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Research Article

Evaluation of sublethal cyanide exposure on plasma biochemical profile in rats and possible protective effect of garlic

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Abstract. Three groups of adult male rats (six per group) were used in order to evaluate the effect of prolonged sublethal exposure to potassium cyanide (KCN) on some circulating biochemical indices, and the possible ameliorating effect of garlic on the considered indices. Cyanide administration caused significant ($p<0.05$) increases in plasma levels of alanine aminotransferase, alkaline phosphatase and urea. On the other hand, plasma levels of aspartate aminotransferase, lactate dehydrogenase, glucose, triglyceride, cholesterol, albumin, total protein, bilirubin and creatinine were not significantly different between the experimental and control groups. The garlic administration modulated the cyanide-induced biochemical alterations, this effect being associated with its organosulfur compounds. Considering both antioxidant properties of garlic organosulfur compounds and their possible ability to elevate the cellular sulfane sulfur level, we suggest garlic as a possible candidate for therapeutic and prophylactic intervention of cyanide poisoning.

Key Words: cyanide poisoning, garlic, plasma biochemistry..

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Introduction

Cyanide is a ubiquitous and potent cytotoxic agent known for its rapid lethal action and toxicity. The sources for cyanide poison are diverse, ranging from fruit pits, nuts, or seeds to industrial-based materials, such as those used in metal processing, electroplating, rubber and plastic production, insecticide and rodenticide production, chemical synthesis, and extraction of gold and silver ores. Furthermore, some drugs of medicinal importance, such as Laetrile and Nitroprusside, can release cyanide (Ellenhorn 1997).

The toxicity of cyanide is a consequence of its high potency as a respiratory poison in all aerobic forms of life. In addition to acute cyanide intoxication, chronic toxicity has frequently been reported in recent years, and it is suggested that the most widespread problems arising from cyanide are from chronic dietary, industrial and environmental sources (Mathangi & Namasivayam 2000). Cyanide has also been shown to induce oxidative stress and damage in a number of biological systems (Okolie & Iroanya 2003; Okolie & Asonye 2004). It has been reported that prolonged sublethal cyanide exposure can cause biochemical and histopathological alterations in different species (Okolie & Osagie 2000; Sousa *et al* 2002; Soto-Blanco & Gorniak 2003; Tulsawani et al 2005).

Several limitations of commonly available cyanide antidotes (e.g. sodium nitrite, 4-dimethyl aminophenol, sodium thiosulphate, dicobalt edetate, etc.) prompted research for better treatments by new mechanistic based antidotes (Baskin *et al* 1999; Bhattacharya & Tulsawani 2009; Satpute *et al* 2010). The conversion of cyanide to thiocyanate is considered to be the main pathway for cyanide detoxification, and is catalysed directly by two sulfurtransferases, rhodanese and 3-mercaptopyruvate sulfurtransferase (Megarbane 2003; Nagahara *et al* 2003). Rhodanese activity level in catalyzing the transformation of thiosulphate to thiocyanate is limited by the availability of sulfur (Bhatt & Linnell 1987). Experimental sulfur donors have been shown to protect mice against exposure to lethal dose of cyanide (Baskin *et al* 1999). It has been suggested that sulfur compounds of garlic (Allium sativum) may have a protective effect against cyanide intoxication (Aslani *et al* 2006; Elsaid & Elkomy 2006).

With respect to serious problems resulted from long-term ingestion of low amounts of cyanide, it is important to carefully assess the effects of sublethal doses of cyanide as well as to identify suitable compounds with potential for protecting against resultant tissue damages. So, the present study was undertaken to assess plasma biochemical alterations following sublethal cyanide exposure and also to evaluate the possible protective effect of garlic in cyanide exposed rats. These studies may be of value for further understanding the pathophysiology of cyanide poisoning and as an aid in diagnosis and supportive therapy of long-term exposure to cyanide.

Materials and methods

Preparation of garlic powder

Garlic bulbs were purchased from a local market in Mashhad, Iran. The cloves were peeled, sliced and dried in the oven at 50 °C, then ground to become powder.

Experimental design and sampling

18 male Wistar rats weighing approximately 180 g were divided randomly into three groups of 6 each. Rats were housed in clean cages at room temperature (22-25 °C) and a photoperiod of 12 h light/12 h dark per day. Animals received standard laboratory balanced commercial diet *ad libitum*. Group I rats received basal diet and tap water throughout the experiment and served as the control. Rats in groups II and III were received tap water containing 200 mg L^{-1} inorganic cyanide in the form of potassium cyanide (KCN, Merck, Germany). Indeed, rats in group II were fed basal diet, while those of group III were fed basal diet supplemented with 5% garlic powder. The experiment was approved by the Animal Welfare Committee of the School of the Ferdowsi University of Mashhad.

At the end of the experimental period (6 weeks), the animals were starved overnight for 12h before the blood was collected. Rats were anaesthetized with ether and venous blood samples were collected by cardiac puncture into vials containing EDTA. Blood plasma separation was done by centrifugation at 750 g for 20 min. The samples were centrifuged at 750×g for 20 min, and then the plasma was pipetted into different aliquots and stored at −70°C until analysis.

Biochemical assays and analysis

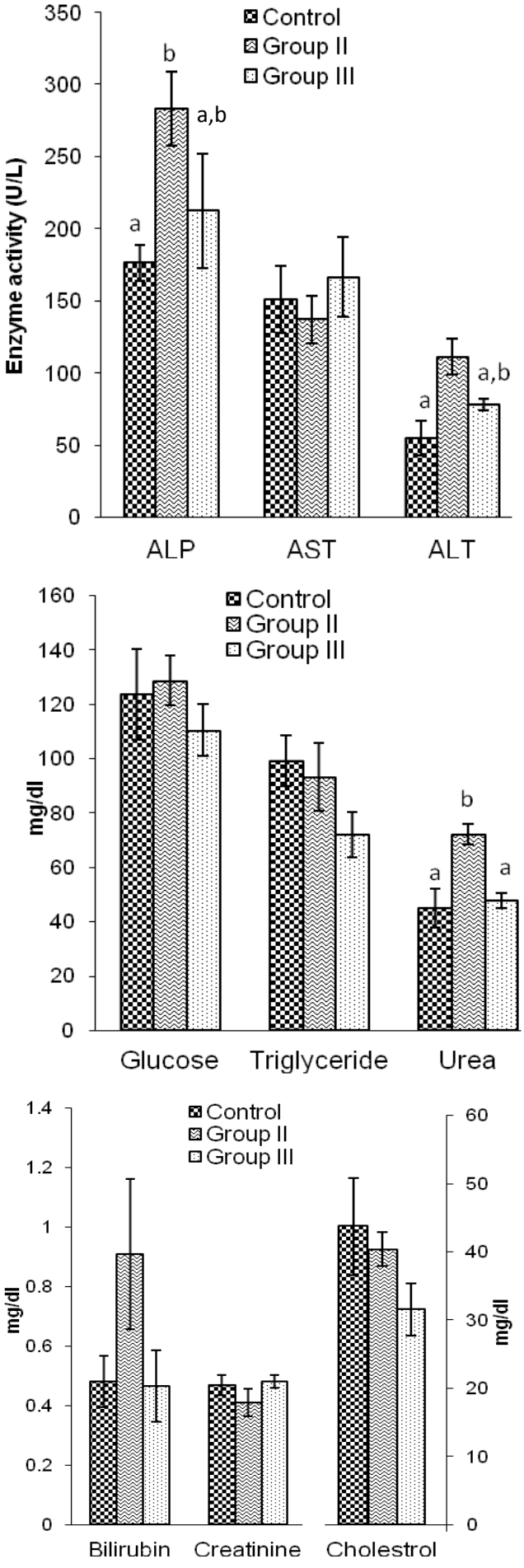
Plasma biochemical analysis including glucose, triglyceride, cholesterol, total protein, albumin, creatinine, urea, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) were done using commercial colorimetric kits (Pars Azmoon, Iran).

Statistical Analysis

All experimental values have been represented as mean ± standard error of mean (SEM). The obtained data were analyzed using one-way analysis of variance followed by Bonferroni's multiple comparisons test. The level of significance was set at $P < 0.05$. All calculations were performed using SPSS/PC software.

Results

The effects of KCN and garlic powder administration on certain plasma biochemical parameters of rats are shown in figure 1. Cyanide exposure caused a significant ($P < 0.05$) increase in the ALT and ALP activities of rats in group II as compared to control values. Moreover, plasma urea concentration in rats from group II was significantly higher than those from controls. On the other hand, the plasma levels of AST, LDH, glucose, triglyceride, cholesterol, albumin, total protein, bilirubin and creatinine were not significantly different between experimental and control groups. Moreover, garlic administration in group III could prevent the cyanide-induced increases in plasma values of ALP, ALT and urea and kept their values almost close to the normal control group.



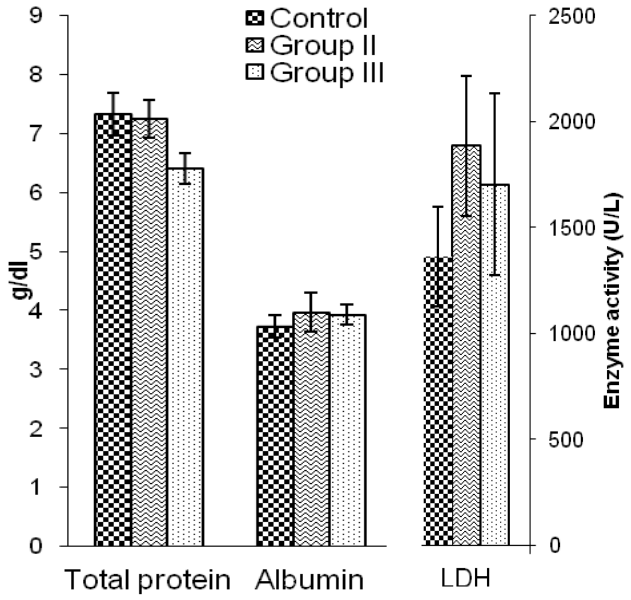


Fig 1. Plasma biochemical parameters in different groups after a 6-week experiment. Data are mean ± SEM of six rats in each group. Values with no common superscript differ significantly ($P<0.05$). Group II: received potassium cyanide; Group III: received potassium cyanide and garlic powder. AST aspartate aminotransferase, ALT alanine aminotransferase, ALP alkaline phosphatase, LDH lactate dehydrogenase.

Discussion

In the present study, ALT and ALP levels were found to be increased following cyanide exposure. In agreement with this finding, significant increases in serum ALP and ALT activities in addition to histopathological derangements in liver, lung and kidney tissues has been reported following chronic cyanide intoxication in rabbits (Okolie & Iroanya 2003). Moreover, increased serum activities of ALP (Okolie & Osagie 2000) and ALT (Elsaid & Elkomy 2006; Manzano *et al* 2007) following sublethal cyanide poisoning has been also reported in some animal species. On the other hand, and as the preset results show, cyanide treatment caused no significant alteration in LDH activity that is different from previously reported results (Okolie & Osagie 2000; Okolie & Iroanya 2003) indicating increased LDH activity due to cyanide intoxication.

Cyanide is also known to alter glucose metabolism (Way 1984). There are some works that report diabetes as a toxic effect produced by ingesting cassava, a cyanogenic plant, in various species (Kamalu 1991; Geldof *et al* 1992; Akanji & Famuyiwa 1993; Petersen 2002). Based on the present results, cyanide exposure caused no significant alteration in glucose concentration that is similar to the previously reported results in goats, rats and rabbits (Okolie & Osagie 2000; Soto-Blanco *et al* 2002; Soto-Blanco & Gorniak 2003). However, increased blood glucose concentration due to cyanide poisoning has been reported in swine and rats (Jackson 1988; Tulsawani *et al* 2005).

Elevation of serum urea and creatinine is usually associated with impairment of renal function (Kumer *et al* 1988). Based on the present study results, increased urea concentration was observed following cyanide exposure. This finding is to some extent in line with those reported in rats (Elsaid & Elkomy 2006) and pigs (Manzano et al 2007), where sublethal cyanide exposure caused significant elevation of serum urea and creatinine concentrations. It has been also reported that degenerative changes in the kidney sections of the cyanide-fed rabbits may be responsible for the significant increases in serum urea and creatinine observed in these animals (Okolie & Osagie 2000). However, the results of Tulsawani *et al* (2005) showed no significant change in the levels of blood urea and creatinine following cyanide exposure (7.0 mg/kg for 14 days) in rats. As mentioned above, some discrepancies exist in the literature concerning the effects of sublethal cyanide poisoning on the biochemical parameters that might be attributed to differences in species, tissue type, utilized dose, exposure time, experimental procedures or other unknown factors.

The increased plasma levels of ALT, ALP and urea in the present study would indicate possible hepatotoxic and nephrotoxic effects of cyanide exposure in rats that is reminiscent of previously reported pathological and biochemical findings in several animal species exposed to cyanide (Okolie & Osagie 2000; Sousa *et al* 2002; Okolie & Iroanya 2003; Tulsawani *et al* 2005). As the results show, the observed changes were mitigated in the rats treated with garlic (group III) to the levels that were not significantly different from control group, which indicates that the garlic powder has capability of providing some protection against cyanide induced tissue damage.

Conclusions

In summary, the results of the present study indicate that cyanide exposure caused a significant increase in the plasma levels of ALT, ALP and urea and garlic supplementation was effective in mitigating resultant alterations. These results suggest that garlic might have some therapeutic and prophylactic effects on cyanide poisoning. However, with respect to the fact that the metabolism of cyanide and its main metabolite, thiocyanate, is species-linked, and toxicokinetic parameters of cyanide compounds vary in different species further research might be needed using other species. Indeed, more studies are required to elucidate the molecular basis of the ameliorative properties of garlic in cyanide poisoning.

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