INVESTIGATING THE EFFECT OF VITAMIN E AND C COMBINED TREATMENT ON PARAQUAT-INDUCED LIVER DAMAGE IN WISTAR RATS (*RATTUS NOVERGICUS*)

Abstract

Paraquat has been referred to as a prominent suicide agent in a number of nations, not only because it is highly acutely poisonous, but also because it is readily available, relatively inexpensive, and has no known antidote. Vitamins C and E are well-known antioxidants that react quickly with free radicals and prevent lipid peroxidation. The aim of this study was to determine the ameliorative effect of vitamin E and C in paraquat induced liver poisoning in rats. A total of 200 male albino rats were used in the study. With 50 rats each, the rats were sorted into four main groups: A, B, C, and D. Each group was divided into two subgroups (0 and VEC), each with 25 rats. The "A" group received no paraquat, while the "B," "C," and "D" groups received 0.02g, 0.04g, and 0.06g of paraquat respectively. All “0” subgroups represented those not treated with Vit E and C combination therapy and those treated with 500mg of vitamin E and 2000mg/dl of vitamin C designated VEC. The paraquat induction frequency was every two weeks for three months, followed by two months of weekly vitamin treatments. Blood was drawn for SGPT, SGOT, ALP, and GGT testing. At p-value 0.05, there was a significant difference in these liver enzymes activities among the "A0", "B0", "C0", and "D0". There was a significant difference in these liver enzymes activities among the "AVEC," "BVEC," "CVEC," and "DVEC," p-value 0.05. As a result, significant differences in intra-group comparisons of SGPT, SGOT, ALP, and GGT p-value 0.05 were observed. This study discovered that combining vitamin E and C had an ameliorative effect on the liver as a result of PQ insult.

Keyword: *Paraquat, Vitamin E, Vitamin C, rat, antioxidant*

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1.0 INTRODUCTION

Paraquat is a man-made substance. Weidel and Russo were the first to describe it in 1882. Paraquat's redox characteristics were found by Michealis and Hill in 1933, and the chemical was given the name methyl viologen. Paraquat (PQ) was first recognized as having herbicidal qualities in 1958, and it became commercially available in 1962 [1]. It has been dubbed a major suicide agent in several nations, not only because it is highly acutely poisonous, but also because it is widely available, relatively inexpensive, and has no known antidote [2]. The majority of research on paraquat toxicity has focused on reducing its absorption or improving its elimination [3]. Currently, there are no effective pharmacological antagonists for paraquat and there are no chelating agents capable of binding the poison in blood or other tissues, and so the management of paraquat poisoning has remained mostly supportive and directed toward decreasing its absorption or enhancing its elimination [3].

PQ is converted to PQ mono-cation free radical by nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P-450 reductase once it enters the cells. The electron delivered to PQ quickly travels to oxygen, resulting in the formation of superoxide anion, and ROS causes severe oxidative damage [3]. The generation of reactive free oxygen radicals (ROS), which induce oxidative stress and lipid peroxidation, is a common underlying mechanism of most hepatotoxicity-inducing contaminants (LPO).. Damage to hepatocyte membranes results in edema, degeneration, necrosis, and fibrosis of hepatocytes as a result of the cumulative consequences of these events [4,5]. ROS and other free radicals are continuously created during normal physiologic processes and damage macromolecules such as proteins, lipids, and DNA, causing tissue harm [6]. The importance of oxidative stress in the etiology and progression of chronic liver disease has long been recognized [7,8]. Antioxidant compounds have previously been shown to protect cells from the damaging effects of a variety of environmental pollutants [9,10].

Vitamins C and E are well-known antioxidants that react quickly with free radicals and prevent lipid peroxidation. As a result, both vitamins C and E are thought to be absorbed during radical reactions [11,12]. For paraquat poisoning, increasing total antioxidant status with high dosages of vitamin C is beneficial as a free radical scavenger. Vitamin C's capacity to quench radicals generated by paraquat redox cycling has been credited with these protective benefits [13]. Although many antioxidants work in concert, ascorbic acid (vitamin C) has the ability to destroy or reactivate other antioxidants [14]. Vitamin C may directly scavenge oxygen free radicals with or without enzyme catalysts, and it can also scavenge them indirectly by recycling others to their reduced state [15].

Vitamin E deficiency potentiated the development of acute paraquat toxicity in rats in multiple experiments, demonstrating its function in paraquat toxicity. One of the most well-known processes is its ability to eliminate free radicals produced by the human body's oxidation response or by foreign substances. In vitro and in vivo, it also suppresses prostaglandin synthesis and prevents platelet aggregation [16].

Antioxidant systems, in general, either prevent ROS from forming or eliminate them before they can harm essential cell components [17].

Glutarate-oxaloacetate-aminotransferase (SGOT), also known as aspartate amino transferase (AST), is an enzyme that catalyzes transamination reaction. It is found in the liver, heart, skeletal muscle, and kidney [18]. Elevations frequently predominate in cirrhosis and even in liver diseases that typically have an increased ALT [19].

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Glutarate-pyruvic-aminotransferase (SGPT), also known as Alanine amino transferase (ALT), is an enzyme found in higher concentrations in the liver than in other tissues of the body [18]. Any type of liver cell injury can cause an increase in ALT levels.

Alkaline phosphatase (ALP) is found in the small intestine's mucosal epithelia, the kidney's proximal convoluted tubule, bone, the liver, and the placenta. It is responsible for lipid transport in the intestine and bone calcification. Its activity is primarily derived from the liver, with bone contributing 50% [18].

(GGT) is a microsomal enzyme found in hepatocytes and biliary epithelial cells, as well as renal tubules, the pancreas, and the intestine. It is also found in the cell membrane, where it transports peptides into the cell and is involved in glutathione metabolism [18]. Because of the relative importance of vitamin C and E as antioxidants, this study focused on how to boost the antioxidant system in the body to counteract the harmful effects of paraquat. Because the liver is the body's energy store and source, the liver enzymes SGOT, SGPT, ALP, and GGT were chosen

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.2.0 MATERIAL AND METHODS

2.1 list 1 : Study Design

Total No of Rats	Groups	Subgroups	Treatment
32 Rats	A (50 rats)	A_0 (25 rats)	Not induced with paraquat and no treatment with Vitamin E and C given
		A_{VEC}(25 rats)	Not induced with paraquat but treated with Vitamin E(500mg) and C(2000mg/dl) given
	B (50 rats)	B_0(25 rats)	Induced with 0.02gc of paraquat (3 months) and no Vitamin E and C treatment given
		B_{VEC}(25 rats)	Induced with 0.02gc of paraquat (3 months) and Vitamin E(500mg) and C(2000mg/dl) treatment given(for 2 months after PQ induction)
	C(50 rats)	C_0(25 rats)	Induced with 0.04gc of paraquat (3 months) with no Vitamin E and C treatment given
		C_{VEC}(25 rats)	Induced with 0.04gc of paraquat (3 months) and Vitamin E(500mg) and C(2000mg/dl) given (for 2

			months after PQ induction elapsed)
	D(50 rats)	D_0(25 rats)	Induced with 0.06gc of paraquat (3 months) with no Vitamin E and C treatment given
		D_{VEC}(25 rats)	Induced with 0.06gc of paraquat(3 months) and Vitamin E(500mg) and C(2000mg/dl) given (for 2 months after PQ induction elapsed)

2.2 Animal source

The Animal House, Rivers State University of Science and Technology's Department of Biology, provided 200 rats weighing an average of 0.20±0.02kg. The rats were brought to the research site and given two weeks to adapt before the trial began. The study was conducted at the Department of Medical Laboratory Science at Rivers State University of Science and Technology.

Treatment Administration

Procedure for Paraquat Administration

Administration of toxicant was via oral gavage route. The dose depended on the treatment group but in all, the treatment was performed every two weeks for three months.

The rats were held at the skin over the head and turned so that the mouth was faced upward and the body lowered towards the holder. The syringe needle bevel was then placed into the mouth of the rat a bit laterally in a way to avoid the teeth which are located centrally. The content in the syringe was then emptied into the mouth of the rat gradually [20].

Procedure for Vitamin Administration

The vitamins (E and C) were given orally every week for two months at doses of 500mg and 2000mg/dl respectively [20].

2.3 Sample Collection method

Blood was collected via cardiac puncture into plain bottles (without anticoagulant) with a 2ml syringe, allowed to clot to obtain serum, and then analyzed for the liver enzymes GGT, SGOT, SGPT, and ALP. The animals were sacrificed under 70 percent chloroform anesthesia. To avoid pollution, the carcasses that remained were incinerated.

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2.4 Laboratory analysis

2.4.1 Serum glutarate-oxaloacetate-aminotransferase (AST/SGOT) method: by Reitman and Frankel, 1957.

Principle: AST is incubated at 37^0C for exactly 60 minutes in a pH 7.5 buffered substrate containing aspartate and α-ketoglutarate. AST catalyzes the transfer of the amino group from aspartate to ketoglutarate, forming oxaloacetate and glutamate. The oxaloacetate reacts with 2, 4-dinitrophenylhydrazine to form 2, 4-dinitrophenylhydrazone which in alkaline medium gives a red-brown colour. The absorbance of the colour produced is measured at 540nm Spectrophotometrically

α-oxoglutarate + L-aspartate SGOT → L-glutamate + Oxaloacetate.

Procedure: In estimating the activity of this enzyme, 0.5ml of buffered-L- aspartate and α-oxoglutarate solution was each added to two glass tubes labeled 'Reagent Blank' and 'Test', followed by 0.1ml each of distilled water and sample to their respective tubes, mixed and incubated for exactly 30 minutes at 37^0C. After which, 0.5ml of 2, 4-dinitrophenylhydrazine (2mmol/L) solution was added to each of the test tubes, mixed again and allowed to stand for exactly 20 minutes, at 20 – 25^0C. At the end of the time, 5.0mls of Sodium hydroxide (0.4mol/L) was added to enhance colour development at alkaline pH. The tubes were mixed and the absorbance of 'Test' (A_{test}) read against that of the 'Reagent blank' after 5 minutes.

Calculation: Obtain the activity of the enzyme AST in the serum from the table of values previously plotted against activities. Haemolysis interferes with the assay.

2.4.2 Serum glutarate-pyruvic-aminotransferase (SGPT) by Reitman and Frankel, 1957.

Principle: ALT is incubated at 37^0C for exactly 60 minutes in a pH 7.5 buffered substrate containing L-alanine and α-ketoglutarate. ALT catalyzes the transfer of the amino group from alanine to ketoglutarate, forming pyruvate and glutamate. The pyruvate reacts with 2, 4-dinitrophenylhydrazine to form 2, 4-dinitrophenylhydrazone which in alkaline medium gives a red-brown colour. The absorbance of the colour produced is measured at 540nm Spectrophotometrically

(α-oxoglutarate-L-alanine GPT → L-glutamate + pyruvate

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Procedure: In estimating the activity of this enzyme, 0.5ml of buffered-L- alanine and α-oxoglutarate solution was each added to two glass tubes labeled 'Reagent Blank' and 'Test', followed by 0.1ml each of distilled water and sample to their respective tubes, mixed and incubated for exactly 30 minutes at 37^0C. After which, 0.5ml of 2, 4-dinitrophenylhydrazine (2mmol/L) solution was added to each of the test tubes, mixed again and allowed to stand for exactly 20 minutes, at 20 – 25^0C. At the end of the time, 5.0mls of Sodium hydroxide (0.4mol/L) was added to enhance colour development at alkaline pH. The tubes were mixed and the absorbance of 'Test' (A_{test}) read against that of the 'Reagent blank' after 5 minutes.

Calculation: Obtain the activity of the enzyme ALT in the serum from the table of values previously plotted against activities. Haemolysis interferes with the assay.

2.4.3 Alkaline phosphatase (ALP) method by Englehardt, *et al*., 1970.

Principle: This is an optimized standard method where ALP is measured by monitoring the concentration of p-nitrophenol formed with p-nitrophenlphosphate.

Procedure: Fresh double distilled water (ddH_2O) was aspirated and used to perform a new Gain calibration in flow cell mode. This zero the equipment from previous sample run. ALP was selected in the Run Test Screen and a water blank test run was carried out, after which 0.02ml of sample and 1.0 ml of reagent (Diethanolamine buffered p-nitrophenylphosphate) was dispensed into a test tube and mixed for 2 minutes. The mixture was then aspirated into the Rx Monza. After about 2 minutes the result of the test sample was then printed out from a printer connected to the machine.

The advantage of this machine procedure is that up to 200 samples can be processed and results produced within one hour in S.I. unit = IU/L.

Manual calculation: To calculate the ALP activity, using the manual method, the following formula was utilized: IU/L = 2760 x ΔA 405 nm/min.

2.4.4 Gamma-Glutamyltransferase (GGT) method: (Szasz and Bergmeyer, 1974 and Teitz, 1987)

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The substrate L-V-glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine is converted by GGT in the sample to 5-amino-2-nitrobenzoate which can be measured spectrophotometrically at 405nm.

L-V-glutamyl-3-carboxy-4-nitroanilide + glycylglycine

L-V-glutamylglycylglycine + 5-amino-2-nitrobenzoate.

Procedure: 0.1ml of sample and 1.0ml of reagent (Buffered Glycylglycerine and L-gammaglutamyl-3-carboxy-4-nitrolide) were dispensed into a cuvette, mixed and the initial absorbance read at 400 – 420nm with simultaneous timer initiation. The absorbance was read again after 1, 2 and 3 minutes.

Calculation: IU/L = 1158 X ΔA (405nm/minute).

2.5 Statistical analysis

The data generated from this study was analyzed using SPSS version 23.0 for descriptive and inferential statistics (ANOVA) for inter-group comparison and T-test for intra-group (sub-group) comparison at test significance, P-value<0.05.

3.0 RESULTS

Table 1 : ***Changes in Liver enzymes biochemical data after two months treatment period*.**

Subgroup	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	GGT (IU/L)
A_0	6.17 ± 1.38	4.18 ± 0.03	1.57 ± 0.05	13.33 ± 0.17
A_{VEC}	4.12 ± 1.39	4.33 ± 0.06	1.55 ± 0.01	8.48 ± 0.47
B_0	15.24 ± 1.39[a]	41.15 ± 0.44[a]	100.30 ± 1.00[a]	63.00 ± 1.39[a]
B_{VEC}	19.72 ± 1.35[a,b]	34.25 ± 0.38[a,b]	66.03 ± 1.14[a,b]	35.98 ± 0.48[a,b]
C_0	99.65 ± 1.64[a]	147.00 ± 1.52[a]	130.68 ± 0.84[a]	76.25 ± 1.30[a]
C_{VEC}	74.60 ± 2.02[a,b]	91.50 ± 0.64[a,b]	80.38 ± 1.84[a,b]	42.75 ± 0.46[a,b]
D_0	193.00 ± 2.70[a]	258.75 ± 3.10[a]	315.53 ± 1,63[a]	99.00 ± 0.60[a]
D_{VEC}	238.75 ± 3.95[a,b]	111.25 ± 2.86[a,b]	164.40 ± 5.83[a,b]	70.25 ± 1.86[a,b]

Statistical significance: $P \leq 0.05$.

- Index (a) = represents a statistically significant difference between the test subgroups and the control subgroups at each treatment month.

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- Index (b) = represents a statistically significant difference observed within each group (i.e. Group B: B_0 Vs B_{VE}) at each month.

A_0 – Not induced with paraquat and no treatment with Vitamin E and C given
A_{VEC} – Not induced with paraquat but Vitamin E and C treatment given
B_0 - Induced with 0.02gc of paraquat and no Vitamin E and C treatment given
B_{VEC} – Induced with 0.02gc of paraquat and Vitamin E and C treatment given
C_0 - Induced with 0.04gc of paraquat with no Vitamin E and C treatment given
C_{VEC} - Induced with 0.04gc of paraquat with Vitamin E and C treatment given
D_0 – Induced with 0.06gc of paraquat with no Vitamin E and C treatment given
D_{VEC} – Induced with 0.06gc of paraquat with Vitamin E and C treatment given
Thus, from the analysis,

Table 2: SGOT (IU/L) – Extrapolated table illustrating simplified statistical interpretation (Vit E + C therapy) at $P \leq 0.05$.

GROUPS	DECISION
A_0	6.17 ± 1.38
B_0	15.24 ± 1.39^{a} - Significant difference
C_0	99.65 ± 1.64^{a} - Significant difference
D_0	193.00 ± 2.70^{a} –Significant difference
A_{VEC}	4.12 ± 1.39
B_{VEC}	19.72 ± 1.35^{a} –Significant difference
C_{VEC}	74.60 ± 2.02^{a} –Significant difference
D_{VEC}	238.75 ± 3.95^{a} –Significant difference
B_0	15.24 ± 1.39
Vs	Significant difference
B_{VEC}	19.72 ± 1.35^{b}
C_0	99.65 ± 1.64
Vs	Significant difference
C_{VEC}	74.60 ± 2.02^{b}
D_0	193.00 ± 2.70
Vs	Significant difference
D_{VEC}	238.75 ± 3.95^{b}

Tabe 3: SGPT (IU/L) - Extrapolated table illus trating simplified statistical interpretation (Vit E + C therapy) at $P \leq 0.05$.

GROUPS	DECISION
A_0	4.18 ± 0.03

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B_0 C_0 D_0	41.15± 0.44[a] - Significant difference 147.00 ± 1.52[a] -Significant difference 258.75 ± 3.10[a] –Significant difference
A_{VEC} B_{VEC} C_{VEC} D_{VEC}	4.33 ± 0.06 34.25 ± 0.38[a] –Significant difference 91.50 ± 0.64[a] –Significant difference 111.25 ± 2.86[a] –Significant difference
B_0 Vs B_{VEC}	41.15± 0.44 34.25 ± 0.38[b] } Significant difference
C_0 Vs C_{VEC}	147.00 ± 1.52 91.50 ± 0.64[b] } Significant difference
D_0 Vs D_{VEC}	258.75 ± 3.10 111.25 ± 2.86[b] } Significant difference

Table 4: ALP (IU/L) - Extrapolated table illustrating simplified statistical interpretation (Vit E + C therapy) at P ≤ 0.05

GROUPS	DECISION
A_0 B_0 C_0 D_0	1.57 ± 0.05 100.30 ± 1.00[a] - Significant difference 130.68 ± 0.84[a] - Significant difference 315.53 ± 1,63[a] –Significant difference
A_{VEC} B_{VEC} C_{VEC} D_{VEC}	1.55 ± 0.01 66.03 ± 1.14[a] – Significant difference 80.38 ± 1.84[a] –Significant difference 164.40 ± 5.83[a] –Significant difference
B_0 Vs B_{VEC}	100.30 ± 1.00 66.03 ± 1.14[b] } Significant difference
C_0 Vs C_{VEC}	130.68 ± 0.84 80.38 ± 1.84[b] } Significant difference
D_0 Vs D_{VEC}	315.53 ± 1.63 164.40 ± 5.83[b] } Significant difference

Table 5: GGT (IU/L) – Extrapolated table illustrating simplified statistical interpretation (Vit E + C therapy) at P ≤ 0.05

GROUPS	DECISION
A_0 B_0 C_0 D_0	13.33 ± 0.17 63.00 ± 1.39[a] 76.25 ± 1.30[a] 99.00 ± 0.60[a] } Significant difference
A_{VEC}	8.48 ± 0.47

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B_{VEC} C_{VEC} D_{VEC}	35.98 ± 0.48[a] 42.75 ± 0.46[a] 70.25 ± 1.86[a]	Significant difference
B_0 Vs B_{VEC}	63.00 ± 1.39 35.98 ± 0.48[b]	Significant difference
C_0 Vs C_{VEC1}	76.25 ± 1.30 42.75 ± 0.46[b]	Significant difference
D_0 Vs D_{VEC}	99.00 ± 0.60 70.25 ± 1.86[b]	Significant difference

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4.0 DISCUSSION

Despite widespread international calls for a global ban on PQ use, its high weed-control effectiveness and low cost are compelling arguments for its continued use in most developing nations [21,22].

This study focused on serum hepatic enzymes like ALT, AST, ALP, and GGT for the study of hepatotoxicity and repair. When compared to the control subgroups (A_0 and A_{VEC}), the enzymes activities were found to be higher in the test subgroups (B0, BVEC, C0, CVEC, D0, and DVEC), there was an interaction between the dose given and the concentration of enzyme activity; thus, the increase was found to be directly related to the dose, as seen in [23,24]. PQ studies and the toxicity effects of this chemical on the body's organs, as well as its mechanism of toxicity, have been reported [25]. Following a hepatocellular injury, liver enzymes are secreted into the bloodstream, resulting in an increase in their activity in serum samples. These abnormal increases in enzymes are sensitive indicators of hepatotoxicity or disease. In general, these findings may point to liver degeneration and hypofunction. Lipid peroxidation is known to disrupt the integrity of cellular membranes, resulting in cytoplasmic enzyme leakage. As a result, increased activity of these enzymes in the serum could be the result of severe hepatocellular damage. This is also consistent with several studies [24, 26, 27, 28, 29, 30] that found PQ to be a hepatotoxin.

Furthermore, within-group comparisons of all enzymes revealed a well-defined reduction in the values of enzyme activities of the vitamin C and E treated subgroups (BVEC, CVEC, and DVEC) when compared to the PQ insult only subgroups (B0, C0 and D0). In this case, the combined effect of Vitamin E and C restored the above-mentioned serum parameters. Vitamin E is well known for regulating all oxidation processes in the body and thus acting as

a powerful antioxidant. It works as an antioxidant by preventing chain propagation by transferring phenolic hydrogen to a peroxyl free radical of a polyunsaturated fatty acid, thereby limiting the extent of lipid peroxidation. The majority of the peroxytocopherol formed is reconverted to tocopherol by vitamin C (synergistic combined therapy/protective effects), while Vitamin C, to a large extent, reduced the toxic insults and acted as a substrate for the antioxidant enzyme ascorbate peroxidase, a function that is especially important in stress resistance, thereby maintaining and repairing cellular integrity and function, which resulted in the lower values.

The capacity of vitamin C to quench radicals created by PQ's redox cycling before they harm other biomolecules could potentially be linked to this protective function. This is consistent with earlier research [24, 31, 32, 33, 34, 35, 36].

Surprisingly, the findings of this study contradict those of [37, 38, 39, 40, 41], who claimed that vitamin E and C had no effect on the biochemical reaction to paraquat, implying that they had no protective effect against paraquat intoxication.

During the PQ toxic insult, all enzyme activities in the subgroups on vitamin C and E improved in general (BVEC, CVEC, and DVEC). Despite the fact that these values were high as compared to the control subgroups (A0 and AVEC) and above the reference ranges for rat liver enzymes, they are nonetheless a sign of improved health. These findings demonstrate that if the vitamin C and E combined treatment had been continued for a longer period of time, the liver cells may have been completely healed, and the health of the rats would have improved much better.

5.0 CONCLUSION

Vitamin C and E are powerful antioxidants that should be considered as a first-line treatment (emergency operation) for toxic injury. In PQ toxicity, its administration should not be overlooked, and it should be continued even after the patient has recovered to ensure enough intracellular repairs.

The findings of this study show that a combination Vitamin C and E treatment regimen can enhance the health of animals that have been exposed to PQ. PQ exposure caused a significant increase in the activities of the liver enzymes SGOT, SGPT, ALP, and GGT, which were dose-dependent. After receiving the vitamins, the activity levels were reduced throughout the study, indicating their efficacy as a supplement to the treatment.

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As a result, more well-designed prospective studies are required to compare the outcomes and mortality rates of various treatment procedures. The failure of present treatments for paraquat poisoning necessitates not just extensive prospective clinical studies for paraquat poisoning treatment, but also immediate prevention actions.

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