ORIGINAL ARTICLE

Paracetamol like antipyretic activity of lyophilized succulent of *Aloe vera* leaves in rats

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Abstract

Aims: To evaluate the antipyretic activity of lyophilized succulent of *Aloe vera* (AVS) as well as its interaction with conventional antipyretic drug paracetamol (PCM) against experimentally induced pyrexia in rats. **Materials and Methods:** Pyrexia was induced experimentally by either Brewer's yeast or misoprostol injection after recording basal rectal temperature in rats. Animals were treated with lyophilized AVS at the dose of 100, 200, and 300 mg kg and standard drug PCM 150 mg/kg, 18 h after injection of Brewer's yeast suspension in one set of experiment and combination of sub-effective dose of AVS with that of PCM 1 h after injecting misoprostol in other set of experiment. Rectal temperature was recorded at different time intervals after drug administration. AVS was also phytochemically screened for the presence of tannins, saponins, flavonoids, coumarins, sterols, reducing sugar, glycosides, starch, alkaloids, and triterpenoids. **Results:** Pyrexia was observed at 18 h and at 1 h after Brewer's yeast and misoprostol injection subcutaneous, respectively. AVS (200 and 300 mg/kg) treated groups showed significant fall in rectal temperature ($P < 0.05$, $P < 0.01$, respectively) in Brewer's yeast-induced pyrexia as compared to control and AVS 100 mg/kg treated groups at different time intervals. The antipyretic activity of AVS at 300 mg/kg was comparable ($P > 0.05$) with standard drug PCM at 150 mg/kg. The combination of sub-effective dose of AVS (100 mg/kg) with that of PCM (50 mg/kg) produced significant ($P < 0.05$) antipyretic activity against misoprostol-induced pyrexia at different time intervals as compared with control, AVS 100 mg/kg and PCM 50 mg/kg treated groups when used alone. Phytochemical tests showed the presence of flavonoids, saponins, tannins, reducing sugar, glycoside, starch, and steroids. **Conclusion:** Lyophilized AVS possesses antipyretic activity. It enhances the antipyretic activity of PCM suggesting its role in inhibition of prostaglandin synthesis.

Key words: *Aloe vera*, antipyretic, Brewer's yeast, misoprostol, rectal temperature

INTRODUCTION

Pyrexia or febrile response is characterized by an elevation of body temperature above the normal range due to an increase in the temperature regulatory set-point.[1] It is important medical signs of: Bacterial infections, viral infections, immunological diseases, tissue destruction, cancers, reaction to incompatible blood products, and metabolic disorders.[2] The high fever often increases faster disease progression by increasing tissue catabolism, dehydration, and existing complaints.[3] Antipyretic drugs commonly used at present to treat fever such as paracetamol (PCM), aspirin, and nimesulide have toxic effects like hepatic damage and agranulocytosis.[4] Medicinal plants contain many chemical compounds which are the major source of therapeutic agents to cure human diseases. Various medicinal plants such as Neem, Arjuna, Ashwagandha, Tulsi, etc., are traditionally used to treat fever. Recently, search for safe herbal remedies with potent antipyretic activity received momentum.[5-7]

Aloe vera (L.) in synonym *Aloe barbadensis* is a cactus-like succulent plant with green, lance-shaped fleshy leaves with spiny margins, has been used for a variety of medicinal purposes and plays an important role in the international

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market.[8] *A. vera* gel present in the inner central zone (parenchyma) of the leaf is reported to have analgesic, anti-inflammatory, antioxidant, anxiolytic, anti-diabetic, antiulcer, and wound healing properties.[9-14]

The literature survey revealed that no scientific investigation has been made regarding antipyretic activity of *A. vera*; hence, the present study is undertaken to evaluate the antipyretic activity of lyophilized succulent of *A. vera* (AVS) leaves using Brewer's yeast-induced pyrexia model and to find its interaction with conventional antipyretic drug PCM to elucidate its role in cyclooxygenase enzyme inhibition using misoprostol-induced pyrexia model in rats.

MATERIALS AND METHODS

Plant Material Collection

The fresh leaves of *A. vera* were collected from Haryali Nursery, Palace Road, Gwalior, Madhya Pradesh, India during May 2012. The plant leaves were identified by Dr. K. K. Koul Professor and Head, Department of the Botany, Jiwaji University, Gwalior, India as *A. vera* and sample specimen (AV12) was deposited in the Department of Pharmacology, G. R. Medical College, Gwalior, India.

Preparation of Lyophilized AVS

The collected leaves were washed in cold water. The lower 1″ of leaf base and tapering 2-4″ inch of leaf top along with spines on the side wall were cut using a knife. The outer green rind was separated from the inner succulent by introducing knife between the rind and succulent. 2 kg of the succulent were cut into pieces and were blended in an electric blender for 10 min. The blended material was squeezed through muslin cloth. The filtrate was freeze dried under vacuum using lyophilizer. Six g of semisolid material (yield 0.3% w/w) were obtained and stored in amber colored bottles in refrigerator at 4°C until use for the study.

Drugs and Chemicals

Misoprostol (Pfizer) and PCM (Cipla) were purchased from the market and were used in the study.

Phytochemical Screening

The lyophilized AVS was subjected to phytochemical screening for the presence of bioactive compounds such as tannins, saponins, flavonoids, coumarins, sterols, reducing sugar, glycosides, starch, alkaloid, and triterpenoids by standard methods as described by Harborne.[15]

Animals

Adult Albino rats of either sex, weighing between 200 and 250 g, were procured from the animal house at G. R. Medical College, Gwalior, India. Animals were kept in polypropylene cages and fed on a standard laboratory diet (Ashirwad Industries, Mohali, Chandigarh, India) with water *ad libitum*, maintained at an ambient temperature of 25°C ± 2°C and 45-55% relative humidity. The animals were exposed to 12 h of darkness and light each. The ethical clearance was obtained by the Institutional Animal Ethics Committee (Registration number 846/PO/Ac/04/CPCSEA).

Brewer's Yeast Induced Pyrexia Model

Animals were fasted for 18 h and then after recording rectal temperature of the rats by introducing 1.5 cm of digital thermometer in the rectum, pyrexia was induced by injecting 20% suspension of dried yeast in 2% gum acacia in normal saline at a dose of 20 ml/kg of body weight subcutaneously in back below the nape of the neck.[16] After 18 h of yeast injection, rats which showed a rise in temperature of at least 2°F were selected for the study. Rectal temperature was recorded at 1 h interval till 4 h after drug administration.

Experimental design

Animals with pyresis were randomly divided into five groups of six animals each and received treatments as follows:
Group 1: (Pyrexia control) 2% gum acacia 5 ml/kg, p.o.
Group 2: AVS 100 mg/kg, p.o.
Group 3: AVS 200 mg/kg, p.o.
Group 4: AVS 300 mg/kg, p.o.
Group 5: PCM 150 mg/kg, p.o.

Each rat was fed respective drug orally as gum acacia suspension.

Misoprostol (prostaglandin E1 [PGE1]) Induced Pyrexia Model

Animals were fasted overnight. After recording the initial rectal temperature with a digital thermometer, pyrexia was induced by subcutaneous injection of 100 µg/kg of misoprostol in back below the nape of the neck.[17] After 1 h of injection of misoprostol, the animals having risen of at least 2°F temperature to the normal temperature were included in the study. Rectal temperature was recorded at 30, 60, 90, and 120 min of drug administration.

Experimental design

Animals with pyresis were randomly divided into five groups of six animals each and received treatments as follows:
Group 1: (Pyrexia control) 2% gum acacia 5 ml/kg, p.o.
Group 2: AVS 100 mg/kg, p.o.
Group 3: PCM 50 mg/kg, p.o.

Group 4: AVS 100 + PCM 50 mg/kg, p.o.
Group 5: PCM 150 mg/kg, p.o.

Each rat was fed respective drug orally as gum acacia suspension.

Doses of AVS were selected on the basis of study conducted by Ghosh *et al*. to explore the analgesic activity of *A. vera* in rats.[18] On this background, we conducted a pilot study to get optimal doses for the present study.

RESULTS

Effect of AVS against Brewer's Yeast Induced Pyrexia in Rats

AVS at the doses of 100 mg/kg did not cause significant ($P > 0.05$) fall in temperature as compared with control group. AVS at 200 and 300 mg/kg caused significant ($P < 0.05$, and $P > 0.01$) fall of temperature following its administration at 1, 2, 3, and 4 h intervals, respectively, as compared to pyrexia control group. Standard drug PCM at the dose of 150 mg kg caused significant fall of temperature at 1, 2, 3, and 4 h, respectively, as compared with control AVS 100 and AVS 200 mg/kg treated groups. The antipyretic activity of AVS 300 mg/kg was comparable ($P > 0.05$) with that of PCM 150 mg/kg doses at each time interval [Table 1].

Effect of Combination of Sub-effective Dose of AVS with that of PCM against Misoprostol Induced Pyrexia in Rats

AVS 100 mg/kg and PCM 50 mg/kg following its administration caused non-significant ($P > 0.05$) fall of temperature respectively when used alone as compared to control group. This suggests that AVS 100 mg/kg and PCM 50 mg/kg doses to be sub-effective. The combination of sub-effective doses of AVS with that of PCM caused significant ($P < 0.05$) fall of temperature respectively as compared to control, AVS 100 and PCM 50 mg/kg treated groups when used alone. These results suggest that AVS potentiates the action of PCM. The antipyretic activity of combination due to AVS 100 + PCM 50 was comparable ($P > 0.05$) with that of PCM 150 mg/kg doses at 60, 90, and 120 min time interval [Table 2].

Phytochemical Analysis

AVS revealed presence of flavonoids, saponins, tannins, reducing sugar, glycoside, starch, and sterols [Table 3].

Table 1: Effect of lyophilized succulent of *Aloe vera* leaves on Brewer's yeast induced pyrexia in rats

Groups	Temperature before Brewer's yeast administration (°F)	Temperature after 18 h of Brewer's yeast administration (°F)	Temperature after treatments (°F)			
			1 h	2 h	3 h	4 h
GA5	97.8±0.29	100.05±0.33	100.12±0.3	100.02±0.28	99.57±0.27	99.55±0.26
AVS100	97.7±0.26	99.9±0.35	99.31±0.32	99.08±0.33	98.88±0.34	98.85±0.37
AVS200	97.67±0.27	99.84±0.19	99.21±0.18*	98.87±0.18**	98.58±0.16**	98.08±0.15**
AVS300	97.4±0.29	99.5±0.31	98.7±0.3***	97.98±0.27***	97.82±0.25***	97.73±0.24***
PCM150	97.75±0.26	99.85±0.22	98.33±0.25***	98.15±0.27***	97.92±0.28***	97.87±0.26***

*P<0.05, **P<0.01, ***P<0.001 as compared to pyrexia control. n=6, all values are represented as mean±SEM. One-way ANOVA followed by Tukey's comparison test; GA5: 2% gum acacia at the dose of 5 ml/kg; AVS100: Lyophilized succulent of *Aloe vera* at the dose of 100mg/kg; AVS200: Lyophilized succulent of *Aloe vera* at the dose of 200 mg/kg; AVS300: Lyophilized succulent of *Aloe vera* at the dose of 300 mg/kg; PCM150: Paracetamol at the dose of 150 mg/kg; SEM: Standard error of the mean

Table 2: Effect of combination of sub-effective dose of AVS with that of paracetamol on misoprostol-induced pyrexia in rats

Groups	Temperature before misoprostol administration (°F)	Temperature after 1 h of misoprostol administration (°F)	Temperature after treatments (°F)			
			30 m	60 m	90 m	120 m
GA5	97.63±0.12	99.90±0.10	100.20±0.09	100.23±0.08	100.24±0.07	100.30±0.01
AVS100	97.20±0.03	100.69±0.02	100.19±0.02	100.15±0.06	100.01±0.11	100.18±0.05
PCM50	97.78±0.03	100.72±0.01	100.00±0.07	99.99±0.01	100.06±0.09	100.11±0.01
AVS100+PCM50	98.40±0.01	100.78±0.01	99.00±0.10	98.90±0.04[a,b,c]	98.72±0.024[a,b,c]	98.27±0.01[a,b,c]
PCM150	97.36±0.02	100.55±0.04	98.91±0.03[a,b,c]	98.73±0.06[a,b,c]	98.45±0.03[a,b,c]	98.24±0.03[a,b,c]

[a]P<0.05 as compared to pyrexia control; [b]P<0.05 as compared to AVS100 mg/kg control; [c]P<0.05 as compared to PCM50 mg/kg. n=6, values are represented as mean±SEM. One-way ANOVA followed by Tukey's comparison test; GA5: 2% gum acacia at the dose of 5 ml/kg; AVS 100: Lyophilized succulent of *Aloe vera* at the dose of 100 mg/kg; AVS2100+PCM50: Lyophilized succulent of *Aloe vera* at the dose of 100 mg/kg+paracetamol at the dose of 50 mg/kg; PCM150: Paracetamol at the dose of 150 mg/kg

Table 3: Phytochemical screening of lyophilized succulent of *Aloe vera* leaves

Chemical class	Result
Alkaloids	–
Flavonoids	+
Saponins	+
Tannins	+
Reducing sugar	+
Cardiac glycoside	–
Glycoside	+
Starch	+
Sterols	+
Triterpenoids	–
Coumarins	–

Statistical Analysis

The results were expressed as mean ± standard error of the mean statistical analysis was carried out using ANOVA followed by Tukey's multiple comparison test. $P < 0.05$ were considered significant.

DISCUSSION

The Brewer's yeast suspension induced pyrexia model is more commonly used to study the antipyretic potential of test drug. Pyrexia is produced due to enhanced formation of proinflammatory mediator's like interleukin 1β, α, β, and tumor necrosis factor-α, from infected or damaged tissue which stimulate synthesis of PGE2 near preoptic hypothalamus area and triggers to elevate body temperature.[19] Exploration of antipyretic potential of AVS was preferred against Brewer's yeast induced pyrexia than against misoprostol induced pyrexia in present study because this treatment caused sustained rise of temperature, however there is delayed onset of pyresis (18 h) and pathological lesion at the site of infiltration. The misoprostol-induced pyrexia model was preferred to study the mechanism of antipyretic activity of AVS over Brewer's yeast induced pyrexia model because of quick onset of pyresis (1 h) with lesser discomfort to the animal as misoprostol is rapidly metabolized in 2-3 h hence study period is short.[20]

It is a known fact that pyrogens trigger fever, through the release of PGE2[21] and PCM inhibits PG synthesis through cyclooxygenase inhibition and produces antipyretic effect. Fall of temperature observed at the dose of 300 mg/kg AVS in the present study was comparable with standard drug PCM, and this confirms antipyretic activity of *A. vera*. Earlier studies on plant extracts of *Ocimum sanctum* has been shown to possess antipyretic activity by decreasing synthesis of PG through inhibition of enzymes of the cyclooxygenase pathway.[22] In the present study, antipyretic activity achieved at a sub-effective dose of AVS in combination with that of PCM suggest the involvement of AVS in inhibiting cyclooxygenase enzyme.

The changes in temperature after AVS treatment may be attributed to several compounds found in *A. vera* leaves. Salicylic acid, an aspirin-like chemical known to possess antipyretic property by inhibiting enzymes of cyclooxygenase pathway has been isolated in leaf of *A. vera*.[23] β sitosterols is another phytochemical present in the leaf of *A. vera* as reported in earlier studies found to lower PG synthesis by preventing conversion of linoleic acid to arachidonic acid a substrate required for PG synthesis and possess antipyretic activity.[24,25] β sitosterols might be involved in the antipyretic activity of AVS but this need to be investigated by further studies. Flavonoids are important bioactive principles reported to inhibit PGs and thus produce the antipyretic effect.[26] Phytochemical analysis revealed the presence of flavonoids in AVS suggestive of its contribution to the antipyretic activity.

Thus, it is concluded that lyophilized AVS possesses the antipyretic activity, and it acts possibly by inhibiting cyclooxygenase enzyme and PG synthesis. This is the first study, which investigated the antipyretic activity of *A. vera*. The results found are encouraging for further studies to isolate phytochemicals involved and to elucidate mechanisms of antipyretic activity.

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REFERENCES

1. Karakitsos D, Karabinis A. Hypothermia therapy after traumatic brain injury in children. N Engl J Med 2008;359:1179-80.
2. Begum TN, Ilyas MH, Anand AV. Antipyretic activity of *Azima tetracantha* in experimental animals. Int J Curr Biomed Pharm Res 2011;1:41-4.
3. Veugelers PJ, Kaldor JM, Strathdee SA, Page-Shafer KA, Schechter MT, Coutinho RA, *et al.* Incidence and prognostic significance of symptomatic primary human immunodeficiency virus type 1 infection in homosexual men. J Infect Dis 1997;176:112-7.
4. Pirmohamed M, James S, Meakin S, Green C, Scott AK, Walley TJ, *et al.* Adverse drug reactions as cause of admission to hospital: Prospective analysis of 18 820 patients. BMJ 2004;329:15-9.

5. Ghule BV, Murugananthan G, Yeole PG. Analgesic and antipyretic effects of *Capparis zeylanica* leaves. Fitoterapia 2007;78:365-9.
6. Jalalpure SS, Habbu PV, Patil MB. Analgesic and antipyretic activity of *Pergolaria extensa* in rats. Indian J Pharm Sci 2002;64:493-5.
7. Patel R, Mahobia N, Upwar N, Waseem N, Talaviya H, Patel Z. Analgesic and antipyretic activities of *Momordica charantia* Linn. fruits. J Adv Pharm Technol Res 2010;1:415-8.
8. Boudreau MD, Beland FA. An evaluation of the biological and toxicological properties of *Aloe barbadensis* (miller), *Aloe vera*. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2006;24:103-54.
9. Egesie UG, Chima KE, Galam NZ. Anti-inflammatory and analgesic effects of aqueous extract of *Aloe vera* (*Aloe barbadensis*) in rats. Afr J Biomed Res 2011;14:209-12.
10. Yagi A, Kabash A, Okamura N, Haraguchi H, Moustafa SM, Khalifa TI. Antioxidant, free radical scavenging and anti-inflammatory effects of aloesin derivatives in *Aloe vera*. Planta Med 2002;68:957-60.
11. Sultana N, Najam R. Anxiolytic activity of *Aloe vera* (L.) Burm. F tested in rodents. Pak J Pharmacol 2012;29:7-15.
12. Jafri SA, Hasan SS, Nadeem A, Kalsoom, Iqbal J. Hypoglycemic effect of *Aloe vera* extract in alloxan induced diabetic Albino rats. Med J Islam World Acad Sci 2011;19:127-30.
13. Yusuf S, Agunu A, Diana M. The effect of *Aloe vera* A. Berger (*Liliaceae*) on gastric acid secretion and acute gastric mucosal injury in rats. J Ethnopharmacol 2004;93:33-7.
14. Oryan A, Naeini AT, Nikahval B, Gorjian E. Effect of aqueous extract of *Aloe vera* on experimental cutaneous wound healing in rats. Vet Arh 2010;80:509-22.
15. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 2nd ed. London: Chapman and Hall; 1983.
16. Singh N, Khatri P, Samantha KC, Damor R. Antipyretic activity of methanolic extract of *Quisqualis indica*. Int J Pharm Res Dev 2010;2:122-6.
17. Tirumalasetty J, Ubedulla S, Chandrasekhar N, Kishan PV, Rasamal K. Evaluation of antipyretic activity of alcoholic extract of *Vitex nigundo* leaves in PGE1 induced pyrexia model in Albino rats. J Chem Pharm Res 2012;4:3015-9.
18. Ghosh AK, Banerjee M, Mandalb TK, Mishra A, Bhowmik MK. A study on analgesic efficacy and adverse effects of *Aloe vera* in Wistar rats. Pharmacologyonline 2011;1:1098-108.
19. Saper CB, Breder CD. The neurologic basis of fever. N Engl J Med 1994;330:1880-6.
20. Wallace JL, Sharkey KA. Pharmacotherapy of gastric acidity, peptic ulcers and gastroesophageal reflux disease. In: Goodman and Gilman's, The Pharmacological Basis of Therapeutics. 12th ed. New York: McGraw-Hill Medical Publishing Division; 2011. p. 1309.
21. Dinarella CA, Porat R. Harrison's Principles of Internal Medicine. 18th ed. New York: McGraw-Hill Medical Publishing Division; 2012. p. 143-7.
22. Kelm MA, Nair MG, Strasburg GM, DeWitt DL. Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum* Linn. Phytomedicine 2000;7:7-13.
23. Surjushe A, Vasani R, Saple DG. *Aloe vera*: A short review. Indian J Dermatol 2008;53:163-6.
24. Yamamoto M, Masui T, Sugiyama K, Yokota M, Nakagomi K, Nakazawa H. Anti-inflammatory active constituents of *Aloe arborescens* Miller. Agric Biol Chem 1991;55:1627-9.
25. Gupta MB, Nath R, Srivastava N, Shanker K, Kishor K, Bhargava KP. Anti-inflammatory and antipyretic activities of beta-sitosterol. Planta Med 1980;39:157-63.
26. Rajnarayana K, Reddy MS, Chaluvadi MR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutical potential. Indian J Pharm Sci 2006;68:380-4.

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