Investigation of the effect of N-acetylcysteine on aluminum phosphide toxicity in rats

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Abstract

Aluminum Phosphide (ALP) is one of the most dangerous pesticides. When it comes into contact with water, it emits Phosphine (PH_3) gas, which causes poisoning and death in many people. The purpose of this study is to look into the role of N-acetylcysteine in the treatment of aluminum phosphide toxicity in rats. In this study, 30 male Wistar rats were fed with aluminum phosphide orally. After 15 minutes, N-acetylcysteine was administered intraperitoneally. The antioxidant enzymes glutathione S-Transferase (GST), Superoxide Dismutase (SOD), Catalase (CAT), glutathione (GSH), Aspartate Aminotransferase (AST), Alanine Transaminase (ALT), and Alkaline Phosphatase (ALK) were studied in blood plasma. CAT, GST, and GSH concentrations in plasma, liver, and kidneys of rats infected with aluminum phosphide decreased, while AST, ALT, and ALK concentrations increased. The levels of all enzymes studied approached normal after N-acetylcysteine administration, and the rats survived for up to 12-15 hours. According to the findings of this study, N-acetylcysteine at a dose of 10 mg/kg improves hepatic manifestations and prevents liver necrosis, so it can be considered a potential therapeutic agent in the treatment of this poisoning.

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Introduction

One of the most hazardous pesticides is Aluminum Phosphide (ALP), which is also known as rice tablets in Iran.[1] This chemical, which was first presented as a perfect pesticide in 1973, is now used in the form of yellow or dark gray crystals under the brand names pesticide, insecticide, and rodenticide.[2] Humans cannot absorb this substance through their skin, so oral ingestion and inhalation are the two main ways it can poison an individual. Additionally, the main mechanism of poisoning by this substance is the release of the gas Phosphine (PH_3), which is created when aluminum phosphide comes into contact with water. As ALP is easily absorbed from the gastrointestinal tract and lung epithelium, the severity of poisoning is high at both gastrointestinal and respiratory level.[3] The electron transfer chain, which is inhibited by this gas, ultimately causes cellular respiration to cease. These modifications cause the production of free radicals, which can harm organs. Because phosphine gas directly affects the myocardium, the major lethal effect of aluminum phosphide typically manifests as a cardiovascular disorder and arrhythmia.[4] Phosphine gas has a density of 1.52 grams per liter, a molecular weight of 34, is colorless, flammable, and smells like rotting fish. The presence of impurities like diphosphine (P_2H_4), methane, arsenic, phosphine, and hydrogen released from it is what gives it its odor. ALP poisoning is associated with a high death rate in the first 24-48 hours[4] and there is no known antidote for treatment. ALP poisoning, in general, is among the most frequent causes of poisoning-related deaths in many nations, particularly developing nations.[5,6] The substance was initially marketed in India, where it results in about 15,000 accidental or intentional poisonings annually, of which two-thirds are fatal. As a result of the high prevalence of ALP use in Iran, this drug accounts for 18.6 to 24% of all deaths from intentional poisoning, which frequently involve suicidal intent and young age groups.[7] N-acetylcysteine (NAC) is a dietary and pharmaceutical supplement used to treat acute acetaminophen poisoning and as a mucolytic agent. This substance is well-

known for its liver-protective qualities and antioxidant capacities.[8,9] The disulfide bonds in mucosa and fluids are broken by acetylcysteine, making it easier for sputum to leave the airways.

The aim of this study was to investigate the effect of NAC on the toxicity induced by ALP in rats.

Materials and Methods

This is an experimental study conducted during 2019. The chemicals used in this study, such as ALP and NAC, were prepared and provided by Sigma Company.

Animals used in research

In this study, 30 male Wistar rats weighing 200-250 g were used and divided into 3 experimental groups.

Group 1 (control group) consisted of 10 rats; to group 2, 10 mg/kg of ALP (n=10) were administered; to group 3, 10 mg/kg of ALP and 10 mg/kg of NAC 15 minutes after ALP (n=10) were administered. ALP was administered orally by gavage to the rats and, after 15 minutes, NAC was administered by intraperitoneal injection. Then 5 rats from each group were anesthetized for investigating the biochemical tests and the rest of the rats were kept for survival time.

Blood collection and tissue preparation

The rats were anesthetized with ether. Liver and kidney tissues of the rats were removed and transferred to liquid nitrogen. Blood samples were also taken from the rats' hearts. Blood samples were collected from the rats and poured into tubes containing heparin as an anticoagulant and immediately placed in ice. Plasma of the blood samples was obtained by the standard method using a centrifuge at 2500 rpm for 20 minutes.[10] Plasma was used for the activity of antioxidant enzymes glutathione S-transferase (GST), Superoxide Dismutase (SOD), Catalase (CAT). The rats' livers and kidneys were washed with cold saline.

Tissues were carefully weighed and homogenized in phosphate buffered saline in a ratio of 1:10. The samples were then centrifuged at 14000 g at 4°C for 15 minutes. The supernatant was used to measure the biochemical parameters.

Aspartate aminotransferase (AST), Alanine Transaminase (ALT) and Alkaline phosphatase (ALK) enzymes activity in tissues and plasma was measured according to the standard protocol.[11] GST, SOD, glutathione (GSH) and CAT were also determined and compared with the control group.

Results

Laboratory findings

The results of chemical study on plasma of the infected rats showed that ALP increased the activity of AST, ALT, and ALK in the rats' plasma and livers compared to the control group. However, the effect of NAC on the rats treated with ALP reduced the concentrations of AST, ALT, and ALK in the rats' plasma and livers to the normal level, which is fully described in Table 1.

The results also showed that the concentrations of CAT, GST, and GSH in plasma, kidneys, and livers of the rats treated with ALP decreased, while the activity of SOD did not change much, but the use of NAC increased the concentrations of CAT, GST, and GSH in plasma, kidneys, and livers of the rats treated with ALP to the normal range (Table 2).

Survival time

In group 2 ALP, behavioral changes were observed in the rats after ALP gavage. The animals showed the first signs after 15-20 minutes: increased activity and anxiety, followed by a gradual decrease in activity, and finally very decreased activity, occasional standing posture and eventually death. The average survival time of the animals was 50-65 minutes. But in group 3 ALP + NAC, on average, the animals survived 12-15 hours after contact with the drug.

Discussion

The current study was designed to look into the effect of N-acetylcysteine on ALP toxicity in rats. ALP poisoning is one of the deadliest types of poisoning in Iran, with an increase in recent years, and despite the ban on its use, some farmers still use it to store crops.[12] The majority of reported pesticide poisonings are intentional and with the intent to commit suicide.[13] ALP works by releasing the poisonous gas phosphine. Unfortunately, no specific antidote for the treatment of phosphine poisoning has been identified; thus, poisoning prevention and aggressive and correct treatment, particularly in the early hours after consumption, play a critical role in patient outcomes.[14]

Chugh *et al.*[15] reported that in the presence of ALP, cellular superoxide and peroxide radicals are produced, with subsequent cellular damage due to lipid peroxidation. Oxidative degradation of lipids, known as lipid peroxidation, and other oxidative mechanisms damage biological macromolecules, especially cell membranes, and ultimately lead to cell death. The mechanism of phosphine-induced

Table 1. Mean values of ALT, AST, and ALK enzymes in the studied groups (SD reported in brackets).

Enzyme	Control	Experimental groups Aluminum phosphide	Aluminum phosphide + NAC
Plasma (U/mL)			
ALT	64.24(3.76)	78.52 (1.45)	68.21 (3.01)
AST	123.09 (3.80)	141.71 (4.44)	129.48 (5.43)
ALK	557.47 (7.66)	613.21 (3.73)	561.21 (6.62)
Liver (U/mg tissue)			
ALT	94.44 (7.37)	109.49 (3.34)	96.45 (3.21)
AS	T151.65 (3.23)	170.41 (2.65)	156.06 (4.66)
ALK	169.09 (4.16)	191.03 (3.94)	174.04 (3.97)

lipid peroxidation may include ROS due to inhibition of cellular respiration or a direct reaction between phosphine and H_2O_2. A direct relationship with mortality based on SOD, CAT, and malondialdehyde has been observed in postmortem studies in patients poisoned with ALP.[16] Rahbar Taramsari *et al.* in a study on changes in liver enzymes in ALP poisoning in patients admitted to Rasht hospital in 2008-2009 mentioned an increase in liver enzymes in one third of the subjects.[17] Also, other studies mentioned changes in liver enzymes in up to two-thirds or more in the subjects.[18]

While in this study it was found that ALP reduces GST, CAT, and GSH in plasma, liver, and kidney, and increases SOD and liver enzymes and also the activity of AST, ALT, and ALK in rat plasma and liver.

The liver is one of the most important organs in the human body for phosphine poisoning. After ingestion, phosphine gas is rapidly absorbed in the gastrointestinal tract and partially transported to the liver via the portal vein.

It has also been shown that administration of NAC reduces the toxic effects of ALP. Some studies have reported that phosphine causes cytotoxicity by inhibiting SOD activity and by affecting cellular antioxidants.[18]

NAC is regarded as one of the most important antioxidants and free radical-fighting agents. It boosts intracellular glutathione, which is one of the cell's oxidative stress defense systems. In this study, it was discovered that combining NAC with ALP prevents an increase in the activity of liver enzymes, indicating a protective effect on the membrane.[19]

Azad *et al.* demonstrated in an experimental study that using NAC in animals poisoned with ALP had a statistically significant effect on reducing heart disorders. This treatment has also reduced recovery time. Furthermore, there was a decrease in malondialdehyde levels and an increase in glutathione peroxidase.[12]

In a clinical study conducted by Tehrani *et al.*, it was shown that the intravenous administration of NAC in patients with acute ALP poisoning significantly reduces oxidative stress indices in patients' serum and reduces hospitalization time.[20]

Chugh *et al.*[15] discovered malondialdehyde as an indicator of lipid peroxidation and that SOD activity, as a criterion for evaluating the antioxidant system in the serum of patients with ALP poisoning, increases in the first and second days after the onset of intoxication, with the more severe the intoxication, the greater the difference. The researchers concluded that increased serum malondialdehyde levels and SOD activity were linked to the severity of intoxication. According to these and other new clinical studies, antioxidant-based therapies are one of the new strategies for treating ALP poisoning.

Male white rats were used in a study by Moghadam Nia *et al.* to demonstrate the effect of various treatments on ALP-poisoned rats. Sodium selenite has no effect on mortality or death time. It does, however, improve pathological findings like lung and liver complications. NAC postponed death and significantly improved liver complications. Vitamin C, on the other hand, delayed death, while magnesium sulfate did not alter survival rate in ALP-poisoned rats.[21]

At 10 mg/kg, NAC improves liver manifestations and prevents liver necrosis. This finding has also been demonstrated in other studies. NAC could also postpone death by up to 13813 minutes.[21] The death time in our study was delayed by 12 hours. It has been proposed that NAC could be used as an effective treatment in ALP-poisoned patients.[22,23]

Conclusions

Although NAC administration delayed death in the treatment group compared to the control group, death occurred in all rats at various time intervals after administration; however, given the improvement in laboratory values evaluated and the survival time of NAC-treated rats, it can be considered as a potential treatment for ALP poisoning. However, more research on the dose and frequency of NAC administration is required before it can be used as the standard treatment for this poisoning.

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Table 2. Mean values of CAT, GST, SOD and GSH concentrations in plasma, kidneys and livers of the studied rats (SD reported in brackets).

Parameter	Experimental groups		
	Control	Aluminum phosphide	Aluminum phosphide + NAC
Plasma			
GST (µmol/hr/mL)	168.75 (3.14)	147.26 (2.90)	165.40 (3.45)
GSH (µmol/ mg tissue)	181.03 (2.32)	168.55(2.35)	174.25 (2.95)
SOD (U/ml)	0.83 (0.06)	0.78 (0.03)	0.85 (0.04)
CAT (µmolH_2O_2/min/mL)	24.36 (2.53)	19.21 (0.54)	21.80 (0.56)
Liver			
GST (µmol/hr/mg)	146.14 (3.86)	133.62(3.10)	148.09 (2.34)
GSH (µmol/ mg tissue)	427.30 (3.76)	394.40 (2.66)	426.59 (3.50)
SOD (U/mg protein)	7.54 (0.47)	7.25 (0.37)	7.66 (0.52)
CAT (µmolH_2O_2/min/mg protein)	7.15 (0.31)	6.13 (0.29)	6.77 (0.47)
Kidney			
GST (µmol/hr/mg protein)	391.13 (3.15)	355.42 (4.51)	381.80 (5.12)
GSH (µmol/ mg tissue)	144.47 (4.03)	135.58 (4.22)	142.20 (4.46)
SOD (U/mg protein)	8.05 (0.75)	7.87 (0.60)	7.91 (0.57)
CAT (µmolH_2O_2/min/mg protein)	48.05 (3.91)	35.13 (4.78)	46.68 (4.60)

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