

**REVERSAL OF SODIUM NITRITE INDUCED IMPAIRMENT OF
SPONTANEOUS ALTERATION BY ALOE VERA GEL: INVOLVEMENT OF
CHOLINERGIC SYSTEM**

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Summary

The present investigation was aimed at determining the spectrum of activity of aloe vera gel on CNS. Effect of aloe vera gel was studied on sodium nitrite induced hypoxia on elevated plus maze with pyritinol as positive control. Aloe vera gel attenuates the sodium nitrite induced memory impairment ($p < 0.001$). To study the effect of aloe vera gel peripheral cholinergic system, concentration response curve of acetylcholine (Ach) was plotted in presence and absence of aloe vera gel. Dose ratio of Ach with aloe vera gel (1 and 2mg/ml) was found to be 0.846 and 0.692 respectively. This shows muscarinic receptor sensitizing effect of aloe vera gel. Moreover, sodium nitrite induced elevation of brain acetylcholinesterase (AchE) activity was significantly ($P < 0.05$) lowered by pyritinol (100 mg/kg, p.o.) and aloe vera gel (100 and 200 mg/kg, p.o.), indicating the counteracting action on the cholinergic system. Attenuation of sodium nitrite induced memory impairment and cholinergic muscarinic receptors sensitization suggest the role of aloe vera gel for inflammatory memory disorders like alzheimer's.

Keywords: Aloe vera gel, muscarinic receptors, memory disorders, sodium nitrite

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Introduction

Aloe barbadensis miller (Aloe vera) belongs to a *Liliacea* family, of which there are about 360 species. Aloe vera has been promoted for large variety of conditions and has come to play a prominent role as a contemporary folk remedy[1]. The fresh leaves of aloe vera are used to obtain bitter yellow latex from peripheral bundle sheath, known for its laxative properties due to presence of anthraquinone glycosides called aloe vera gel sap, aloe vera gel juice.

It also contains amino acid, auxins, gibberellins, minerals, vitamins, an aspirin like compound, magnesium lactate, various enzymes like SOD [2] & β -sitosterol[3]. Aloe vera gel, beginning in the 50's, has gained respect as a commodity used as a base for nutritional drinks, as a moisturizer, and a healing agent in cosmetics and OTC drugs. Aloe products have long been used in health foods, medical and cosmetic purposes. Reports credit aloe with anti-inflammatory [4] and antidiabetic [5] properties in addition to efficacy in treatment of gastric ulcers [6]. Despite the extensive history and popular acceptance of dermatological products containing aloe, only few articles in the literature have discussed aloe as antioxidant and its physiological effects in biological systems. In this communication, we describe the role of aloe vera gel in sodium nitrite induced hypoxia and involvement in brain cholinergic system.

Its efficacy as an antioxidant was evaluated on elevated plus maze. Hypoxia is reported to cause memory disorders [7]and to evaluate the potential of aloe vera gel in the memory disorders its effect on dose response curve (DRC) of the acetylcholine (Ach) was evaluated & the results were compared with positive reference, pyritinol that is an established nootropic with cholinergic activity. To further explore, effect on brain acetylcholinesterase (AchE) was also evaluated.

Materials and Methods

Animals: Swiss mice (200-250 g) supplied by the central animal house facility (Reg. 173/CPCSEA), Jamia Hamdard were kept in polypropylene cages under standard conditions of temperature ($25 \pm 1^\circ \text{C}$) with 12 hr light / 12 hr dark cycle and had a free access to commercial pellet diet (Amrut laboratory rat & mice feed, Navmaharashtra Chakan oil mills Ltd., Pune, India) and water ad libitum. Study was approved by the Institutional Animal Ethical Committee (approval no.-123/CPCSEA/2005). Animals were kept as per guidelines of CPCSEA, Dept. of Animal Welfare, Govt. of India.

Drugs and Chemicals: Aloe vera gel was purchased from Nature, Forever Living Products International (USA) and Ach was purchased from Sigma Chemicals Co., USA. All other chemical were of standard analytical grade.

Experimental Section

1. Dose response curve (DRC): DRC of Ach ($10\mu\text{g/ml}$) was plotted in the presence and absence of aloe vera gel (1mg/ml and 2mg/ml) and pyritinol (1mg/ml). DRC was plotted using isolated rat ileum preparation (3 cm) isolated from an overnight fasted rat. Log dose Vs % response curve of Ach was plotted to calculate the dose ratio of Ach with aloe vera gel (1mg/ml and 2mg/ml) [8].

Dose ratio of Ach = $\frac{\text{EC}_{50} \text{ of Ach presence in of aloe vera gel}}$

$\frac{\text{EC}_{50} \text{ of Ach in absence of aloe vera gel}}$

2. Spontaneous alteration behavior (SAB). The study comprised of four groups (n=6) respectively

Group I. normal saline (1ml/kg , p.o.)+ sodium nitrite (75mg/kg , s.c)

Group II. aloe vera gel (100mg/kg , p.o.) + sodium nitrite (75mg/kg , s.c)

Group III. aloe vera gel (200mg/kg , p.o.) + sodium nitrite (75mg/kg , s.c)

Group IV. pyritinol (100mg/kg , p.o.) + sodium nitrite (75mg/kg , s.c)

All the treatment were given for 10 days and on the 10th day sodium nitrite was given and 1 hr after sodium nitrite mice were subjected to the behavioral studies (spontaneous alteration behavior). After 24 hours of the sodium nitrite, mice were again subjected to spontaneous alteration behavioral [9].

3. Estimation of brain AChE activity. Swiss mice of either sex weighing 20- 25 g were used.

Group I. (n=6), served as control and treated with normal saline

Group II. (n=6), was treated with sodium nitrite (75mg/kg, s.c.)

Group III. (n=6) with pyritinol (100 mg/kg, p.o.)

Group IV and Group V. (n=6) were treated with Aloe vera gel (100 mg/kg and 200 mg/kg, p.o.) respectively for 10 days

On the 11th day the animals were euthanized by cervical dislocation carefully to avoid any injuries to the tissue. The whole brain AChE activity was measured [10]. This was measured on the basis of the formation of yellow color due to the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The sample was first treated with 5, 5'- dithionitrobenzoic acid (DTNB) and the optical density (OD) of the yellow color compound formed during the reaction at 412 nm every minute for a period of three minutes was measured. Protein estimation was done using Folin's method. AChE activity was calculated using the following formula:

$$R = \frac{\Delta O.D \times \text{Volume of Assay (3 ml)}}{E \times \text{mg of protein}}$$

Where

R= rate of enzyme activity in 'n' mole of Δ acetylthiocholine iodide hydrolyzed / minute / mg protein

$\Delta O.D$ = Change in absorbance / minutes

E = Extinction coefficient = 13600 /M/cm

Statistical Analysis: Statistical analysis was carried out using Graph pad Prism 3.0 (Graph pad software; San Diego, CA). All results were expressed as Mean \pm S.E. Data analysis was done using ANOVA followed by Student-Newman-Keul's test for multiple comparisons. A $p \leq 0.05$ level of probability was used as criterion for significance.

Results

To evaluate the potential of the aloe vera gel on cholinergic system, DRC of Ach was plotted in presence and absence of aloe vera gel. Dose ratio of Ach was found to be 0.841 (aloe vera gel 1mg/ml) and 0.692 (aloe vera gel 2mg/ml), indicating left shift of the percentage response curve of Ach in presence of aloe vera gel.

Dose ratio of Ach was found to be 0.631 in presence of the pyritinol (1mg/ml), showing maximum potentiation of Ach activity (Fig: 1). Results show the dose dependent potentiation of the Ach action on peripheral muscarinic receptors by aloe vera.

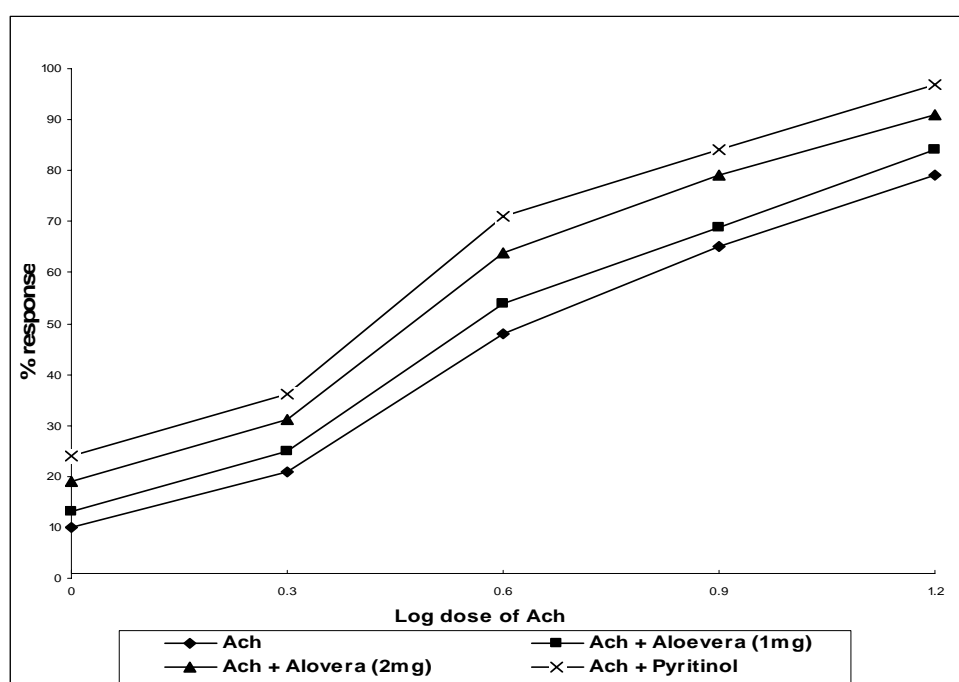


Fig 1: Log dose Vs % response curve of acetylcholine in presence and absence of aloe vera gel and pyritinol

Sodium nitrite resulted in decreased memory scores. The aloe vera gel (100mg/kg) pretreated group showed a moderate decrease in severity of hypoxia and increase in SAB as compared to the normal control. Administration of aloe vera gel (100mg/kg) resulted in moderate increase in percent alterations, when compared with saline treated group but not up to a significant level (Table: 1).

Table 1: Effect of aloe vera gel on sodium nitrite induced hypoxic cognition deficit in rats on elevated plus maze test.

S. No.	Treatment	Zero day	Third day	1 hr after Sodium nitrite	24 hr after Sodium nitrite
Group 1	Normal saline (1ml/kg, p.o.) + Sodium nitrite (75mg/kg, s.c.)	78.32 ± 1.08	76.15 ± 1.25	49.00 ± 2.42	54.44 ± 1.77
Group 2	Aloe vera gel (100mg/kg, p.o.) + Sodium nitrite (75mg/kg, s.c.)	73.62 ± 1.84	73.37 ± 1.07	51.10 ± 2.58	53.84 ± 2.09
Group 3	Aloe vera gel (200mg/kg, p.o.) + Sodium nitrite (75mg/kg, s.c.)	70.28 ± 3.06	71.86 ± 2.00	54.08 ± 3.04 ^c	66.11 ± 1.77 ^c
Group 4	Pyritinol (100mg/kg, p.o.) + Sodium nitrite (75mg/kg, s.c.)	81.65 ± 2.94	80.01 ± 0.92	68.45 ± 2.11 ^a	75.51 ± 3.27 ^a

(Values are Mean ± SEM), each group contains 6 animals

All groups were compared to the group 1 by Students-Newman-Keul's-test.

(^a P<0.001, ^b P<0.01, ^c P<0.05)

Aloe vera gel (200mg/kg) treatment significantly (p<0.05) increased memory scores both after 1 hour and 24 hours of the sodium nitrite treatment. Pyritinol (100mg/kg) treated group taken as positive control, showed a significant (p<0.001) increased percentage alternations as compared to the normal saline group.

Table 2: Effect of Aloe vera gel and Pyritinol on AChE activity in aged mice

Treatment	Dose	AChE (μ moles)
Control	10 (mg/kg, p.o.)	118.45 \pm 6.20
Sodium nitrite	75 (mg/kg, s.c.)	192.2 \pm 1.84*
Pyritinol	100 (mg/kg, p.o.)	90.55 \pm 8.68*
Aloe vera gel	100 (mg/kg, p.o.)	93.27 \pm 8.52*
Aloe vera gel	200 (mg/kg, p.o.)	86.71 \pm 8.10*

Values are mean \pm SEM, AChE- whole brain AChE activity

P<0.05 Vs control (Students Newmans Keul's Test - Multiple comparison test)

The whole brain AChE activity with sodium nitrite (75mg/kg, s.c.) demonstrated significant rise in AChE activity as compared to control and pyritinol (200 mg/kg, p.o.) treated groups. Aloe vera gel (100 and 200 mg/kg, p.o.) significantly (P<0.05) lowered AChE activity (Table: 2).Diarrhea was observed in animals after seven days treatment with aloe vera gel.

Discussion

The present study indicates that aloe vera is a potential anti-cholinesterase agent. It also possesses nootropic activity in view of its facilitatory effect on retention of acquired learning. A deficient cholinergic system has been implicated in the impairment of memory [11]. Hypoxia is reported to cause memory disorders. Many diseases e.g. alzheimer's disease, that are associated with memory deficits, also manifest a deficient cholinergic system with inflammatory lesions in the cortical regions [12].

Aloe vera gel potentiates the action of Ach on isolated rat ileum, which enumerates its role in cholinergic system. Sodium nitrite per se (75 mg/kg, s.c.) significantly elevated brain AchE activity. Pyritinol (100 mg/kg, p.o.) and aloe vera gel (100 and 200 mg/kg, p.o.), on the other hand, significantly ($P < 0.05$) lowered this activity indicating the counteracting action against sodium nitrite on the cholinergic system. Aloe vera gel also reversed the sodium nitrite impairment in learning and memory, when assessed on elevated plus maze, showing its antioxidant potential.

Immunohistochemical studies suggested the existence of chronic inflammation in certain regions of the brain in Alzheimer's disease patients. Since inflammation can be damaging to host tissue, it was hypothesized that anti-inflammatory drugs might be inhibiting both the onset and the progression of Alzheimer's disease. This hypothesis is supported by the observation that indomethacin (NSAID) halted the progressive memory loss seen in Alzheimer's disease patients [13]. Anti-inflammatory action of Aloe vera might also be contributing to the observed memory-enhancing activity [14]. Oxygen free radicals, the harmful by-products of oxidative metabolism are known to cause organic damage to the living system, which may be responsible for the development of Alzheimer's disease in the elderly [15]. Aloe vera is reported to possess antioxidant activity due to presence of anthraquinones [16] and have anti-inflammatory activity due to presence various auxins, gibberellins and other aspirin like compounds. Thus, a combination of acetyl cholinesterase inhibition, anti-inflammatory and antioxidant role of aloe vera could all be leading to the net memory enhancing effect.

Hence Aloe vera may be useful as a nootropic agent in the treatment of various cognitive disorders. Further investigations using more experimental paradigms are required for further confirmation of nootropic potential of aloe vera in the treatment of various cognitive disorders.

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