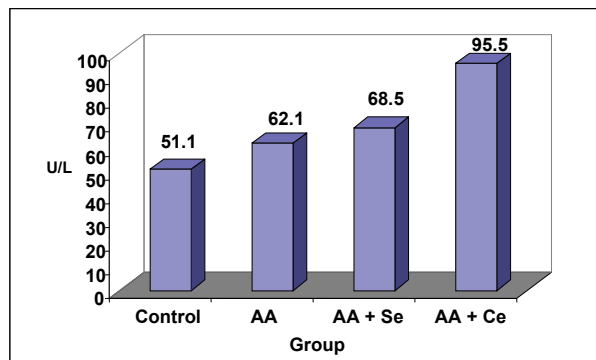


Fig. 2. Blood levels of ALT

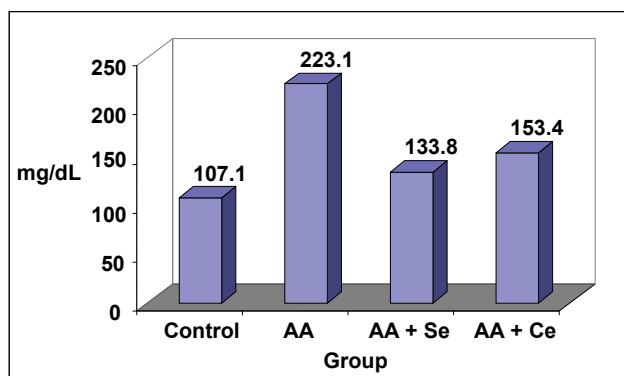


Fig. 3. Blood levels of Iron

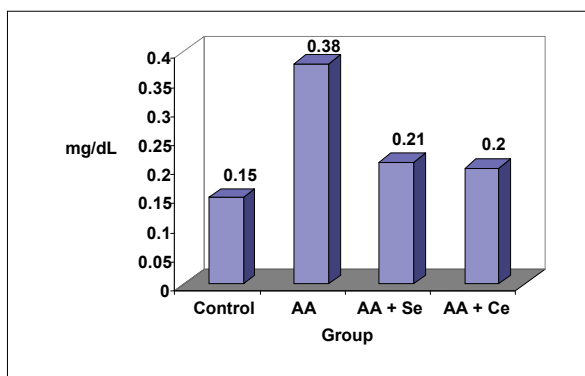


Fig. 4. Blood levels of total bilirubin

Acrylamide produced a significant increase (108.3 %) in iron blood levels, in group 2, compared to the control group. Sodium selenite and Celnium® had a beneficial effect, the blood levels of iron being decreased by 40.02 % in group 3 and by 31.24 % in group 4, compared to group 2 (Fig. 3). As opposed to the results of the present study, Allam A.A. et al. (2010) found that acrylamide produced a significant decrease in serum iron levels in new born Albino rats, but it must be considered that in this case acrylamide was administered during the perinatal period.

Activities of the liver

Acrylamide produced a radical increase (1800 %) in direct bilirubin blood levels, compared to the control group (Fig. 5). A less spectacular increase (153.33 %) was also obtained in the case of total bilirubin (Fig. 4). In the case of total bilirubin, selenium selenite and Celnium® produced a 44.73 %, respectively 47.36 % decrease, compared to group 2 (Fig. 4). Compared to group 2, sodium selenite decreased the direct bilirubin levels by 92.1 %, bringing it almost to the baseline value, recorded in the control group. In group 4 the decrease was less pronounced (65.78 %) (Fig. 6). The liver breaks

down hemoglobin, creating metabolites such as bilirubin and biliverdin. Supplementation with sodium selenite and Celnium shows the protective action on catabolic activities of the liver.

In group 2, blood levels of total cholesterol and LDL-cholesterol were increased by 65.98 %, respectively 19.28 %, compared to the control group. In group 3 the decrease of the total cholesterol level was 34.5 % and that of the LDL-cholesterol level was 17.71 %, compared to group 2. In group 4 the decrease of total cholesterol and LDL-cholesterol levels was less pronounced – 19.95 % and 11.81 %, respectively (Figs. 6, 7).

Acrylamide, sodium selenite and Celnium® had a similar effect on the blood levels of triglycerides (Fig. 8) to that observed in the case of total- and LDL-cholesterol. In group 2, the increase was 14.58 %, reported to the control group. In groups 3 and 4, the decrease was 21.57 % and 15.82 %, respectively, reported to group 2. The liver performs several roles in lipid metabolism, including cholesterol synthesis and the production of triglycerides. The results that were obtained can be explained by acrylamide involvement in lipid peroxidation.

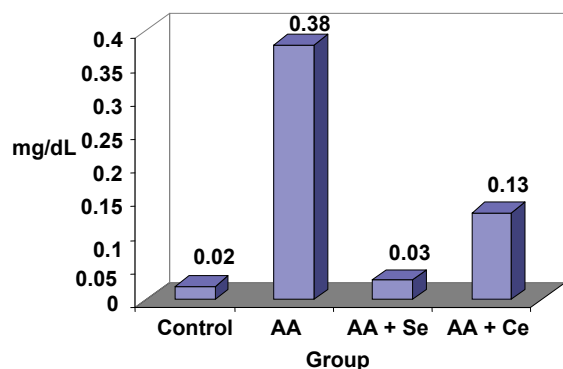


Fig. 5. Blood levels of direct bilirubin

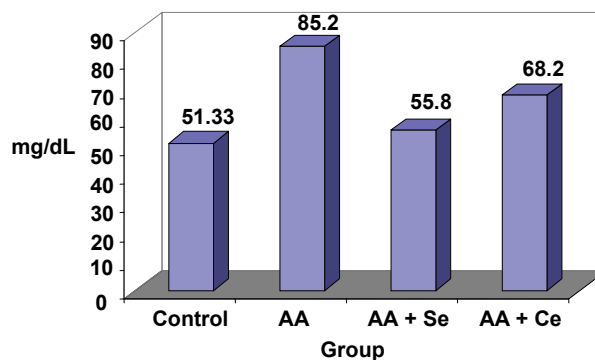


Fig. 6. Blood levels of total cholesterol

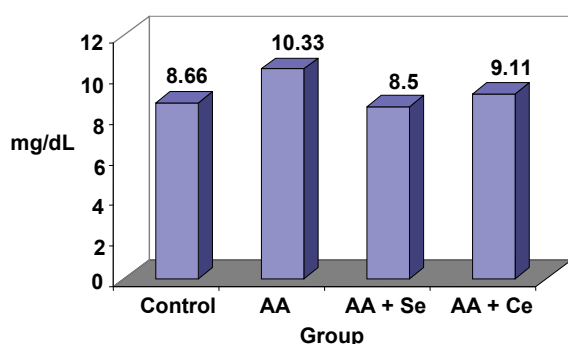


Fig. 7. Blood levels of LDL-Cholesterol

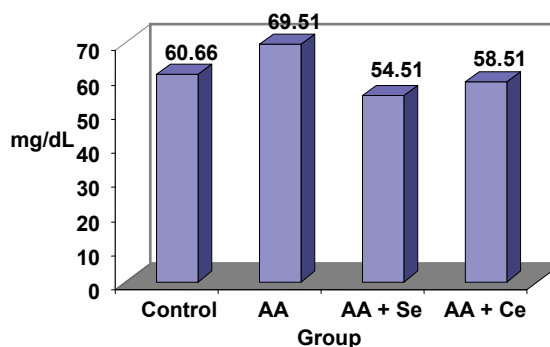


Fig. 8. Blood levels of triglycerides

The data obtained from this study regarding the influence of acrylamide on serum total cholesterol, LDL-cholesterol and triglycerides levels come partially in contradiction to those obtained by Totani et al. (2007), who observed a decrease in the levels of these biochemical parameters after trace acrylamide exposure. A possible explanation could reside in the different toxicodynamic mechanisms, due to the dose difference. At the level of exposure described by Totani et al., (2007), a decrease in insulin level takes place, which indirectly can lead to a decrease in triglyceride levels and eventually cholesterol; the capacity of the liver to process circulating lipoproteins was probably not affected seriously at that dose, unlike the dose of 50 mg/kg/day used in this experiment, which leads to certain hepatic damage.

Renal function

Clinical parameters for the renal function are urea and creatinine; a secondary parameter for assessing the renal function is uric acid. Acrylamide induced a 91.42 % increase in creatinine blood levels, compared to the control group. Both sodium selenite and Celnium® brought the creatinine almost to baseline levels, a 49.25 %, respectively 41.79 % decrease being observed, reported to group 2 (Fig. 9). Acrylamide had a similar effect on uric acid levels to that on creatinine levels, but less significant, the increase being of 35.11 %, compared to the control group.

In group 3 and 4, uric acid levels decreased by 42.73% and 11.45%, respectively, compared to group 2 (Fig. 10).

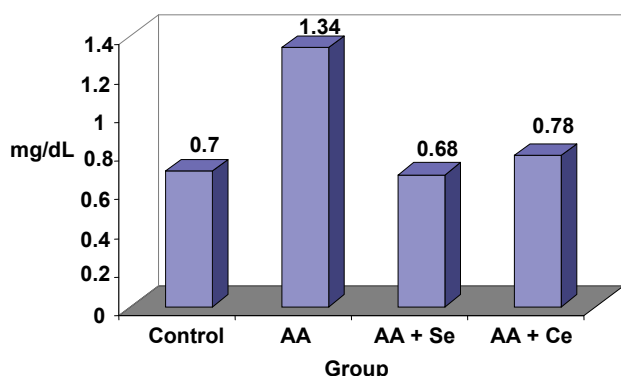


Fig. 9. Blood levels of creatinine

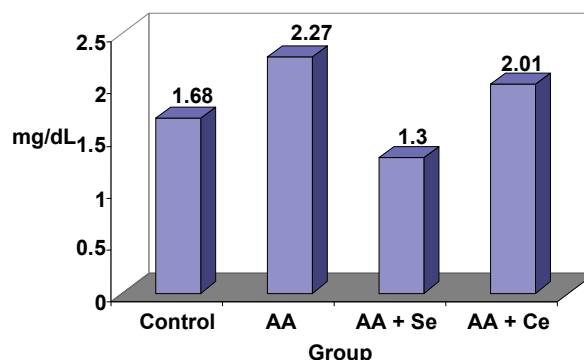


Fig. 10. Blood levels of uric acid

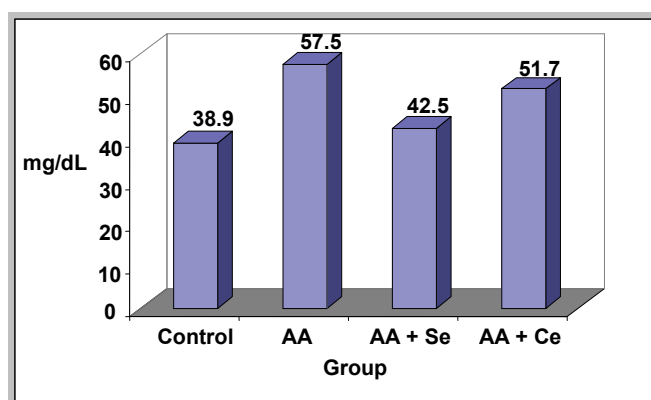


Fig. 11. Blood levels of urea

Kesik et al. (2008) also showed that the supplementation with selenium contributed to the decrease of uric acid levels.

Urea blood levels were 47.81 % higher in group 2, compared to the control group. Sodium selenite and Celnium® produced a 26.8 %, respectively 10.08 % decrease in urea blood levels, compared to group 2 (Fig. 11). These data suggest that sodium selenite and Celnium® had a protective effect on the renal function.

CONCLUSIONS

The results show that a 50 mg/kg acrylamide intake in laboratory animals (Wistar rats) resulted in severe perturbation of some biochemical parameters. The simultaneous intake of selenium as sodium selenite or as a dietary supplement (Celnium) partially prevented some of the biochemical changes in rats which received high doses of acrylamide, evidencing its beneficial role in case of acrylamide intoxication.

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