Research Paper Medical Science

Evaluation of Analgesic Activity of Lyophilized Aloe Vera Succulent in Albino Swiss Mice

Dr. Pritesh Goutam Assistant Professor, Dept. Of Psychiatry, Laxmi Narayan Medical College, Bhopal, Madhya Pradesh.

Dr. Rajesh Kumar Assistant Professor, Dept. Of Pharmacology, N.S.C.B. Medical College,Jabalpur.

ABSTRACT

Pain is a protective and unpleasant sensation. Long-term use of NSAIDs and opioid drugs is associated with serious adverse effects. Aloe vera have been used a remedy for pain and some studies shown anti-inflammatory activities. Lyophilized succulent Aloe vera (AVS) have shown analgesic property in tail flick latency (TFL) and writhing model (acetic acid induced writhing method). Lyophilized succulent Aloe vera (AVS) in the doses of 200 and 300 mg/kg have shown significant analgesic activity and tail flick latency model in mice of radiant heat method. The analgesic effect of AVS with dose 300 mg/kg was comparable to that of 10 mg/kg dose of Diclofenac sodium (standard drug). AVS showed significant reduction in writhes with 200 and 300 mg/kg of dose. Analgesic activity of AVS at 300mg/kg in writhing method was comparable with that of Diclofenac sodium (standard drug) at 10 mg/kg of dose.

KEYWORDS Analgesic activity, Lyophilized Aloe vera succulent, Albino Swiss mice.

Introduction

Pain is a protective and unpleasant sensation. It is produced in response to noxious stimuli. Pain involves complex pathophysiology. Peripherally stimulation of nociceptors being sensitized and stimulated by low pH, Substance P, Histamine, Bradykinins and most importantly Prostaglandins and Leukotrienes play major role. Cycloxygenase (COX) enzyme is key enzyme that produces an array of all these inflammatory cytokines that produce pain. COX also present in spinal cord where it produces Prostaglandins and facilitate pain transmission. Along with pain pathway there are present opioid receptors and monoaminergic pain modulating circuits that play role in pain modulation.[1,2,3]

Drugs commonly used in modern medicine for suppression of pain and inflammation like non-steroidal anti-inflammatory drugs and corticosteroids provide only symptomatic relief. Long-term use of these drugs is associated with serious adverse effects. Hence, the search for a new, safe analgesic and anti-inflammatory drug is ongoing.[4]

Medicinal plants are the basis for the treatment of various ailments for centuries.[5] Almost four out of five persons from developing countries still depend on plant-based traditional medicine for their primary health care and almost three-fourths of the herbal drugs used worldwide are derived from medicinal plants.[6] However, the quality control of herbal medicine remains a challenge owing to the fact that there is a high variability in the active constituents involved.[7] Hence, World Health Organization (WHO) has approved fingerprint technique or standardized extract for quality assurance of herbal medicines.[8]

Aloe vera (L.) Burm.f. (***Aloe barbadensis*** Miller) is a perennial xerophytic succulent plant. 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], antiulcer [koo], antitumor [saito], and antidiabetic [bunyaprabhatsara] activities[9-13]. 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 acid[14]. ***Aloe vera*** have been used a remedy for pain and some studies shown anti-inflammatory activities. Thus preclinical experimental study was planned to evaluate its analgesic potential of ***Aloe vera***.

Material and methods:

The study was conducted in Department of Pharmacology, Gajra Raja Medical College, Gwalior (M.P.) during author's residency. The experimental protocol was approved by Institutional Animal Ethical Committee of the institution. ***Aloe vera*** plant purchased from a private nursery and lyophilized Succulent of ***Aloe vera*** (AVS) was prepared and used. Tab. Voveran (Diclofenac sodium) 50mg tablet – (Novartis Pharmaceuticals Pvt. Ltd.) purchaged from market and Morphine Sulphate was used from lab. Albino mice (Swiss strain) weighing 25-30 gm of either sex were kept under 12 hr light dark cycle, and were provided with food and water ***ad libitum***.

Analgesic effect was evaluated by the method described by **Devies**, **Reventos and Walpole (1946)** by using hot wire type of analgesiometer for evaluation of central analgesic activity with some modifications[15,16] and acetic acid induced writhing model for peripheral analgesic activity. 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(MPH1), was treated with standard centrally acting drug, Morphine 1 mg/kg p.o. for tail flick latency model while designated DCF10 was treated with standard drug Diclofenac 10 mg/kg p.o. in acetic acid induced writhing. All test and standard drugs used as fresh made suspension with 2% gum.

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

Tail Flick Latency (TFL) in mice :

All drug treated animal groups shown increased TFL as compared to their basal readings and **Control** group. The increase in TFL was not significant for **AVS100** (**p>0.05**) while it was significantly increased in cases of **AVS200** (at 3 and 4 hr) and **AVS300** (at 1,2,3 and 4 hr) and **DCF10** (1,2,3 and 4 hr), with **p<0.001** when compared with Basal reading group TFL. Different doses group of AVS, shown dose dependant increase in TFL, with significant difference between **AVS100 vs. AVS200**

(**p<0.05**) and between **AVS200 vs. AVS300** (**p<0.05**). Increase in TFL in **AVS300** group was comparable to that of **DCF10** at ½, 1, 3, and 4 hr (**p>0.05**). Maximaum effect of **MPH1** on TFL is seen at 3 hrs then it falls to 8.18 sec at 4 hrs.

Graph-1: Effect of AVS on TFL in mice

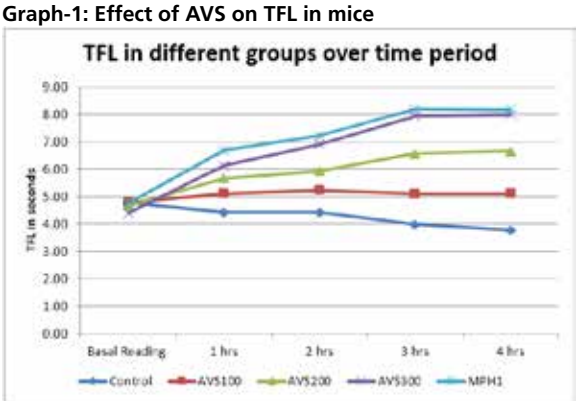

Table 5: Effect of AVS in Acetic acid induced writhing in mice

Groups	Number of writhes in 20mins	Percent inhibition (%)
Control	20.33 ± 1.48	----
AVS100	17 ± 1.18	16.38
AVS200	12.83 ± 0.65	36.89[a,b]
AVS300	7.17 ± 0.48	64.73[a,b,c]
DCF10	5.5 ± 0.76	72.95[a,b,c]

n=6, number of writhes represented as **Mean±SEM**.

One way ANOVA followed by tukey's multiple comparison test

df=, F= 41.30

a = p<0.001 as compared to Control group.

b = p<0.05 as compared to AVS100.

c = p<0.05 as compared to AVS200.

Acetic acid induced writhing in different groups:
All the drug treated groups have shown inhibition in writhing. Percent inhibition in **AVS100** group (**16.38 %**) was not significantly different (**p>0.05**) than Control group, while was found to be significant in **AVS200** (**36.89 %, p<0.001**) and **AVS300** (**64.77 %, p<0.001**). There was significant inhibition of writhes (**72.95 %, p<0.001**) in **DCF10** group (standard drug). Different groups of AVS have shown dose dependent inhibition of writhing, with significant difference between **AVS100 vs. AVS200** (**p<0.05**) and **AVS200 vs. AVS300** (**p<0.01**). The percent inhibition with **AVS300** was comparable with **DCF10** (**p>0.05 %**).

Discussion:
AVS have shown analgesic property in tail flick latency (TFL) (radiant heat method) and writhing model (acetic acid induced writhing method).

AVS in the doses of 200 and 300 mg/kg have shown significant analgesic activity and tail flick latency model in mice of radiant heat method. The analgesic effect of AVS with dose 300 mg/kg was comparable to that of 10 mg/kg dose of Diclofenac sodium (standard drug). In a study by **Ghosh *et al.* (2011)** using lyophilized aqueous extract of ***Aloe vera*** succulent found that ***Aloe vera*** extract have shown analgesic activity at 100, 200 and 300 mg/kg in rats.[17] These results were similar to our study except the increase in TFL in our study was not significant at the dose of 100 mg/kg. They also evaluated analgesic activity on Hot plate model, in which they found significant analgesic activity only on 300 mg/kg of dose. This slight difference in results may be because of possible variation in plant constituents due to climatic and seasonal variation.

AVS showed significant dose dependent analgesic activity in mice with acetic acid induced writhing. Writhing model for analgesic activity considered to be a valid model for accessing analgesic activity of peripherally acting drugs. Introduction of irritant compound in peritoneal cavity induced production and secretion of PGE_2 and $PGF_{2\alpha}$ into the peritoneal cavity, which is the reason for pain induction and writhing.[18,19] AVS showed significant reduction in writhes with 200 and 300 mg/kg of dose. Analgesic activity of AVS at 300mg/kg in writhing method was comparable with that of Diclofenac sodium (standard drug) at 10 mg/kg of dose. A Similar kind of study done by **Ghosh *et al.* (2011)** shown significant analgesic activity only with 300 mg/kg of dose in rats.[17] This difference in results may because of possible variation in plant constituents due to climatic and seasonal variation, different animal used in their study (rat) or because of fact that they have used 4% of NaCl as writhes inducing agents while in our study we use 1% (v/v) acetic acid to induce writhes.

Aloe vera have anti-inflamatory activity which is considered to be due to inhibition of arachidonic acid pathway by inhibiting COX. Various compounds present in ***Aloe vera*** was found to have COX inhibiting activity i.e. salicylic acid and carbohydrate fraction. Bradykinase present in ***Aloe vera*** degraded Bradykinin which is one of the most important mediators of pain after PGs. It also known to inhibit histamine production.[1,20] Some other componates with analgesic effect are present in ***Aloe vera*** like cinnamic acid and Isobarbaloin.[21] Evidence suggest most possible pharmacodynamic target for ***Aloe vera***'s analgesic activity is arachidonic pathway and bradykinin degradation but there may be involve central pathways of pain modulation. Further pharmacodynamic studies are needed in this regard.

REFERENCES

1. Ito S, Teradaira R, Beppu H, Obata M, Nagatsu T, Fujita K. Properties and pharmacological activity of carboxypeptidase in Aloe arborescens Mill. var. natalensis Berger. Phytotherapy Res 1993; 7: S26-S29. | 2. Fields HL, Martin JB. Harrison's principles of internal medicine. 17th ed. New York : McGraw-Hill Companies Inc.; 2008. Chapter 12, Pain: Pathology and management; p81-86. | 3. Smyth EM, Grosser T, FitzGerald GA. Goodman & Gilman's The Pharmacological basis of therapeutics. 12th ed. New York: McGraw-Hill Companies Inc.; 2011. Chapter 33, Lipid-Derived Autocoids: Eicisanoids and Platelet-Activaing Factor; 937-955. | 4. Sparrow CP, Burton CA, Hernandez M, Mundt S, Hassing H, Patel S, et al. Simvastatin has anti-inflammatory and antiatherosclerotic activities independent of plasma cholesterol lowering. Arterioscler Thromb Vasc Biol. 2001;21:115–21. | 5. Ridtitid W, Sae-Wong C, Reanmongkol W, Wongnawa M. Antinociceptive activity of the methanolic extract of Kaempferia galanga Linn. in experimental animals. J Ethnopharmacol. 2008;118:225–30. | 6. Verma S, Singh SP. Current and future status of herbal medicine. Veterinary World. 2008;1:347–50. | 7. Lijuan M, Xuezhu Z, Haiping Z, Yiru G. Development of a fingerprint of Salvia miltiorrhiza Bunge by high-performance liquid chromatography with a coulometric electrode array system. J Chromatogr B Analyt Technol Biomed Life Sci. 2007;846:139–46. | 8. WHO Guidelines for the Assessment of Herbal Medicine. Munich: World Health Organization; 1991. | 9. 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. | 10. 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. | 11. Koo, M. W. L. (1994). Aloe vera: antiulcer and antidiabetic effects. Phytotherapy Research, 8(8), 461–464. doi: 10.1002/ptr.2650080805. | 12. 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. | 13. Bunyapraphatsara, N., Yongchaiyudha, S., Rungpitarangsi, V., & Chokechaijaroenporn, 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. | 14. Hamman, J. H. (2008). Composition and applications of Aloe vera leaf gel. Molecules, 13(8), 1599–1616. | 15. Kothari S, Kushwah A, Kothari D. Involvement of opioid and monoaminergic pain pathways in Aegle marmelos induced analgesia in mice. Indian J Pharmacol. 2013 Jul-Aug;45(4):371-5. | 16. Jain NK, Singh A, Kulkrni SK. Analgesic, anti inflammatory in mice and rats. Pharma Pharmacol Commun 1999;5:599-602. | 17. Ghosh AK , Banerjee M, Mandal TK, Mishra A, Bhowmik MK. A Study on Analgesic Efficacy and Adverse Effects of Aloe vera in Wistar Rats. Pharmacol on line [Internet]2011;1:1098-1108. | 18. Collier HDJ, Dinnin LC, Johnson CA, Schneider C. Abdominal response and its suppression by analgesic drugs in mouse. Br J Pharmacol 1968; 32:295-310. | 19. Bentley GA, Newton SH, Starr J. Studies on anti nociceptive action of drugs and their interaction with opioid mechanism. Br J Pharmacol 1983;79:125-34. | 20. Canigueral S, Vila R. Aloe. Br J phytother1993;3:p67-75. | 21. Basseti A, Sala S. The great Aloe book. 1st ed. Trento: Zuccari Pvt. Ltd; 2001. Chapter 2, The Boatany and Chemical composition;53-55. |