Enhancement Potential of *Aloe vera* on Permeation of Drugs with diverse Lipophilicites across Rat Abdominal Skin

Ramesh Gannu, Vamshi Vishnu Yamsani and Madhusudhan Rao Yamsani*
Centre for Biopharmaceutics and Pharmacokinetics, University College of Pharmaceutical Sciences, Kakatiya University, Warangal-506 009 (A.P). India
*For Correspondence: ymrao123@yahoo.com

**Abstract**

The current work aims to study the effect of *Aloe vera* juice on skin permeation of drugs with diverse molecular weight and lipophilicity. Saturated solutions of lisinopril, diclofenac sodium and lacidipine in *Aloe vera* juice and water or water containing 40 % v/v polyethylene glycol 400 (control) were used to dose rat abdominal skin mounted in Franz diffusion cells. No significant difference (p>0.05) was observed in solubilities of drugs investigated in aloe juice and control. The *Aloe vera* juice showed significant (p<0.05) improvement in the permeation of drugs. The flux of lisinopril, diclofenac sodium and lacidipine in *Aloe vera* juice was increased, respectively, 1.11, 1.11 and 1.93 times than the respective control. The penetration enhancement order was lisinopril ≤ diclofenac sodium < lacidipine. Enhancement potential was dependent upon the molecular weight and lipophilicities of the drug in formulation, with the enhancement effect attributable to as yet unidentified components within the *Aloe vera*. These results demonstrated that the *Aloe vera* juice have penetration enhancement effect on skin permeation of drugs studied.

**Keywords:** *Aloe vera*, lisinopril, diclofenac sodium, lacidipine, skin permeation

**Introduction**

*Aloe vera* (*Aloe barbadensis* Miller) is part of the Lilaceal family and is a perennial succulent cactus-like plant, which grows in hot and dry climates (1). It has been used in the traditional medicinal practices of many cultures for a host of curative purposes and in the treatment of a variety of disorders including wounds and burns (2). It is often referred to as a ‘healing’ plant and is the source of two products. First one is a bitter yellow juice (exudates) from cut leaf base, which contains a high concentration of anthraquinone compounds and when dried is used as a potent cathartic (3) and lacquer to inhibit nail biting. The second is mucilaginous juice from the leaf parenchyma, which has been used as a remedy for a variety of pathological states, such as arthritis, gout, acne, dermatitis, burns, and peptic ulcers induced by epithelial alterations (4, 5). It was reported to have anti-inflammatory and analgesic activites by inhibiting pain-producing substances such as bradykinin or thromboxane and cyclooxygenase-2 (6, 7); antibacterial, antifungal and antiviral properties (8, 9). Polysaccharides are another group of juice constituents to which activity has been ascribed, particularly in immunomodulatory reactions (10). It is also claimed to have hepatoprotective, anti-proliferative, anti-carcinogenic, anti-aging, and laxative effects (11, 12). These effects are thought to be the result of radical scavenging and immuno-modulatory mechanisms. *Aloe vera* is thus incorporated into many products and is used for various medicinal, cosmetic and nutraceutical purposes (13) with topical applications featuring prominently.
Transdermal delivery is one of the more successful drug delivery approaches that have been investigated as an alternative to parenteral or oral delivery. Barrier function of skin restricts the entry of drugs into systemic circulation. To improve the permeation of drugs through skin, various strategies such as (i) physical enhancement techniques, iontophoresis (14), electroporation (15), sonophoresis (16) and microneedles (17); (ii) chemical (18) have been reported. Enhancement with chemical enhancers have received much attention and an enhancement system based upon a product such as *Aloe vera* appears an attractive prospect due to its purported ‘skin friendly’ and humectant properties (19). There are very few evidences to suggest that *Aloe vera* has skin penetration enhancement properties. One recent paper reported that *Aloe vera* juice have an enhancement property on drugs with different molecular weights (20) and other mentions its use as a vehicle for other substances (5). Two recent United States patents have been filed which claim that *Aloe vera* is responsible for increased skin penetration of co-formulated drugs. A 2004 US patent concerned the use of fresh macerated leaves of *Aloe barbadensis* formulated with fentanyl and claimed that when *Aloe vera* oil (*Aloe vera* extract macerated in soybean oil) was incorporated into formulation, the amount of fentanyl permeating the skin was increased by up to 50%, compared to formulations lacking *Aloe vera* oil (21). A further patent described the skin permeation enhancement of oxybutynin by *Aloe vera* (22).

The aim of present investigation is to study the effect of *Aloe vera* juice on in vitro skin permeation potential of drugs from saturated solutions, across rat abdominal skin. Water soluble (*Lisinopril; LSP*), partially soluble (*Diclofenac sodium; DFS*) and insoluble (*Lacidipine; LCDP*) drugs were used as model drugs with diverse molecular weight and lipophilicity.

### Materials and Methods

#### Materials

*Lisinopril* and *Lacidipine* were obtained as gift samples from Dr Reddy’s Laboratories, Hyderabad, India. *Diclofenac sodium* was purchased from Nihal Traders, Hyderabad, India. Polyethylene glycol 400 (PEG 400) was purchased from Merck, India. All other chemicals and reagents used were of analytical grade.

#### Preparation of *Aloe vera* juice

*Aloe vera* juice was prepared from the full size mature leaves of *Aloe vera*. They were cut from the plant and the green rind was removed. From the cut leaf bases the yellow juice was allowed to drain into a container to remove the exudates. The colorless parenchyma was ground in a blender and centrifuged at 3000 rpm for 30 min to separate the fibers. The supernatant (*Aloe vera* juice) was used for experiments.

#### Preparation of saturated solutions

An excess of drug was added to glass vials containing either 10 mL of *Aloe vera* juice or distilled water for LSP and DFS; *Aloe vera* juice or water containing 40% v/v PEG 400 for LCDP. Water or water containing 40% v/v PEG 400 formulations were used as control. The suspensions were kept for shaking at room temperature on a rotary shaker (Remi Scientifics, India) for 24 hr. The suspensions were filtered through 0.45 μm membrane filters (Millipore, India). About 100 μL of the filtrate was diluted suitably and the drug content was estimated by UV visible spectrophotometer (Elico 169 SL, India). The remainder of solutions was used for permeation studies. All experiments were performed by using the same batch of *Aloe vera* juice.
Determination of partition coefficient

The partition studies were conducted by shake flask method. n-octanol was saturated with distilled water for 24 hours before the experiment. Accurately weighed amount about, 1000 mg of lisinopril, diclofenac sodium and lacidipine was added to each 20 mL of n-octanol and water mixture (1:1 ratio). The solution was kept for shaking for 24 hours at room temperature on rotary shaker. Two phases were separated and filtered through 0.45 µ membrane filter. The concentration of the drug in the aqueous phase was analyzed spectrophotometrically; concentration of the drug in n-octanol was calculated from the difference between the initial amount and amount in water phase. Similarly the partition study was conducted using Aloe vera juice. The partition coefficient was calculated using following equation (I).

\[
\text{Partition coefficient (P)} = \frac{[\text{Drug}]_{\text{Octanol}}}{[\text{Drug}]_{\text{Aqueous}}} \quad \text{(I)}
\]

Preparation of rat abdominal skin

The animal study protocol was reviewed and approved by the Ethics Committee at the University College of Pharmaceutical Sciences, Kakatiya University. Albino rats weighing 150-200 gm were selected for permeation studies. The animals were sacrificed using anesthetic ether, hairs of test animal were carefully removed with electrical clippers and full thickness skin was removed from the abdominal region. The epidermis was prepared surgically by heat separation technique (23), which involved soaking the entire abdominal skin in water at 60° C for 45 sec, followed by careful removal of the epidermis. The epidermis was washed with water and used for in vitro permeability studies.

In vitro skin permeation experiments

Permeation studies were carried out using Franz diffusion cell with a diffusion surface area of 3.56 cm². The rat abdominal skin was mounted between the compartments of the diffusion cell with stratum corneum facing the donor compartment and clamped into position. Magnetic stirrer bars were added to the receptor chambers and filled with the receptor phase. Phosphate buffer saline (PBS) pH 7.4 was used for LSP and DFS, where as PBS pH 7.4 containing 40% v/v PEG 400 was used as receptor medium for LCDP. The entire setup was placed over magnetic stirrer and temperature was maintained at 37° C. Samples of 1 mL was collected from receptor compartment at predetermined intervals over 24 h period and replaced with fresh buffer.

Determination of drug content in the samples

The samples were filtered through 0.45 µ filter, diluted suitably and the amount of permeated drug was measured by UV visible spectrophotometer and the concentration was corrected for sampling effects according to the equation (II) (24). The absorbencies were measured, respectively, at 208 nm, 276nm and 282 nm for LSP, DFS and LCDP.

\[
C_i^n = C_i^n \left(\frac{V_T}{V_T - V_S}\right) \left(\frac{C_{i-1}^n}{C_{i-1}^n}\right) \quad \text{(II)}
\]

Where \(C_i^n\) is the corrected concentration of the \(n^{th}\) sample, \(C_i^n\) is the measured concentration of drug in the \(n^{th}\) sample, \(C_{i-1}^n\) is the measured concentration of the drug in the \((n - 1)^{th}\) sample, \(V_T\) is the total volume of the receiver fluid and \(V_S\) is the volume of the sample drawn.

Data analysis

Cumulative amounts of LSP, DFS and LCDP permeated over the 24 h period were plotted against time. Steady state flux (Jss) and permeability coefficient (kp) were calculated using equations (III) and (IV). Lag times were
determined by extrapolating the steady state line back to the x-axis of the permeation profile. Enhancement ratio was obtained when the flux of a drug in the saturated Aloe vera formulation was divided by the flux of the drug in the saturated water formulation.

\[
J_{ss} = \frac{dQ}{dt}_{ss} \left( \frac{1}{A} \right) \quad (III)
\]

\[
K_p = \frac{dQ}{dt} \left( \frac{1}{A} \right) \left( \frac{1}{C_s} \right) \quad (IV)
\]

Where, A is the effective diffusion area; Cs, the concentration of drug in saturated solution and \(\frac{dQ}{dt}_{ss}\) is the steady state slope.

**Statistical Analysis**

The saturation solubilities and flux values obtained from the various systems were tested for significant differences using unpaired 't' test. The statistical analysis was performed using SigmaStat software version 1.0 (Jandel Corp., California).

**Results**

**Solubilities**

The results of solubility study were shown in Table 1. Water, Aloe juice were used for LSP and DFS, where as water containing 40 % v/v PEG 400 and Aloe juice containing 40% v/v PEG 400 was utilized for LCDP. PEG 400 (40 % v/v) was incorporated in water (control) as co solvent to improve the solubility of LCDP and same concentration was incorporated in Aloe vera juice. LSP showed maximum solubility of 107.91 ± 10.53 in Aloe vera than water formulation, however the difference was not significant (p>0.05). LCDP showed lowest solubility among the drugs investigated. As Aloe vera contains more than 98.5 % water, the appropriate control vehicle was water for LSP and DFS. Aloe vera is a succulent plant and the juice is comprised mostly of water, with only less than 1.5 % (w/v) of solids, thus very similar solubilities would be expected and the same result was obtained.

**Partition studies**

The results (Table 1) of partition studies reveal that there is not much difference in the distribution. It indicates that components of Aloe vera juice did not show any effect on drug’s distribution in the vehicles. The results obtained in the study are comparable to that, were reported in the literature (25).

**In vitro permeation studies**

The permeation profiles of LSP, DFS and LCDP were shown in Fig 1. Saturated solution in aloe juice (LSP-A) showed highest steady state flux of 25.02 ± 0.90 µg/h/cm² (permeability coefficient 2.32 x 10⁻⁴ cm/h), which was statistically significant (P = 0.0391) different, compared to the flux obtained with LSP from

<table>
<thead>
<tr>
<th>Code</th>
<th>MW</th>
<th>log P</th>
<th>Solubility <em>(mg/mL)</em></th>
<th>(Q_{ss}) (µg/cm²/hr)</th>
<th>Flux* (µg/cm²/hr)</th>
<th>Permeation Coefficient* x 10⁻⁴ (cm/h)</th>
<th>Lagtime* (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSP-W</td>
<td>405.5</td>
<td>-1.22</td>
<td>95.16 ± 5.34</td>
<td>2420.68 ± 206.70</td>
<td>22.52 ± 1.35</td>
<td>2.367 ± 0.0160</td>
<td>2.10 ± 0.18</td>
</tr>
<tr>
<td>LSP-A</td>
<td>405.5</td>
<td>-1.27</td>
<td>107.91 ± 10.53</td>
<td>2712.71 ± 231.63</td>
<td>25.02 ± 0.90</td>
<td>2.319 ± 0.0062</td>
<td>1.75 ± 0.12</td>
</tr>
<tr>
<td>DFS-W</td>
<td>318.4</td>
<td>3.68</td>
<td>18.83 ± 2.57</td>
<td>1952.21 ± 167.73</td>
<td>21.43 ± 1.14</td>
<td>11.390 ± 0.1100</td>
<td>3.42 ± 0.33</td>
</tr>
<tr>
<td>DFS-A</td>
<td>318.4</td>
<td>3.44</td>
<td>13.61 ± 2.17</td>
<td>2437.27 ± 146.52</td>
<td>22.81 ± 1.37</td>
<td>12.123 ± 0.1200</td>
<td>1.50 ± 0.08</td>
</tr>
<tr>
<td>LCDP-W*</td>
<td>455.5</td>
<td>5.02</td>
<td>0.83 ± 0.03</td>
<td>248.98 ± 37.27</td>
<td>0.54 ±0.08</td>
<td>0.015 ± 0.0010</td>
<td>0.83 ± 0.04</td>
</tr>
<tr>
<td>LCDP-A†</td>
<td>455.5</td>
<td>4.37</td>
<td>0.99 ± 0.03</td>
<td>198.98 ± 29.79</td>
<td>0.28 ±0.04</td>
<td>0.003 ± 0.0004</td>
<td>0.80 ± 0.02</td>
</tr>
</tbody>
</table>

Note: * values presented are mean ± S.D (n=3); W- Saturated solution in water; A Saturated solution in Aloe vera juice; b Saturated solution in water containing 40 %v/v PEG 400; † Saturated solution in Aloe vera juice containing 40% v/v PEG 400.
water formulation (LSP-W). The water formulation gave a flux $22.52 \pm 1.35 \mu g/h/cm^2$ and a permeability coefficient of $2.33 \times 10^{-1} \text{cm/h}$ with an enhancement ratio of 1.11. For DFS, the highest steady state flux of $22.81 \pm 1.37 \mu g/h/cm^2$ (permeability coefficient 12.12 cm/h) was observed from saturated solution of Aloe vera juice (DFS-A), which was statistically significant ($P = 0.0294$), compared to the flux obtained with DFS from water formulation (DFS-W). The aqueous formulation gave a flux $21.43 \pm 1.14 \mu g/h/cm^2$ and a permeability coefficient of 11.39 cm/h with an enhancement ratio of 1.11. For LCDP, the highest steady state flux of $0.54 \pm 0.081 \mu g/h/cm^2$ (permeability coefficient $0.015 \times 10^{-1} \text{cm/h}$) was observed from saturated solution of Aloe vera juice (LCDP-A), which was statistically significant ($P = 0.0013$), compared to the flux obtained with LCDP from control (LCDP-W). The control formulation gave a flux $0.28 \pm 0.04 \mu g/h/cm^2$ and a permeability coefficient of 0.003 cm/h with an enhancement ratio of 1.93. The penetration enhancement order was LSP<DFS<LCDP. The lag time was found to decrease in all the cases with aloe formulation.

Discussion

Present study reports the skin permeation effect of Aloe vera juice on drugs with diverse molecular weight and lipophilicity. As a natural product, Aloe vera contains a diverse array of components, including anthraquinones, saccharides, polysaccharides, lignin, and numerous low molecular weight compounds such as vitamins and salicylic acid (26); in addition to recently discovered compounds such as bioactive maloyl glucans (27). The more lipophilic components of the Aloe vera may penetrate the stratum corneum more readily and modulate it in some way. The following mechanisms may be proposed for penetration enhancement effect of Aloe vera juice. Given the general impermeability of skin it is reasonable to assume there can only exist finite availability of permeation pathways across skin at any given time. In this investigation it was observed that the penetration was influenced with the molecular weight and log P of drug (Fig. 2). The higher the drug size/ molecular weight, the higher the enhancement ratio was observed. Similar reports were also observed in previous reports by Louise and Charles (20). It appears that along with molecular weight, the lipophilic drugs were also showed good penetration through skin. It indicates that the Aloe vera juice influences the permeation of lipophilic drugs. This phenomenon can be rationalized in terms of the scope for interaction and complexation of the drug with the (unidentified) enhancing factor. Another possible mechanism is that the lipophilic components of Aloe vera juice might have entered into stratum corneum and altered the membrane structure so that lipophilic components can penetrate easily. Therefore, lipophilic drug LCDP might have showed more E.R among the drugs investigated. LSP showed similar enhancement to that of DFS, which may be due to higher concentration of the LSP in the vehicle might have showed permeation comparable to that of DFS.
Conclusions

The present study demonstrates that the Aloe vera juice enhances permeation of drugs studied. The skin permeation potential appears to be attributable to one or more components of the juice. The results suggest that Aloe vera juice may be used as penetration enhancer in the development of topical and transdermal formulations.

Acknowledgements: One of the author thank AICTE, New Delhi, India for providing financial assistance in the form of National Doctoral Fellowship (NDF).

References


