Herbal Medicines Journal **2020; 5(3):113-8**
DOI: 10.22087/herb%20med%20j.v5i3.783 **ISSN:** 2538-2144

Original Article

Effects of Allium Hirtifolium Hydroalcoholic Extract and N-acetylcysteine on the Oxidative Toxic Stress Biomarkers in Aluminum Phosphide and Paraquat Toxicity in Rats

Hassan Ghasemi[1], Seyed Mostafa Hosseini Zijoud[2], Nejat Khiripour[3], Omid Heidari Shayesteh[4], Amir Nili-Ahmadabadi[4], Akram Ranjbar[4,5]*

[1]Abadan School of Medical Sciences, Abadan, Iran
[2]Department of Biochemistry, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.
[3]Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran
[4]Medical Plants and Natural Products Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
[5]Nutrition Health Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

Received: 30.12.2019; Accepted: 13.10.2021

Abstract

Background and Aim: It has been indicated that aluminum phosphide (ALP) and paraquat (PQ) poisoning generate oxidative stress through the production of free radicals. The aim of the present study was to examine the effect of hydro alcoholic extract of Persian shallot and N-acetyl cysteine (NAC) against induced oxidative stress by PQ and ALP.

Materials and Methods: Adult male Wister rats were divided into ten groups of 7. Animals received ALP and PQ alone and also in combination with NAC and the hydro alcoholic extract of Persian shallot. After twenty-four hours of treatment, the animals were scarified and their blood as well as liver samples were collected. Then, alanine aminotransferase (ALT), aspartate aminotransferase (AST), catalase (CAT) activity, as well as total antioxidant capacity (TAC) and lipid peroxidation (LPO) were measured.

Results: The PQ and ALP significantly increased ALT and AST leakage compared to the control group ($P<0.05$). The TAC of serum and liver remarkably increased in the groups treated with NAC and hydro alcoholic extracts of Persian shallot in comparison with the control group ($P < 0.05$).

Conclusion: As a natural antioxidant, the hydro alcoholic extract of Persian shallot can counteract with oxidative stress induced by ALP and PQ to ameliorate liver function.

Keywords: Persian Shallot, Oxidative stress, N-acetyl cysteine, Aluminum Phosphide, Paraquat

***Corresponding Author:** Akram Ranjbar, Hamadan University of Medical Sciences, Hamadan, 6517838678. Iran. Tel/Fax: (+98) 813 8380031. Email: akranjbar2015@gmail.com.

Please cite this article as: Ghasemi H, Hosseini Zijoud SM, Khiripour N, Heidari Shayesteh O, Nili-Ahmadabadi A, Ranjbar Subchronic A. Effects of Allium Hirtifolium Hydroalcoholic Extract and N-acetylcysteine on the Oxidative Toxic Stress Biomarkers in Aluminum Phosphide and Paraquat Toxicity in Rats. Herb. Med. J. 2020; 5(3):113-8.

Introduction

As a metal phosphide, aluminum phosphide (ALP) is frequently used throughout the world due to its protective effects in grain storage (1). Following the contact of the ALP with moisture environments such as gastric hydrochloric acid, phosphine gas (PH3) is released. Phosphine could cause competitive inhibition of cytochrome c oxidase enzyme in the mitochondrial respiratory chain (2). Inhibition of mitochondrial

respiratory chain leads to incomplete reduction of molecular oxygen and subsequently super oxide radical generation which causes lipid peroxidation and oxidative stress (3).

Paraquat (PQ) is a herbicide which is extensively used in agriculture (4). Exposure to PQ and its absorption through airway and systemic route leads to PQ accumulation in lung and other organs that result in pulmonary edema, bronchial and alveolar damage, and eventually lung fibrosis. PQ toxicity mechanism include radical chain reaction through its quaternary ammonium nitrogen atoms and bipyridyl ring that lead to reactive oxygen species (ROS) generation and oxidative stress (4). According to obtained results, PQ poisoning is one of the fatal poisoning in emergency ward.

Antioxidant system plays a significant role in preventing the formation of free radicals and their harmful effects in the oxidative stress condition. This system is divided into three sub-categories, including enzymatic antioxidants (glutathione peroxidase, catalase and superoxide dismutase), small molecules (such as glutathione) and proteins (such as albumin) (5). Given that ALP and PQ poisoning pathogenesis are based on oxidative stress induction, prescription of antioxidant compound is one of the main strategies in the treatment of ALP and PQ poisoning (6).

N-acetyl cysteine (NAC) is an amino acid derivative that has antioxidant properties due to direct removal of free radicals and also restoration of the active form of glutathione (7). Previous studies demonstrated that reduced thiol moiety in NAC structure can scavenge free radicals and thereby counteract with oxidative stress (8). Persian shallot or *Allium hirtifolium* is a plant species that could be found in Iran. According to previous reports, this plant has antioxidant properties (9). Antioxidant property of Persian shallot is mainly due to a flavonoid known as quercetin (10). Since the natural compounds have fewer side effects compared to synthetic forms, the use of natural products in treatment of various diseases by patients has been increasing. Due to the high rate of aluminum phosphide and paraquat poisoning and lack of any report associated with the role of Persian shallot in improving this condition, this study examined the impacts of the hydro alcoholic extract of Persian shallot compared to NAC on oxidative stress parameters induced by ALP and PQ acute poisoning.

Materials and Methods

Chemicals

We purchased trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), and 2,4,6-tripyridyl-s-triazine (TPTZ) from the Sigma Chemical Co. (United States). AST and ALT kits used in the present study were purchased from Pars Azmun Co. (Iran), NAC (Avicenna, Iran) and PQ (Ariashimi, Iran). All other chemicals were puchased from the Sigma.

Preparation of Persian Shallot Hydro Alcoholic Extract

One hundred g of fresh and dried Persian shallot whose genus and species were confirmed by the Department of Botany, Bu-Ali Sina University, Hamedan-Iran, was well powdered and then mixed with 400 ml distilled water/ethanol (25/75). The prepared solution was incubated for 48h and then filtered using a filter paper and a buchner funnel.The filtered solution was well evaporated, dried and converted to dry powder using a rotator evaporator. The obtained dry powder was used for preparing other concentrations (11).

Animals and Treatments

Seventy adult male Wistar rats weighing 180-250g were recruited in the present study. The animals were kept on a 12-hour light/dark cycle with free access to tap water and standard laboratory chow. Then animals were randomly divided into 10 group (n=7):

Group1: healthy controls received normal saline.

Group2: received aluminum phosphide (2 mg/kg).

Group3: received 50mg/kg of paraquat

Group 4: received N-acetyl cysteine (6.25 mg/kg).

Group 5: received 100mg/kg of the hydro alcoholic extract of Persian shallot

Group 6: received 300mg/kg of the hydro alcoholic extract of Persian shallot

Group 7: received 2 mg/kg of aluminum phosphide and 300mg/kg of the hydro alcoholic extract of Persian shallot

Group8: received 2 mg/kg of aluminum phosphide and 100mg/kg of the Persian shallot hydro alcoholic extract

Group9: received 50mg/kg of paraquat and 100mg/kg

of the hydro alcoholic extract of Persian shallot

Group10: received 50mg/kg of paraquat and 300mg/kg of the hydro alcoholic extract of Persian shallot

Treatment in all the groups was carried out by oral gavage**.** Twenty-four hours after the treatment, the rats were anesthetized with ketamine and then were sacrificed. Then blood and liver samples were collected. The animals in the treatment groups were approved by the Research Committee of Hamedan University of Medical Sciences, Iran.

Biochemical Analysis

Lipid Peroxidation Measurement

Lipid peroxidation level was measured using colorimetric methods. In this method, during the acid heating condition, malondialdehyde – as a lipid peroxidation byproduct- reacts with TBA and produces a pink complex that can absorb light in 532nm. We used the calibration curve of tetraethoxypropane standard solutions to measure the concentrations of TBA + MDA adducts in the samples (12).

Total Antioxidant Capacity Assay

Total antioxidant capacity was measured using the ferric reducing ability (FRAP) method. This method is based on Fe^{3+} to Fe^{2+} reduction in the presence of TPTZ reagent. In the next step, Fe^{2+} and TPTZ form a blue color complex with maximum absorbance in 593 nm (13).

Catalase (CAT) Measurement Activity

Catalase activity measurement of the samples was based on the decrease of absorbance in the reaction medium containing H_2O_2 (10mM) and sodium phosphate buffer (50 mM, pH. 7.0) at 240nm. One unit of enzyme activity is considered as the amount of enzyme needed to degrade 1 mol H2O2 as a substrate within one minute (14).

ALT and AST Activity Assay

ALT and AST activity were measured using Pars Azmun kit, Tehran, Iran.

Statistical Analysis

Statistical analysis was conducted using SPSS version 16. Quantitative data were expressed as Mean ± SD. One-way ANOVA followed by Tukey post hoc test was used for the comparison of variables mean value between the studied groups. P values < 0.05 were considered to be significant. The research project was approved by the ethical committee of the Hamadan University of Medical Sciences (ethical approval No. 9209052832).

Results and Discussion

Effect of Treatment on Serum Biochemical Parameters

The results of serum biochemical parameter measurement have been summarized in Table1. ALP and PQ remarkably increased ALT and AST activities in comparison with the control group ($p < 0.05$). Co-administration of ALP with 100mg/kg of the hydro alcoholic extract of Persian shallot also significantly increased AST activity compared to the control. TAC in the groups treated with NAC, 300mg/kg of the hydro alcoholic extract of Persian shallot and co-administration of ALP with 100mg/kg of the hydro alcoholic extract of Persian shallot, compared to the control group, significantly increased ($p < 0.05$). On the other hand, TAC level in the group treated with NAC swas ignificantly higher than in the groups treated with ALP and PQ ($p < 0.05$). Co-administration of ALP with 300mg/kg of the hydro alcoholic extract of Persian shallot and PQ with 100mg/kg of the hydro alcoholic extract of Persian shallot caused a significant decrease in TAC compared the group treated with NAC ($p < 0.05$).

Effect of Treatment on Liver Biochemical Parameters

Table 2 shows the level of liver biochemical parameters. NAC and 300mg/kg of the hydro alcoholic extract of Persian shallot significantly increased TAC in comparison with the control group ($p < 0.05$). The TAC level in the groups treated with ALP and PQ also significantly decreased compared to the group treated with NAC ($p < 0.05$). Co-administration of ALP and PQ with the hydro alcoholic extract of Persian shallot caused a significant decrease in TAC level compared to the group treated with NAC ($p < 0.05$).

The ALP and PQ poisoning induce tissue damages mainly by the induction of oxidative stress. Therefore, the use of antioxidant compounds can be effective in modulating the damage caused by these poisoning. Accordingly, our study examined the NAC and hydro alcoholic extract of Persian shallot effects on the oxidative stress induced by ALP and PQ poisoning.

The present study demonstrated that ALP and PQ

Table 1: The effects of treatments on serum biochemical parameters in rats.

Groups	ALT (U/L)	AST (U/L)	TAC (µmol/ml)	LPO (nmol/ml)	CAT (U/ml)
Control	20.4 ± 6.4	33.7 ± 6.4	1.73 ± 0.1	3.2 ± 0.1	1.29 ± 0.4
ALP 2 mg/kg	43.8 ± 6.4*	63.1 ± 10.2*	1.33 ± 0.1**	6.1 ± 0.1	1.64 ± 0.4
PQ 50 mg/kg	43.3 ± 6.7*	68.9 ± 8.8*	1.41 ± 0.1**	5.6 ± 0.2	1.21 ± 0.2
NAC 6.25 mg/kg	23.9 ± 3.8	48.4 ± 6.7	4.78 ± 0.6*	4.6 ± 0.1	1.40 ± 0.6
PS 100 mg/kg	34.6 ± 3.2	51.05 ± 8.9	3.70 ± 0.2	1.0 ± 0.5	9.60 ± 4.2
PS 300 mg/kg	33.1 ± 2.5	52.4 ± 8.7	4.60 ± 0.2*	1.2 ± 0.03	2.50 ± 0.9
ALP + PS 100 mg/kg	43.1 ± 7.5	68.4 ± 6.7*	3.60 ± 0.4*	8.1 ± 0.1	4.01 ± 1.5
ALP + PS 300 mg/kg	32.7 ± 5.5	66.9 ± 12.5	1.07 ± 0.2**	4.0 ± 0.09	3.50 ± 2
PQ + PS 100 mg/kg	40.8 ± 10.4	59.7 ± 8.7	2.31 ± 0.4**	3.4 ± 0.2	1.43±0.6
PQ + PS 300 mg/kg	33.3 ± 5.4	51.78 ± 8.08	3.21 ± 0.2	3.8 ± 0.1	1.88 ± 0.6

Data are presented as mean ± SD.ALP: Aluminium phosphide, PQ: Paraquat, PS: Persian shallot, ALT: Alanin aminotransferase, AST: Aspartate aminotransferase, TAC: total antioxidant capacity, LPO: Lipid peroxidation, CAT: Catalase.* significantly different compared with control group (P< 0.05). ** Significantly different compared with NAC group (P< 0.05).

could induce oxidative stress in the liver and increase liver enzyme leakage. On the other hand, it was shown that Persian shallot hydro alcoholic extract and NAC modulate oxidative stress parameters probably through increasing the TAC.

Based on the proposed mechanism, ALP increased the free radicals concentration and induced oxidative stress through mitochondrial respiratory chain inhibition that caused multi-organ damage (15). Moreover, as an herbicide, the PQ produced reactive oxygen species (ROS) and caused oxidative stress through its ability to undergo redox-cycling (16). Since the liver has a central role in detoxification, induced oxidative stress and subsequently liver damage by PQ and ALP are not unexpected. Increased activity of the ALT and AST in the present study also confirmed the hypothesis that PQ and ALP induce liver damage. Consistent with our results, another study by Mathai *et al*. demonstrated that ALP poisoning increased ALT and AST leakage (17). The study conducted by Louriz *et al*. also showed that ALP poisoning could significantly increase liver enzyme activity (18). In agreement with our results, Pasha *et al*. showed that oxidative stress induced by acute paraquat poisoning could increase liver damage and liver enzymes leakage (19).

On the other hand, the present study indicated that NAC and Persian shallot hydro alcoholic extract counteract with oxidative stress and improve liver

Table 2: The effects of treatments on liver biochemical parameters in rats.

Groups	TAC (µmol/ml)	LPO (nmol/ml)	CAT (U/ml)
Control	2.23 ± 0.4	1 ± 0.07	4.5 ± 1.7
ALP 2 mg/kg	1.52 ± 0.1**	1.6 ± 0.4	7.3 ± 4.9
PQ 50 mg/kg	1.72 ± 0.2**	2.4 ± 0.1	6.8 ± 3.3
NAC 6.25 mg/kg	6.01 ± 0.9*	0.6 ± 0.03	4.8 ± 0.76
PS 100 mg/kg	4.22 ± 0.8	0.2 ± 0.08	6.4 ± 2.24
PS 300 mg/kg	5.97 ± 0.7*	0.1 ± 0.01	3.9 ± 1.9
ALP + PS 100 mg/kg	2.74 ± 0.4**	0.5 ± 0.02	2.03 ± 0.6
ALP + PS 300 mg/kg	2.51 ± 0.2**	0.1 ± 0.09	2.67 ± 0.44
PQ + PS 100 mg/kg	2.22 ± 0.9**	0.3 ± 0.01	10.93 ± 9.1
PQ + PS 300 mg/kg	1.77 ± 0.4**	0.8 ± 0.03	6.39 ± 1.75

Data are presented as mean ± SD.ALP: Aluminium phosphide, PQ: Paraquat, PS: Persian shallot, TAC: total antioxidant capacity, LPO: Lipid peroxidation, CAT: Catalase.* significantly different compared with control group (P< 0.05). ** Significantly different compared with NAC group (P< 0.05).

function. Our finding is supported by the results of the study carried out by Tehrani *et al*. which showed that NAC counteract with harmful effects of oxidative stress by ALP poisoning (5). Another study conducted by Oghabian *et al*. revealed that NAC could modulate oxidative stress and improve acute liver failure induced by zinc phosphide (20). Indeed, the antioxidant properties of NAC are associated with its ability in GSH biosynthesis facilitating by extracellular cysteine reduction to cysteine and stimulating GSH synthesis by providing sulfhydryl (-SH) groups and subsequently enhancing glutathione-S-transferase activity (21, 22). These findings suggested that NAC counteract with ALP and PQ poisoning probably by TAC strengthening.

Another interesting point in this study was the capability of the hydro alcoholic extract of Persian shallot in reducing the liver injury induced by PQ and ALP poisoning by increasing the TAC. Indeed, the non-significant difference in the TAC of the NAC treated group compared to the hydro alcoholic extract of Persian shallot treated group indicated that Persian shallot has a remarkable therapeutic potential. Although there is not any data about the effect of the Persian shallot hydro alcoholic extract on ALP and PQ poisoning, other studies about Persian shallot confirmed our results. Ramaiah *et al*. demonstrated that the hydro alcoholic extract of Persian shallot could improve oxidative stress caused by STZ in diabetic rats and significantly reduce the liver enzymes leakage (23). The results of the study conducted by Hosseini *et al*. revealed that in oxidative stress induced by alloxan, the hydro alcoholic extract of Persian shallot could decrease the serum concentration of ALT and AST (24). Persian shallot has some antioxidant compounds in its structure such as phenolic and sulfur groups (9). These compounds increase antioxidant enzymes activity and thereby protect the cells against depletion of reduced glutathione. On the other hand, polyphenolic compounds and flavonoids are able to scavenge free radicals and neutralize their harmful effects (25). The results of this study showed that the hydro alcoholic extract of Persian shallot and NAC could improve the oxidative stress induced by PQ and ALP, mainly through strengthening the total antioxidant capacity.

Conclusion

As a natural antioxidant, the hydro alcoholic extract of Persian shallot can counteract with oxidative stress induced by ALP and PQ to ameliorate liver function.

Acknowledgment

This project was supported by a dissertation grant from Hamadan University of Medical Sciences for a student thesis (No: 9209052832).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Singh Y, Joshi SC, Satyawali V, Gupta A. Acute aluminium phosphide poisoning, what is new? Egypt J Intern Med. 2014;26(3):99.
2. Batra K, Taneja O, Khemani L. Acute oral toxicity of aluminum phosphide in male albino rats (Wistar). Bull Environ Contam Toxicol. 1994;52(5):662-6.
3. Chugh S, Arora V, Sharma A, Chugh K. Free radical scavengers & lipid peroxidation in acute aluminium phosphide poisoning. Indian J Med Res. 1996;104:190-3.
4. He X, Wang L, Szklarz G, Bi Y, Ma Q. Resveratrol inhibits paraquat-induced oxidative stress and fibrogenic response by activating the nuclear factor erythroid 2-related factor 2 pathway. J Pharmacol Exp Ther. 2012;342(1):81-90.
5. Tehrani H, Halvaie Z, Shadnia S, Soltaninejad K, Abdollahi M. Protective effects of N-acetylcysteine on aluminum phosphide-induced oxidative stress in acute human poisoning. Clinical Toxicology. 2013;51(1):23-8.
6. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaiee A. Pesticides and oxidative stress: a review. Medical Science Monitor. 2004;10(6):RA141-RA7.
7. Lukaszewicz-Hussain A. Role of oxidative stress in organophosphate insecticide toxicity–Short review. Pestic Biochem Physiol. 2010;98(2):145-50.
8. Pereira-Filho G, Ferreira C, Schwengber A, Marroni C, Zettler C, Marroni N. Role of N-acetylcysteine on fibrosis and oxidative stress in cirrhotic rats. Arq Gastroenterol. 2008;45(2):156-62.
9. Javad H, Seyed-Mostafa H-z, Farhad O, Mehdi M, Ebrahim AO, Nader RG, et al. Hepatoprotective effects of hydroalcoholic extract of Allium hirtifolium (Persian shallot) in diabetic rats. J Basic Clin Physiol Pharmacol. 2012.
10. Moradi Y, Moradi-Sardareh H, Ghasemi H, Mohamadi N, Moradi M-N, Hosseini-Zijoud S-M. Medicinal properties of Persian shallot. European Journal of Experimental Biology. 2013;3(1):371-9.
11. Mahmoodi M, Hosseini-Zijoud S-M, Arababadi MK, Khorramdelazad H, Moradi-Sardareh H, Moradi Y, et al. Effect of Persian shallot (Allium hirtifolium Boiss.) extract on glucokinase (GCK), glycogen phosphorylase and phosphoenolpyruvate carboxykinase (PEPCK) genes expression in diabetic rats. Afr J Pharm Pharmacol. 2013;7(7):389-96.
12. Zarrinkalam E, Ranjbar K, Salehi I, Kheiripour N, Komaki A.

Resistance training and hawthorn extract ameliorate cognitive deficits in streptozotocin-induced diabetic rats. Biomed Pharmacother. 2018;97:503-10.
13. Zanganeh N, Siahpoushi E, Kheiripour N, Kazemi S, Goodarzi MT, Alikhani MY. Brucellosis Causes Alteration in Trace Elements and Oxidative Stress Factors. Biol Trace Elem Res. 2017. 1-5. doi: 10.1007/s12011-017-1102-3. [Epub ahead of print]. 2017.
14. Johansson LH, Borg LH. A spectrophotometric method for determination of catalase activity in small tissue samples. Anal Biochem. 1988;174(1):331-6.
15. Moghadamnia AA. An update on toxicology of aluminum phosphide. DARU. 2012;20(1):25.
16. Gawarammana IB, Buckley NA. Medical management of paraquat ingestion. British journal of clinical pharmacology. 2011;72(5):745-57.
17. Mathai A, Bhanu MS. Acute aluminium phosphide poisoning: Can we predict mortality? Indian J Anaesth. 2010;54(4):302.
18. Louriz M, Dendane T, Abidi K, Madani N, Abouqal R, Zeggwagh A. Prognostic factors of acute aluminum phosphide poisoning. Indian journal of medical sciences. 2009.
19. Pasha SA, Prabodh S, Rao N, Suhasini T, Sripad DV, Chowdary N. Role of Liver Function Tests and Serum Lactate Levels in Predicting the Severity of Acute Paraquat Poisoning. Int J Sci Study. 2015;3(8).
20. Oghabian Z, Afshar A, Rahimi HR. Hepatotoxicity due to zinc phosphide poisoning in two patients: role of N- acetylcysteine. Clin Case Rep. 2016;4(8):768-72.
21. Issels RD, Nagele A, Eckert K-G, Wllmanns W. Promotion of cystine uptake and its utilization for glutathione biosynthesis induced by cysteamine and N-acetylcysteine. Biochem Pharmacol. 1988;37(5):881-8.
22. Nakata K, Kawase M, Ogino S, Kinoshita C, Murata H, Sakaue T, et al. Effects of age on levels of cysteine, glutathione and related enzyme activities in livers of mice and rats and an attempt to replenish hepatic glutathione level of mouse with cysteine derivatives. Mech Ageing Dev. 1996;90(3):195-207.
23. Ramaiah SK. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. Food and chemical toxicology. 2007;45(9):1551-7.
24. Kazemi S, Asgary S, Moshtaghian J, Rafieian M, Adelnia A, Shamsi F. Liver-protective effects of hydroalcoholic extract of Allium hirtifolium Boiss. In rats with alloxan-induced di-abetes mellitus. ARYA Atheroscler. 2010;6(1):11-5.
25. Leelarungrayub N, Chanarat N, Rattanapanone V. Potential activity of Thai shallot (Allium Ascalonicum L.) extract on the prevention of hemolysis and glutathione depletion in human erythrocyte from oxidative stress. CMU J. 2004;3(3):225-34.