

Spectrophotometric assessment of effect of aqueous extract of *Aloe vera* on rabbit erythrocytes in varying concentrations of saline

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ABSTRACT

Background: Hemolytic disorders are one of the prime reasons for frequent blood transfusions which involves lots of costs and sufferings to the patient. This study was undertaken to determine the effect of water soluble extract of *Aloe vera* on rabbit erythrocytes in varying concentrations of NaCl from 0.9% (isotonic) to 0.15% (hypotonic).

Methods: Aqueous extract of *Aloe vera* (AVE) 200mg/kg was orally administered to rabbits in the test group while control group was given 1ml of distilled water (DW). Blood was withdrawn from rabbits, centrifuged and suspension in 1ml of normal saline was made. 20 microliter of red blood cells suspension from both control and test groups was added to normal saline of varying concentrations from 0.9% to 0.15% NaCl which were quantitatively analysed for hemolysis by UV spectrophotometer. Data was analysed by unpaired t test and P <0.05 was considered statistically significant.

Results: The difference in percentage of hemolysis in both test and control groups was not statistically significant. Therefore, acute administration of water soluble extract of *Aloe vera* (200mg/kg) did not have protective effect on rabbit erythrocytes against hypotonic solution of normal saline.

Conclusions: *Aloe vera* might be useful for the treatment of oxidative stress-related human disorders by virtue of its antioxidant activity and may have a role in prevention of hemolysis which needs to be explored by further studies.

Keywords: *Aloe vera*, Antioxidant, Erythrocytes, Hemolysis, Hypotonic saline

INTRODUCTION

Traditional medicines are still used by about more than 80% of the world's population for their ailments. These medicines are not only used by the rural masses for their primary health care but are also used by urban population who mostly use modern medicines.¹

Aloe vera is the oldest medicinal plant ever known and the most applied medicinal plant worldwide.² *Aloe vera* (*Aloe barbadensis*) is a perennial succulent plant belonging to the Aloeaceae family, a sub-family of the Asphodelaceae.³ It is indigenous to Africa and Mediterranean countries. It

is reported to grow wild in the islands of Cyprus, Malta, Sicily, Canary cape, Cape Verde and arid tracts of India.⁴ The plant is composed of turgid green leaves joined at the stem in a rosette pattern. Each leaf consists of an outer green rind (skin) and an inner clear pulp.

Aloe vera contain several active compounds including flavonoids, saponins, tannins, alkaloids, polysaccharides, amino acids, enzymes, vitamins B₁₂, B₁, B₆, C, niacinamide, choline, calcium, iron, lecithin, magnesium, potassium, zinc and other compounds which exhibit medicinal effects.⁵

Aloe vera leaves possess many pharmacological properties including anti-inflammatory, antibacteria, antifungal, antioxidant and anticancer activities.⁶⁻¹⁰ Throughout the world *Aloe vera* has been marketed as a remedy for wound healing, ulcers, eczema, gastritis, arthritis, immune-system deficiencies, cancer and many other conditions.

Hemolytic disorders are one of the prime reasons for frequent blood transfusions which involves lots of costs and sufferings to the patient. *Aloe vera* supplementation can help to prevent oxidative stress and might be useful for the treatment of oxidative stress-related human disorders by virtue of its antioxidant activity. Therefore, this study was undertaken to determine the effect of water soluble extract of *Aloe vera* on rabbit erythrocytes (red blood cells) in varying concentration of NaCl from 0.9% (isotonic) to 0.15% (hypotonic).

METHODS

Principle

Erythrocytes when challenged to hypotonic medium in test tubes start swelling due to osmotic gradient. When the concentration of NaCl decreases below 0.55% erythrocytes start getting rapidly hemolysed. Further hemolysis keeps increasing as the concentration of NaCl decreases and the stored hemoglobin of erythrocytes gets released out. This hemoglobin was estimated at 540 nm using double beam UV-Spectrophotometer. Percentage of hemolysis was then calculated.

Extraction of *Aloe vera* extract

Study was carried out with water soluble dried extract of *Aloe vera* (*Aloe barbadensis*) obtained from botanical garden of Mahatma Gandhi Memorial Medical College, Indore and identified by the botanist.

Fresh *Aloe vera* leaves were collected and washed with clean water. Removal of the outer coat of the *Aloe vera* leaves gives pulp, which was dried in shade and was powdered till it became dry. This powder was dissolved in chloroform and filtered by filter paper. The remaining supernatant lying in the filter paper was subjected to drying and then it was dissolved in distilled water and separated by filter paper. This water-soluble *Aloe vera* extract was dried and kept in air tight glass bottles for experiment.

Experimental animals

Rabbits (*Oryctolagus cuniculus*) weighing 2-2.5kg, were obtained from the central animal house of the institute and divided into two groups of six each. Animals were housed individually in standardized environmental conditions with food and water provided *ad libitum* and were acclimatized for seven days before starting the experiment.

Dose selection was made on the basis of acute oral toxicity study as per OECD guidelines 423 adopted and received

from Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA), Ministry of social justice and empowerment, Government of India.¹¹ One tenth of upper limit dose was selected for the experiment.

Test group was administered aqueous extract of *Aloe vera* (AVE) in a dose of 200mg/kg orally dissolved in 1ml of distilled water for 7 days. Control group was given 1ml of distilled water (DW) by the oral route daily for the same duration.

On the 8th day, the test and control groups were kept in fasting condition for 3 hours and 1 ml of blood samples were withdrawn from marginal ear vein of each rabbit in heparin rinsed test tubes. It was subjected to centrifugation at 2500 rpm for 10 min to separate the plasma.

The blood pellet obtained after discarding the plasma was suspended in 1ml of normal saline to form a suspension. 20 microliter of red blood cells suspension from both control and test groups was added to normal saline of varying concentrations from 0.9% to 0.15% NaCl. All the test tubes were incubated for 30 min at 37°C and then centrifuged to see the hemolysis. The supernatant was quantitatively analysed for assessing the wavelength by UV spectrophotometer. At concentration of 0% NaCl, that is distilled water (DW) there is 100% hemolysis. The absorption spectrum of this 100% hemolysis is noted and the absorption spectrum of other test tubes with varying hemolysis is compared with that test tube which showed 100% hemolysis.

Percentage hemolysis =

$$\frac{\text{wave length absorbed in test tube} \times 100}{\text{wave length absorbed in DW}}$$

Statistical analysis

Data was analysed by unpaired t test and the results were expressed as mean±SEM (standard error of mean), P <0.05 was considered statistically significant.

RESULTS

The mean percentage hemolysis of rabbit erythrocytes (red blood cells) in test and control groups was calculated in varying concentrations of saline ranging from 0.9% NaCl to 0.15% NaCl (Table 1).

The assessment of wave length of hemolysed blood by UV spectrophotometer showed that the difference in percentage of hemolysis in both test and control groups was not statistically significant at any concentration of NaCl. Thus, acute administration of water soluble extract of *Aloe vera* at dose of 200mg/kg did not have protective effect on red blood cells of rabbit against hypotonic solution of normal saline.

Table 1: Mean percentage hemolysis in test and control groups.

NaCl conc. (g/dl)	Mean % hemolysis	
	Control group (DW)	Test group (AVE 200mg/kg)
0.9%	0.17±0.17	0.0±0.0
0.75%	4.00±0.37	3.5±0.22
0.6%	21.67±0.67	20.50±0.62
0.45%	34.17±1.33	33.67±0.95
0.3%	99.33±0.33	98.67±0.33
0.15%	100±0.00	100±0.17

AVE = Aloe vera extract, DW = Distilled water

Data expressed as mean±SEM (standard error of mean), n= 6; unpaired t test, P > 0.05, not significant as compared to control

DISCUSSION

Modern science has brought into light various diseases which were existing in the past but not known to mankind. Blood disorders like hemophilia, sickle cell anemia and many other genetic disorders lead to hemolysis resulting in morbidity.

Few authors have reported antioxidant effect of *Aloe vera* indicating its usefulness in wound healing.¹² Some studies have demonstrated free radical scavenging activity of *Aloe vera* gel extract in the maintenance of the antioxidant status and antioxidant defence in red blood cells, which might be due to the presence of various bioactive compounds present in the extract.^{13,14} Studies also show that *Aloe vera* plant extracts can also be beneficial in the management of sickle cell disease due to preponderance of nutrients, phytochemicals, amino acids and other compounds.¹⁵

In this study, acute administration of water soluble extract of *Aloe vera* at dose of 200mg/kg did not confer protection to red blood cells of rabbit against hypotonic solution of normal saline. Most studies which demonstrated antioxidant activity of *Aloe vera* were *in vitro* studies or involved topical application of *Aloe vera* gel, while this study involved systemic (oral) administration of aqueous extract of *Aloe vera*. Moreover, effect of chronic administration of *Aloe vera* extract needs to be studied. Therefore, further studies are required to completely evaluate the potential of water soluble extract of *Aloe vera* in prevention of hemolysis and its hidden potentials.

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Conflict of interest: None declared

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REFERENCES

- Ballabh B, Chaurasia OP. Traditional medicinal plants of cold desert Ladakh-Used in treatment of cold, cough and fever. *Journal of Ethnopharmacology*. 2007;112(2):341-5.
- Rajeswari R, Umadevi M, Rahale CS, Selvavenkadesh S, Kumar KS, Bhowmik D. Aloe vera: the miracle plant its medicinal and traditional uses in India. *Journal of Pharmacognosy and Phytochemistry*. 2012 Nov 1;1(4).
- Eggl U. *Illustrated Handbook of Succulent plants: Monocotyledons*. Springer; 2001:102-186.
- Yates A. *Yates Garden Guide*. Harper Collins (Australia) Pvt Ltd. 2002:466.
- Yamaguchi I, Mega N, Sanada H. Components of the Gel of Aloe vera (L.) Bunn. f. *Bioscience, biotechnology, and biochemistry*. 1993 Jan 1;57(8):1350-2.
- Rishi P, Rampuria A, Tewari R, Koul A. Phytomodulatory potentials of Aloe vera against Salmonella OmpR-mediated inflammation. *Phytotherapy research*. 2008 Aug 1;22(8):1075-82.
- Tian B, Hua YJ, Ma XQ, Wang GL. Relationship between antibacterial activity of Aloe and its anthraquinone compounds. *Zhongguo Zhong Yao Za Zhi*. 2003;11:1034-7.
- Jasso de Rodriguez D, Hernández-Castillo D, Rodriguez-García R, Angulo-Sánchez JL. Antifungal activity in vitro of Aloe vera pulp and liquid fraction against plant pathogenic fungi. *Industrial Crops and Products*. 2005;21:81-7.
- Hu Y, Xu J, Hu Q. Evaluation of Antioxidant Potential of Aloe vera (*Aloe barbadensis* Miller) Extracts. *Journal of Agricultural and Food Chemistry*. 2003;51(26):7788-91.
- Pecere T, Gazzola MV, Mucignat C, Parolin C, Vecchia FD, Cavaggioni A, et al. Aloe-emodin is a new type of anticancer agent with selective activity against neuroectodermal tumors. *Cancer Research*. 2000;60:2800-4.
- OECD/OCDE, OECD Guidelines for the testing of chemicals, revised draft guidelines 423: Acute Oral toxicity- Acute toxic class method, revised document, CPCSEA, Ministry of Social Justice and Empowerment, Govt. of India; 2000.
- Olaifa AK. Effects of Aloe vera gel application on epidermal wound healing in the domestic rabbit. *Int J Res Med Sci*. 2017;5:101-5.
- Mazzulla S, Sesti S, Schella A, Perrotta I, Anile A, Drogo S. Protective effect of Aloe vera (*Aloe barbadensis* Miller) on erythrocytes anion transporter and oxidative change. *Food and Nutrition Sciences*. 2012 Dec 10;3(12):1697.
- Kammoun M, Miladi S, Ali YB, Damak M, Gargouri Y, Bezzine S. In vitro study of the PLA2 inhibition and antioxidant activities of Aloe vera leaf skin extracts. *Lipids in health and disease*. 2011 Dec;10(1):30.
- Nwaoguikpe RN, Braide W, Ezejiofor TIN. The effect of aloe vera plant (*Aloe barbadensis*) extracts on sickle

cell blood (hbss). *African Journal of Food Science and Technology*. 2010;1(3):058-63.

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