



## The protective effect of *Aloe vera* on paraquat hepatotoxicity

Morteza Pourahmad<sup>1,2,3</sup>, Hossein Kargar Jahromi<sup>1,3\*</sup>, Zahra Kargar Jahromi<sup>3</sup>,  
Mohammad Hadi Sameni<sup>1</sup>, Hassanali Abedi<sup>1</sup>, Zahra Khabbaz Kherameh<sup>4</sup>,  
Sara Mohammadi<sup>3</sup> and Marzieh Alsadat Hosseini<sup>3</sup>

<sup>1</sup>Research Center for Noncommunicable Diseases, Faculty of Medicine, Jahrom University of Medical Sciences, Jahrom, IR Iran

<sup>2</sup>Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, IR Iran

<sup>3</sup>Zoonoses Research Center, Jahrom University of Medical Sciences, Jahrom, IR Iran

<sup>4</sup>Young Researchers and Elite Club, Islamic Azad University, Jahrom Branch, Jahrom, Iran

Corresponding Email: [hossein.kargarjahromy@yahoo.com](mailto:hossein.kargarjahromy@yahoo.com)

---

### ABSTRACT

Paraquat is a common herbicide that is used in agriculture, and Liver damage is a severe complication after its consumption in mammals, which can be prevented by antioxidants. With due attention to the various nutritional materials and antioxidants in aloe vera (a medicinal plant), evaluation of its effect on prevention of paraquat induced hepatotoxicity was the goal of this study. In this experimental study, 72 Wistar male rats were randomly divided into control, sham, and 7 experimental groups. In experimental groups paraquat with various doses of aloe vera is given to the mice for 2 weeks. After that liver enzymes and histologic changes were compared in different groups. In first experimental group (in which paraquat alone was administered), aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) concentrations were significantly elevated in compare to the control and sham groups ( $p < 0.05$ ). In addition histologic destruction was significant in this group. On the other hand, in experimental groups in which paraquat were administered plus aloe vera liver enzymes were significantly low. In addition histologic structure was preserved in groups in which aloe vera was administered in compare with paraquat alone group. In this study; we concluded that, Aloe vera extract, probably due to its strong antioxidant property, could reduce the destructive effects of Paraquat on the rat liver.

**Keywords:** Aloe vera, Paraquat, liver, rats

---

### INTRODUCTION

Nowadays, in modern agriculture many compounds such as pesticides, herbicides and various kinds of fertilizers are used for more and better crop. These materials, while of benefit, cause some problems. Paraquat (one of the herbicides of the bipyridylum group) is an example of these compounds.

Paraquat (with the trade name of Gramoxone) is a nonpersistent herbicide in a dark green liquid formulation that is used as a non-selective contact herbicide in non-agricultural lands, roadsides, fallow land, and orchards. It effectively controls all one-year-old grasses and broadleaf weeds and bedbugs in alfalfa fields <sup>[1]</sup>.

paraquat free radicals after re-oxidation by oxygen has toxic effect on green plants. These products interfere with photosynthesis in plants and cause fade it <sup>[1]</sup>.

On the other hand this toxin can induce cytotoxicity in different organs in human and animals. This toxin can cause toxic effect on lungs, kidneys, myocardium, adrenal glands and liver <sup>[2]</sup>. When it given in acute dose (50 mg/kg) in mice, liver inflammation and necrosis will develop <sup>[3]</sup>. It seems that this toxicity is associated with the production of superoxide anion that can result in substantial production of reactive oxygen species such as hydrogen peroxide and highly toxic peroxy nitrte anion <sup>[4]</sup>. Toxicity with this poison cause disruption of important NADPH requiring biochemical processes <sup>[5]</sup>. Because of availability of this poison in human environment, many cases of acute poisoning and death have been reported over the past few decades <sup>[5]</sup>. In addition there are some report of hepatotoxicity after dermal absorption of paraquat in human <sup>[6]</sup>.

Currently we have no true pharmacological antagonist for this poison, therefore management of paraquat poisoning are directed toward the use of antioxidants <sup>[5]</sup>.

Aloe vera is one of the oldest and most-widely used medicinal plants in the world. This plant belongs to the class Liliopsida, order Asparagales, and family Aloaceae, and there are more than 250 species of it in the world. Aloe vera, which is a shrub with thick and fleshy leaves containing jelly-like transparent, and edible substances, is a native plant of tropical regions, and grows in autumn <sup>[7]</sup>.

This plant is rich in various antioxidants and vitamins including vitamins E, C, B6, B2, B1, and folic acid <sup>[8]</sup>. With due attention to these antioxidants and vitamins it seems that this plant may be useful for management of paraquat poisoning especially its hepatotoxic effects by destroying the free radicals. So we conducted this study to evaluate its protective effect on paraquat hepatotoxicity in male rats.

## MATERIALS AND METHODS

This experimental study is conducted in Jahrom University of Medical sciences.

### 2.1 Extract preparation method

The standard soaking and percolation method was used to prepare Aloe vera extract. Five hundred grams of fresh leaves were ground and soaked for 24 hours in a container with 96% ethanol. The filtered solution was centrifuged at 3000 rpm for 5 minutes to make sure suspended particles were removed from the plant material. The obtained dry extract was water soluble <sup>[9]</sup>.

### 2.2 Animals and their grouping

All ethical points regarding treatment with laboratory animals were considered in this research. Seventy two Wistar male rats with mean weight of 180-200g were used in this study. They were kept for a week in the laboratory animal breeding room of Jahrom University of Medical Sciences to be adapted to the environment. During the research, the rats were kept under 12 h light/12 h dark conditions at 20-25 C° and had free access to food and water. Every day at 10 AM, Paraquat was injected intraperitoneally into the rats at 2 mg/kg B.W. using insulin syringes [10]. Immediately after the injections, the rats were given the extract by gavage. Both Paraquat and the extract were soluble in distilled water.

The rats were randomly divided into nine experimental groups, each of which contained 8 rats. The groups were as follows:

Control group: Mice did not receive any substance and they lived normally as said above.

Sham group: Mice were given distilled water at the volume of 1ml/mice via an intragastric tube daily all the time of study (2 weeks). In addition 0.2 cc of distilled water also was injected intraperitoneally in them daily.

Experimental group 1: Mice were given paraquat 2 mg /kg of body weight, intraperitoneally daily all the time of study (2 weeks).

Experimental groups 2,3 and 4: Mice were given the low, moderate, and high doses of the Aloe vera extract (50, 100, and 200 mg /kg/day, respectively) at the volume of 1ml/mice via an intragastric tube daily all the time of study (2 weeks).

Experimental groups 5,6 and 7: Mice were given the low, moderate, and high doses of the Aloe vera extract (50, 100, and 200 mg /kg/day, respectively) at the volume of 1ml/mice via an intragastric tube daily all the time of study (2 weeks). In addition 2mg/kg/day of paraquat also was injected intraperitoneally in them daily (for 2 weeks).

**2.3 Taking Blood Samples and Doing Biochemical Investigations**

On the last day of the research, the rats were weighed, blood samples were taken directly from their hearts using 5 cc syringes (after the rats were anesthetized with diethyl ether), the samples were centrifuged at 3000 rpm for 15 minutes, and the sera were collected and kept in a freezer at -20 C° until they were tested. Biochemical assessment kits (made in Iran) using the colorimetric method and an autoanalyzer machine (Selectera XL model made in Holland) were used for measuring biochemical factors including ALT, AST, ALP, total protein, and albumin.

**2.4 Tissue studies**

After applying the treatments for 14 days, the rats were anesthetized on the 15<sup>th</sup> day using the standard method, and their liver tissues were quickly removed and put in containers with 10% formalin. After 48 hours, the tissues were taken out of the solution and, following tissue passage, 5µ thick paraffin-embedded tissue sections were prepared in the form of serial arrays. Ten cross-sections including five sections selected from each liver were stained employing the H&E and PAS methods, and were studied using a light microscope. Fifty microscopic fields (magnification 10x40) were randomly selected and pathological changes in the livers were studied. This included disruption in the radial orientation of hepatocytes, polycythemia, varicocele, necrosis of hepatocytes, Kupffer cell aggregation, infiltration of inflammatory cells, and changes in the portal space.

**2.5 Statistical analysis**

SPSS 21 was used for statistical calculations, and the selected level of significance was p<0.05. The data was calculated and compared in the form of Mean± SEM in the section on results.

**RESULTS**

There was no significant difference in the level of liver enzymes (ALT, AST and ALP ) between the control, sham and the experimental groups 2,3 and 4 (in which different doses of the Aloe vera extract were given).

The concentrations of these enzymes were significantly increased in experimental group 1 (in which mice were given paraquat alone) compared to the control and sham groups (P<0.05).

The concentrations of liver enzymes were significantly low in experimental groups 5,6 and 7 (in which paraquat and Aloe vera extract were given) in compared to the experimental group 1 (in which mice were given paraquat alone) (P<0.05). table (1)

**Table 1: the levels of liver enzymes in different groups**

Parameter/group	AST (SGOT) (IU/L)	ALT (SGPT) (IU/L)	ALP (ALK) (IU/L)
Control	183.3750 ± 2.19527ab	84.6250± 4.56672a	128.3750± 5.11279b
Sham	185.5000±2.19578ab	87.2500± 4.52276a	128.6250± 4.47189b
Aloe vera at 50 mg/kg	156.000 ± 1.72171ab	82.0000± 2.43487a	118.2500± 3.922679ab
Aloe vera at 100 mg/kg	115.6250±3.16192a	65.8750± 1.45697a	79.6250± 2.89049a
Aloe vera at 200 mg/kg	142.7500±3.17214ab	73.7500± 2.33567a	107.2500±3.43173ab
Paraquat	600.7500±69.73438d	241.7500± 25.9159e	777.8750±7.08479f
Paraquat+ aloe vera at 50 mg/kg	561.3750±6.71602d	207.5000± 4.29285d	631.2500±36.81893e
Paraquat + aloe vera at 100 mg/kg	297.2500±2.25198c	168.0000± 3.52763c	537.7500±11.83810d
Paraquat + aloe vera at 200 mg/kg	214.0000±15.82465b	128.1250±2.49285b	377.6250±18.48238c
p-value	<0.0001	<0.0001	<0.0001

- Based on Duncan’s test, means in each column with at least one letter in common are not significantly different at the 5% significance level
- The means are presented in the form of Mean ±SEM
- P< 0.05 is considered statistically significant

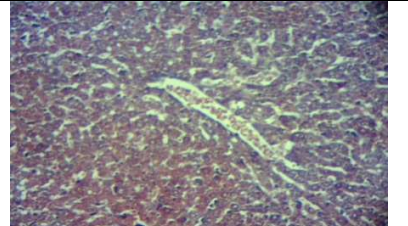
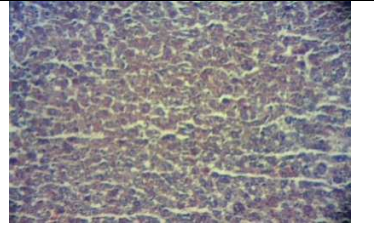
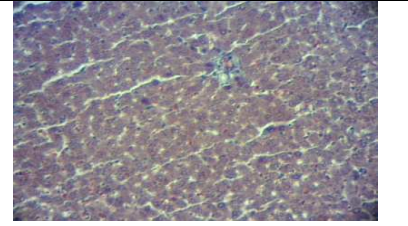
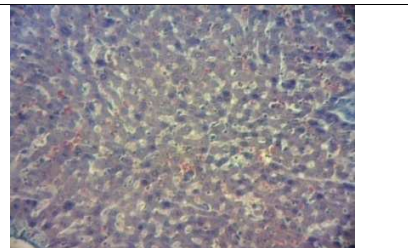
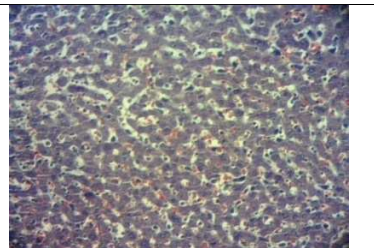
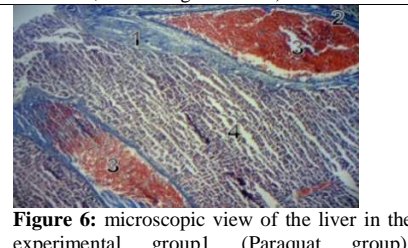

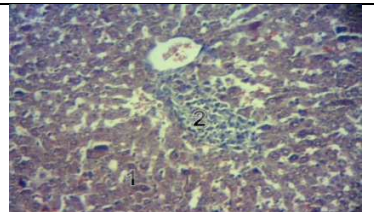
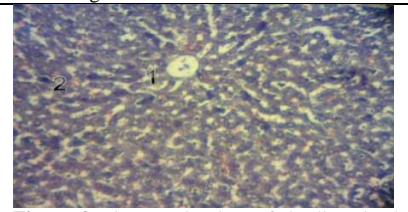
**RESULTS**

**4. Histological results**

Microscopic examination of rat liver tissues in the control group revealed that they were healthy and normal (normal lobular structure, normal central vein and sinusoids and Kupffer cells, normal distribution of glycogen, no lymphocytic infiltration, and no vascular congestion).

In the sham group (in which mice were given distilled water) and the experimental groups 2,3 and 4 (in which different doses of the Aloe vera extract were given), the tissues appeared relatively healthy and showed no special pathological changes.

**figures (1-9) histologic changes of the liver in different study groups**

		
<p><b>Figure 1:</b> microscopic view of the liver in Control Group, with a natural structure (Masson trichrome, 100X magnification)</p>	<p><b>figure 2:</b> microscopic view of the liver in the sham Group ,with a natural structure (Masson trichrome, 100X magnification)</p>	<p><b>Figure 3:</b> microscopic view of the liver in the experimental group 2 ( low aloe vera). Its tissues seem relatively healthy. (Masson trichrome, 100X magnification )</p>
		
<p><b>Figure 4:</b> microscopic view of the liver in the experimental group3 (moderate aloe vera). Its tissues seem relatively healthy. (Masson trichrome, 100X magnification)</p>	<p><b>Figure 5:</b> microscopic view of the liver in the experimental group4 (high aloe vera). Its tissues seem relatively healthy. (Masson trichrome, 100X magnification)</p>	<p><b>Figure 6:</b> microscopic view of the liver in the experimental group1 (Paraquat group): (1)centrilobular vein enlargement (2)Infiltration of inflammatory cells and collagen fibers around the centrilobular vein (3) enlargement of the centrilobular vein (4) the loss of cellular order toward the center (Masson trichrome, 100X magnification)</p>
		
<p><b>Figure 7:</b> microscopic view of the liver in the experimental group5 ( paraquat group and low aloe vera): (1) collagen fibers around the centrilobular vein (2) Infiltration of inflammatory cells around the centrilobular vein (3) Enlarged and congested centrilobular vein (Masson trichrome, 100X magnification)</p>	<p><b>Figure 8:</b> microscopic view of the liver in the experimental group6 (paraquat group and moderate aloe vera): (1) more regular cellular order toward the center (2) Infiltration of inflammatory cells (Masson trichrome, 100X magnification)</p>	<p><b>Figure 9:</b> microscopic view of the liver in the experimental group7 (paraquat group and high aloe vera): (1) more regular cellular order toward the center (2) more decreased vascular congestion (Masson trichrome, 100X magnification)</p>

In the experimental group 1 (in which mice were given paraquat alone), very intensive hepatocyte necrosis, degenerative changes, proliferation and activation of Kupffer cells (in scattered form) were seen. In this group infiltration and proliferation of inflammatory cells around the portal and central vein spaces of the centrilobular region and in the sinusoidal space were also observed. In addition, formation of fibrous bridges between liver lobules, polycythemia and blood congestion in the sinusoids, and cellular ballooning were seen. In this group,

progressive liver fibrosis had taken place as evidenced by the presence of collagen fibers in hepatic parenchyma, around the central vein in the centrilobular region, and in the portal space.

In the experimental groups 5, 6 and 7 (in which paraquat plus Aloe vera extract were given), the destructive effects of Paraquat decreased. figures 1-9

## DISCUSSION

Nitric oxide, which is synthesized by the endothelial nitric oxide synthesizing enzyme, regulates liver blood flow and vascular resistance, and plays an effective role in maintaining hepatic portal blood circulation <sup>[1]</sup>. Superoxide anion produced by Paraquat in the liver reacts with nitric oxide and produces peroxy nitrite anion which is a very toxic agent. It seems that congestion of veins in the centrilobular region and hepatic sinusoids in Paraquat toxicity are due to the destruction of endothelial cells that leads to the production of peroxy nitrite anion and, eventually, disrupts liver blood flow <sup>[1]</sup>. Paraquat also causes changes in transcription of enzymes related to liver, kidneys, and lungs. In addition, Paraquat impairs enzymes which are responsible for esterification and entry of fatty acids into hepatocytes; therefore leading, eventually, to fatty liver <sup>[11]</sup>.

Undoubtedly, oxidative agents play an important role in creating pathological changes in the liver, especially in poisoned livers, because they disrupt the biological membranes in the cellular membrane structure and cause pathological changes through peroxidation of unsaturated fatty acids. Very often, various inhibitory mechanisms in the body cannot fully play a protective role, therefore necessity of some beneficial compounds, especially antioxidants in foodstuff is considered <sup>[12]</sup>.

Aloe vera contains more than 75 nutritious materials and minerals, amino acids, vitamins (including A, E and C), salicylic acid, enzymes, tannins, and various polysaccharides <sup>[7]</sup>. It also includes active pharmaceutical substances and compounds such as sterols, four important sterols (cholesterol, campesterol, lupeol, and beta-sitosterol) <sup>[8]</sup>, anthraquinones (aloin, aloe emodin, coumaric acid), and glycoproteins and prostaglandins <sup>[8, 13]</sup>.

With due attention to these components in aloe vera; this plant has anti-toxic <sup>[12]</sup>, anti-inflammatory <sup>[14, 15]</sup>, and antioxidant properties <sup>[16, 17]</sup>. Results of various studies indicated that aloe vera extract can inhibit liver damage, and elevation of hepatic transaminases. It also can reduce hepatic glutathione concentration <sup>[18, 19]</sup>.

In this research, intraperitoneal injection of Paraquat at 2 mg/kg for two weeks caused extensive destruction of liver tissues and increased serum concentrations of the hepatic enzymes AST, ALT, and ALP. These results agree with those of other studies conducted on Paraquat toxicity. Dere and Polat studied the effects of Paraquat on the activities of some hepatic enzymes in Balb/C rat liver in 2001 and found that Paraquat considerably increased activities of AST and ALT <sup>[11]</sup>. Tahir *et al.* also showed in 2010 that Paraquat caused polycythemia and blood congestion in the veins of the centrilobular region and hepatic sinusoids. It is shown in this study that consumption of aloe vera in the same time can reduce significantly liver damage.

## CONCLUSION

Considering the concentrations of AST, ALT, and ALP and the histopathology of rat liver tissue in this study, it seems that aloe vera extract can reduce the toxic effect of paraquat on rat liver tissues through inhibiting the oxidative stress. Therefore more studies in human may lead to use of this plant in various hepatotoxicity.

## REFERENCES

- [1] Chohan MS, *et al.* Paraquat Induced Hepatotoxicity in Albino Mice. *Pakistan J. Zool.* 42(1): 69-73.
- [2] Fukushima T, *et al.* Mechanism of cytotoxicity of paraquat: I. NADH oxidation and paraquat radical formation via complex I. *Experimental and Toxicologic Pathology*, 1993. 45(5): 345-349.
- [3] Dragin N *et al.* Acute oxidative stress is associated with cell proliferation in the mouse liver. *FEBS letters*, 2006. 580(16): 3845-3852.
- [4] Donnell VB, Freeman BA. Interactions between nitric oxide and lipid oxidation pathways implications for vascular disease. *Circulation Research*, 2001. 88(1): 12-21.
- [5] Suntres ZE. Role of antioxidants in paraquat toxicity. *Toxicology*, 2002. 180(1):65-77.

- 
- [6] PeirA AM, et al. Hepatotoxicity related to paraquat and diquat absorption through intact skin. *Digestive diseases and sciences*, 2007.52(11): 3282-3284.
- [7] Botes L, Van der Westhuizen FH, Loots DT. Phytochemical contents and antioxidant capacities of two *Aloe greatheadii* var. *davyana* extracts. *Molecules*, 2008. 13(9): 2169-2180.
- [8] Surjushe A, Vasani R, Saple D. Aloe vera: a short review. *Indian journal of dermatology*, 2008. 53(4): p. 163.
- [9] KarimiJashni H, Najmadini N, Hooshmand F. Effect of alcoholic extract of aloe vera plant on serum testosterone and gonadotropin levels in rats. *Journal of Jahrom University of Medical Sciences*. 10(2): 2.
- [10] Najafiyani M, et al. The study of the effect of Parquet Herbicide on the Physiology of Breeding in Rats. *Advances in Environmental Biology*.6(3): 1117-1121.
- [11] Dere E, Polat Fky. The effect of paraquat on the activity of some enzymes in different tissues of mice (*Mus musculus*-Swiss albino). *Turkish Journal of Biology*, 2001.25(3): 323-332.
- [12] Ebrahimi S, et al. protective effect of *Zizyphus vulgaris* extract on liver toxicity in laboratory rats . *ArmaghaneDanesh* 2011, 16(2): 172-180.
- [13] Vazquez B, et al. Antiinflammatory activity of extracts from Aloe vera gel. *Journal of ethnopharmacology*, 1996. 55(1): 69-75.
- [14] Etim OE, Farombi EO, Usuh IF, Akpan EJ. The protective effect of aloe vera juice on lindane induced hepatotoxicity and genotoxicity. *Pak. J. Pharm. Sci*, 2006. 19(4): 333-337.
- [15] de Melo, JG, et al. Medicinal plants used as antitumor agents in Brazil: an ethnobotanical approach. *Evidence-Based Complementary and Alternative Medicine*.2011: 1-14.
- [16] Anilakumar K, et al. Effect of Aloe vera gel extract on antioxidant enzymes and azoxymethane-induced oxidative stress in rats. *Indian J Exp Biol*. 2010 Aug;48(8):837-42.
- [17] Gomes de Melo J, et al. Antiproliferative activity, antioxidant capacity and tannin content in plants of semi-arid northeastern Brazil. *Molecules*. 15(12): 8534-8542.
- [18] Kim SH, et al. Protective effect of a mixture of Aloe vera and *Silybummarianum* against carbon tetrachloride-induced acute hepatotoxicity and liver fibrosis. *Journal of pharmacological sciences*, 2009. 109(1): 119.
- [19] Davis RH. Biological activity of Aloe vera. *Sofw Journal*, 1993. 119: 646-646.