



Evaluation of antianxiety activity of lyophilized *Aloe vera* succulent in Albino Swiss mice

KEYWORDS

Antianxiety activity, Lyophilized *Aloe vera* succulent, Albino Swiss mice

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ABSTRACT Anxiety disorders are quite common among the neuropsychiatric problems. Present pharmacological treatment is effective but not devoid of unwanted side effects. *Aloe vera* is a medicinal plant of great importance. In this study potential of *Aloe vera* succulent for antianxiety activity evaluated in Albino mice with Elevated plus maze and Light and Dark box models. It showed significant antianxiety activity in both models with 200mg/kg and 300mg/kg doses. The open arm and light box stay increased in dose dependant manner, while it was not significant with 100mg/kg dose.

INTRODUCTION:

One-eighth of the total population of the world and has been affected by anxiety. It has very important area of interest in psychopharmacology since last decade. Anxiety is characterized by excessive fear, motor tension, sympathetic hyperactivity, apprehension, and vigilance syndromes. Benzodiazepines are the major class of compounds that are used in anxiety and they are the most common prescribed treatment for anxiety, despite the important unwanted side effects that they produce such as sedation, muscle relaxation, ataxia, amnesia, ethanol and barbiturate potentiation and tolerance¹. In quest of finding new therapeutic agents for the treatment of neurological ailments, medicinal plant research worldwide, has progressed constantly demonstrating the pharmacological effectiveness of different plant species in a variety of animal models². Different herbal medicines have been used as anxiolytic drugs in different parts of the world³. *Aloe vera* (L.) Burm.f. (*Aloe barbadensis* Miller) is a perennial xerophytic succulent plant. The inner part of the leaf is a clear, soft, moist, and slippery tissue that consists of large thin-walled parenchyma cells in which water is held in the form of a viscous gel and forms succulent⁴. Apart from *Aloe* being used extensively in the cosmetic industry, it has been described for centuries for its laxative, anti-inflammatory, immunostimulant, antiseptic [okyar], wound and burn healing [chithra], antitumor [koo], antitumor [saito], and antidiabetic [bunyaprabhatsara] activities⁵⁻⁹. More than 75 active ingredients from inner gel have been identified including vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid¹⁰. There have been many *Aloe vera* products in market claiming calming and anxiolytic effect¹¹. Basic preclinical studies suggest anxiolytic and sedative activity¹².

MATERIAL AND METHODS:

The study was conducted in Department of Pharmacology, Gajra Raja Medical College, Gwalior (M.P.). The experimental protocol was approved by Institutional Animal Ethical Committee of the institution.

Aloe vera plant purchased from a private nursery. Leaves were washed with tap water. Succulent from leaves was procured, grinded in electric grinder and filtered through muslin cloth. The filtrate was lyophilized. This lyophilized

Succulent of *Aloe vera* (AVS) was used for study. Tab. Alprex - Alprazolam 0.5mg (Torrent Pharmaceuticals Ltd) and gum acacia was purchased from market.

Albino mice (Swiss strain) weighing 25-30 gm of either sex from institutional Animal House were used. All animals under experiment were kept under 12 hr light dark cycle, and were provided with food and water *ad libitum*.

Animals were divided into five groups of six animals each. Group 1 (GA), was administered 2% Gum acacia suspension 10ml/kg per orally (p.o.). Group 2, 3, and 4 were treated with test drug AVS 100mg/kg p.o. (AVS100), AVS 200mg/kg p.o.(AVS200) and AVS 300mg/kg p.o.(AVS300) respectively. While Group 5(ALP5), was treated with standard anxiolytic drug, Alprazolam 5mg/kg p.o. All test and standard drugs used as fresh made suspension with 2% gum.

Elevated Plus Maze (EPM) and Light and Dark box (LD box) models were used for antianxiety activity.

Study of Anxiolytic activity of AVS in Albino mice using Elevated Plus Maze method:

The animals received treatment as per schedule, 30 minutes before the start of the session, animal was placed at the centre of maze, while head facing the open arm and total time of stay and number entries in different arms were recorded for five minutes. An entry is defined as the presence of all four paws in the arm. The EPM was carefully wiped with 10% ethanol after each trial, to eliminate the possible bias due to the odors of the previous animal^{13,14}.

Study of Anxiolytic activity of ALV in Albino mice using Light and Dark box method:

The animals received treatment as per schedule, 30 minutes before the start of the session, animal was placed at the centre of illuminated white chamber (light box), and its head facing the side wall. Total time of stay in dark and light box was measured and entry from dark to light box was counted, all for five minutes. An entry is defined as the presence of all four paws in the respective box. The LD was carefully wiped with 10% ethanol after each trial, to eliminate the possible bias due to the odors of the previous animal. Drug-induced relative increase in behaviors in

the white part of a two-compartment box, in which white compartment is illuminated and black compartment is darkened, is suggested as an index of anxiolytic activity¹⁵.

One way ANOVA followed by tukey's comparison test was applied for statistical analysis with the help of GraphPad Prism software version 4.0.

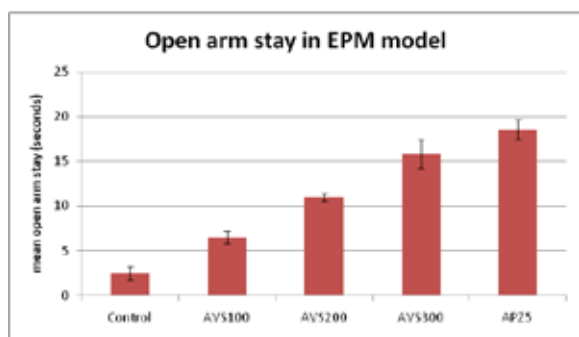
RESULTS:

Evaluation of antianxiety activity on EPM showed increase in open arm stay was increased in all the drug treated groups compared to Control group. Increase for AVS100 was not statistically significant ($p>0.05$) as compare to Control group. While increase in open arm stay in AVS200, AVS300 and APZ5 was statistically significant ($p<0.001$) compare to Control group. AVS treated groups showed dose dependent effect on open arm stays AVS100 vs. AVS200 ($p<0.05$) and AVS200 vs. AVS300 ($p<0.05$). Open arm stay of AVS300 and standard drug group APZ5 was comparable ($p>0.05$). Open arm entries, Rears and head dipping count also showed significant rise compare to Control group. Effect on entries was also dose dependent and significant.

Table-1: Effect of AVS on EPM model parameters in mice (n=6, All values represented as Mean±SEM. a = $p<0.05$ as compared to Control group, b = $p<0.05$ as compared to AVS100, and c = $p<0.05$ as compared to AVS200.)

| Mean±SEM | Open arm stay (seconds) | Closed arm Stay (seconds) | Open arm Entries (numbers) | Rears (numbers) |
|----------|-----------------------------|------------------------------|----------------------------|-----------------------------|
| Control | 2.5±0.76 | 297.5±0.76 | 1.17±0.31 | 8.33±1.12 |
| AVS100 | 6.5±0.72 | 293.5±0.72 | 2.33±0.49 | 12.83±2.68 |
| AVS200 | 11±0.45 ^{a,b} | 289±0.45 ^{a,b} | 5.67±0.67 ^{a,b} | 23.33±2.6 ^{a,b} |
| AVS300 | 15.83±1.62 ^{a,b,c} | 284.17±1.62 ^{a,b,c} | 9±0.97 ^{a,b,c} | 33.33±2.65 ^{a,b} |
| APZ5 | 18.5±1.12 ^{a,b,c} | 281.5±1.12 ^{a,b,c} | 9.33±0.88 ^{a,b,c} | 35.67±2.65 ^{a,b,c} |

Figure-1: Effect of AVS on Open arm stay in EPM model



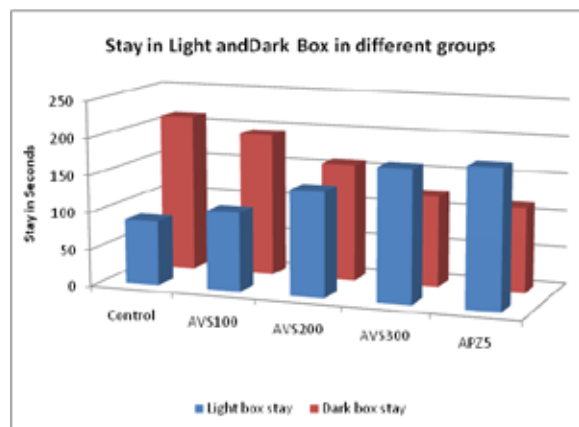
The Light Box stay was increased in all the drug treated groups compared to Control group. AVS100 showed increase in Light box stay which was not statistically significant ($p>0.05$) compare to Control group, while increase in Light box stay in AVS200, AVS300 and APZ5 was statistically significant ($p<0.001$) compare to Control group. AVS treated groups showed dose dependent effect on Light box stays AVS100 vs. AVS200 ($p<0.05$) and AVS200 vs. AVS300 ($p<0.05$). Light box stay of AVS300 and standard drug group APZ5 showed no statistical difference ($p>0.05$). Light box entries and Rearing count also showed rise compare to Control group, which was not significant for

AVS100 group, while significant ($p<0.01$) for AVS200, AVS300 and APZ5. Effect on entries was significant dose dependent, while dose dependant effect that on rearing and head dips was not significant.

Table-2: Effect of AVS on LD box parameters in mice (n=6, All values represented as Mean±SEM, a = $p<0.05$ as compared to Control group, b = $p<0.05$ as compared to AVS100, and c = $p<0.05$ as compared to AVS200.)

| Mean ± SEM | Light box stay (seconds) | Dark box stay (seconds) | Light box entries (number) | Rears (number) |
|------------|-------------------------------|-------------------------------|-----------------------------|----------------------------|
| Control | 87.33±3.04 | 212.67±26.88 | 4.5±0.76 | 8.83±0.87 |
| AVS100 | 106.33±2.58 | 193.67±2.58 | 9.67±0.67 ^a | 12.17±1.19 |
| AVS200 | 141.5±5.21 ^{a,b} | 158.5±5.21 ^{a,b} | 14.5±0.85 ^{a,b} | 15.5±0.96 ^{a,b} |
| AVS300 | 177.5±9.98 ^{a,b,c} | 122.5±9.98 ^{a,b,c} | 19.5±0.99 ^{a,b,c} | 22.5±1.48 ^{a,b,c} |
| APZ5 | 185.33±10.39 ^{a,b,c} | 114.67±10.39 ^{a,b,c} | 23.83±1.92 ^{a,b,c} | 24.5±1.73 ^{a,b,c} |

Figure-2: Effect of AVS on Light and Dark box stay in mice



DISCUSSION:

AVS showed significant dose dependent antianxiety activity in EPM model. AVS not only showed significant increase in open arm stay but also have shown significant increase in numbers of entries, rears and head dips at 200 and 300 mg/kg of dose. AVS also showed significant dose dependent antianxiety activity in Light and dark box (LD) model. AVS showed significant increase in light box stay, number of entries and rear count with 200 and 300 mg/kg of dose. Antianxiety effect of AVS at 300mg/kg was comparable to Alprazolam (standard drug) at 5 mg/kg of dose, in both models. In a study by Sultana and Najam (2012), they have shown antianxiety of *Aloe vera* in mice by using different behavioral models other than ours¹². While in our study we have evaluated antianxiety activity in standard models (EPM and LD) for evaluation of anti anxiety activity. Antianxiety drugs such as Alprazolam (Benzodiazepine) show excellent results in EPM and LD box models. Mechanism of these drugs said to be swinging neurotransmitter balance in favor of GABAergic transmission while decrement in excitatory Glutaminergic neurotransmission. Some drugs with mechanism involving SSRIs and SNRIs also being used in anxiety disorder^{16,17}. Modulation of GABAergic, Glu-

taminergic, or monoaminergic pathway may be involved in antianxiety action of *Aloe vera* that can not be concluded from such a preliminary study further studies are needed to find the answers:

REFERENCE

- Lader, M., & Morton, S. (1991). Benzodiazepine problems. *British Journal of Addiction*, 86(7), 823-828. | 2. Zhang, Z. J. (2004). Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. *Life Science*, 75, 1659-1699. | 3. Gray, A. M., & Flatt, P. R. (1999). Insulin-releasing and insulin-like activity of the traditional anti-diabetic plant *C. sativum* (coriander). *British Journal of Nutrition*, 81(3), 203-209. | 4. Dabai, Y. U., Muhammad, S., & Aliyu, B. S. (2007). Antibacterial activity of anthraquinone fraction of *Vitex doniana*. *Pakistan Journal of Biological Sciences*, 1-3. | 5. Okyar, A., Can, A., Akev, N., Baktir, G., & Sutlupinar, S. (2001). Effect of *Aloe vera* leaves on blood glucose level in type I and type II diabetic rat models. *Phytotherapy Research*, 15(2), 157-161. doi: 10.1002/ptr.719. | 6. Chithra, P., Sajithlal, G. B., & Chandrakasan, G. (1998). Influence of *Aloe vera* on the healing of dermal wounds in diabetic rats. *Journal of Ethnopharmacology*, 59(3), 195-201. doi: 10.1016/S0378-8741(97)00124-4. | 7. Koo, M. W. L. (1994). *Aloe vera*: antiulcer and antidiabetic effects. *Phytotherapy Research*, 8(8), 461-464. doi: 10.1002/ptr.2650080805. | 8. Saito, H. (1993). Purification of active substances of *Aloe arborescens* Miller and their biological and pharmacological activity. *Phytotherapy Research*, 7(7), pp. S14-S19. doi: 10.1002/ptr.2650070707. | 9. Bunyapraphatsara, N., Yongchaiyudha, S., Rungpitarangsi, V., & Chokechajareonporn, O. (1996). Antidiabetic activity of *Aloe vera* L. juice. II. Clinical trials in diabetes mellitus patients in combination with glibenclamide. *Phytomedicine*, 3(3), 245-248. doi: 10.1016/S0944-7113(96)80061-4. | 10. Hamman, J. H. (2008). Composition and applications of *Aloe vera* leaf gel. *Molecules*, 13(8), 1599-1616. | 11. Simpraga, S. (2014). *Aloe vera*: natural approach to mental wellness. Retrieved from <http://everythingcountsmarketing.com/aloee-and-depression/> (cited on 15 Aug, 2014) | 12. Sultana, N., & Najam, R. (2012). Anxiolytic activity of *Aloe vera* (L.) Burm.f tested in rodents. *Pakistan Journal of Pharmacology*, 29(1), 7-15. | 13. Bourin, M., Petit-Demoulière, B., Dhonnchadha, B. N., & Hascoët, M. (2007). Animal models of anxiety in mice. *Fundamental and Clinical Pharmacology*, 21(6), 567-574. | 14. Kothari, S., Minda, M., & Tonpays, S. D. (2010). Anxiolytic and antidepressant activities of methanol extract of *Aegle marmelos*. *Indian Journal of physiology and Pharmacology*, 54(4), 318-328. | 15. Bourin, M., & Hascoët, M. (2003). The mouse light/dark box test. *European Journal of Pharmacology*, 463(1-3), 55-65. | 16. Sharma, H.L., & Sharma, K. K. (2011). Anxiolytics and Hypnotics In Sharma, H.L., & Sharma, K. K. (2nd ed.) *Principles of Pharmacology* (pp. 442-450). Hyderabad: Paras Medical publisher. | 17. Vasile, R. G., Bruce, S. E., Goisman, R. M., Pagano, M., & Keller, M. B. (2005). Results of naturalistic study of benzodiazepine and SSRI in the treatment of generalized anxiety disorder and social phobia. *Depression and Anxiety*, 22(2), 59-67. |