Intestinal Drug Transport Enhancement by *Aloe vera*

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Key words
- *Aloe vera* (L.) Burm. f.
- Asphodelaceae
- gel
- whole leaf extract
- absorption enhancement
- Caco-2

Abstract

The effect of *Aloe vera* (L.) Burm. f. (*Aloe barbadensis* Miller) gel and whole leaf extract on the permeability of Caco-2 cell monolayers was determined. Solutions of gel and the whole leaf extract were applied to the cell monolayers, and the transepithelial electrical resistance was monitored for 2 hours, which was then continued for another 2 hours after removal of the test solutions to measure reversibility of the effect. The transport of insulin in the presence and absence of the *A. vera* gel and whole leaf extract solutions was also investigated. Both the *A. vera* gel and whole leaf extract were able to significantly reduce the transepithelial electrical resistance of the Caco-2 cell monolayers at concentrations above 0.5% w/v and thereby showed the ability to open tight junctions between adjacent cells. This effect was fully reversible, as the electrical resistance of the cell monolayers returned to the original value upon removal of the test solutions. The *A. vera* gel and whole leaf extract solutions significantly enhanced the transport of insulin across the Caco-2 cell monolayers compared with the control. The results suggest that these plant products have a high potential to be used as absorption enhancers in novel dosage forms for drugs with poor bioavailabilities when administered orally. On the other hand, an uncontrolled increase in the bioavailability of drugs that are taken simultaneously with *A. vera* gel and whole leaf extract products may result in adverse effects, and the potential exists that toxic blood plasma levels may be reached.

Introduction

The oral route of administration is preferred by most patients for drug intake, but the low bioavailability of many pharmaceutically active compounds preclude them from being administered orally. Reasons for poor drug absorption include degradation by chemical and enzymatic reactions, low aqueous solubility, large molecular weight, pre-systemic metabolism, and low permeability across the gastrointestinal mucosa. Co-administration of an absorption enhancer formulated into the dosage form is one way to improve oral bioavailability of these drugs [1,2]. Absorption enhancers are compounds that reversibly remove the barrier of the outer layer of body tissues with minimum tissue damage, thus allowing the drug to penetrate across the epithelial cells and enter the blood and lymph circulation [3]. Numerous classes of compounds with diverse chemical properties, including surfactants, bile salts, calcium chelating agents, fatty acids, medium-chain glycerides, acyl carnitine, alkanoyl cholines, *N*-acetylated α-amino acids, *N*-acetylated non-α-amino acids, chitosans, mucoadhesive polymers, and phospholipids, have been reported to enhance the intestinal absorption of small drug molecules and large polypeptide drugs [4], but none of these are currently in clinical use due to toxic effects or insufficient activity [5]. Developing or discovering compounds that act as safe and effective absorption enhancers is still a challenge for the formulation scientist.

Aloe vera (L.) Burm. f. (syn. *Aloe barbadensis* Miller) is a perennial succulent xerophyte belonging to the Asphodelaceae family. The innermost part of its leaves, known as the pulp, consists of large, thin-walled parenchyma cells in which water is held in the form of a viscous mucilage also referred to as the gel [6]. The three distinct parts of *A. vera* that are used for medicinal purposes include the aloe latex (or exudate), the aloe gel, and the whole leaf (or whole leaf extract). The exudate is used mainly for its laxative effect,

received August 15, 2008
revised Nov. 4, 2008
accepted Dec. 14, 2008

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DOI 10.1055/s-0029-1185341
Published online February 12, 2009
Planta Med 2009; 75: 587–595

ISSN 0032-0943

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while the gel is used topically for the treatment of skin ailments such as wound healing, psoriasis, and genital herpes. Internally, the gel has been investigated for the treatment of diabetic patients, for the healing of gastric ulcers, and for use as an immunomodulatory agent and an antioxidant. The whole leaf extract has shown potential in the treatment of cancer and acquired immunodeficiency syndrome [7–9].

While aloe whole leaf extracts are prepared by grinding the entire leaf and then removing the anthraquinones such as aloin through charcoal filtration at the final stage of processing, the gel is extruded from the inner pulp of the fresh leaves [7]. This gel consists primarily of water (> 99%), and the remaining 0.5–1% of solid material consists of a variety of polysaccharides, vitamins, enzymes, lipids, and inorganic and small organic compounds [10]. Acemannan has been identified as the main polysaccharide in *A. vera* gel and has a backbone of β-(1 → 4)-D-mannosyl residues acetylated at the C-2 and C-3 positions that exhibit a mannose monomer : acetyl ratio of approximately 1 : 1 and contain some side chains of mainly galactose attached to C-6 [11–13]. Other polysaccharides such as pectic substances, arabinan, arabinorhamnogalactan, galactan, galactogalacturan, glucogalactomannan, galactoglucoarabinomannan, and glucuronic acid –containing polysaccharides also have been isolated from *A. vera* inner leaf gel [14, 15].

An *in vivo* investigation on the effect of *A. vera* liquid preparations, namely, the gel and whole leaf extract, on the bioavailability of vitamins C and E in human subjects showed that both of the *A. vera* preparations had an increasing effect on the overall bioavailability (area under the curve) of the two vitamins [16]. Furthermore, *A. vera* gel increased the *in vitro* skin penetration of compounds depending on their molecular weights. This penetration-enhancement effect of the aloe gel was explained by a probable pull effect of complexes formed between the compound and the enhancing agent within the aloe gel, but it was stated that the proposed mechanism of action has to be further investigated and confirmed [17]. It is known that polysaccharides of natural origin are capable of enhancing the intestinal absorption of coadministered drugs by means of a transient opening of the tight junctions between adjacent epithelial cells to allow for paracellular transport across the intestinal epithelium [18, 19]. Advantages of using polymeric absorption enhancers over small-molecular-weight compounds reside in the fact that the polymeric substances are not absorbed themselves and therefore have a reduced risk of systemic toxic or adverse effects. In addition, many of these high-molecular-weight polymers are mucoadhesives that act synergistically with their other permeability-enhancing effects because of ensuring a longer contact time with the surface of the intestinal epithelium [20].

In this study, the potential of *A. vera* gel and whole leaf extract to enhance the transport of the poorly absorbable model drug, insulin, across intestinal epithelial cell monolayers was investigated. The reversibility of the effect of *A. vera* gel and whole leaf extract on the transepithelial electrical resistance of the cell monolayers as well as the mechanism of drug-transport enhancement are elucidated.

Materials and Methods

Plant material

Both *Aloe vera* gel and whole leaf extract were received as donations from the International Aloe Science Council (IASC, 051309, Texas, USA) in dry powder form and were originally obtained from companies that produce certified *A. vera* gel and whole leaf materials for commercial purposes that are used in different products available on the market.

Nuclear magnetic resonance (^{1}H-NMR) spectroscopic characterization of plant materials

To record ^{1}H-NMR spectra of the plant materials, approximately 50 mg of each of the *A. vera* powders and approximately 5 mg internal standard (nicotinic acid amide or NSA) were dissolved in 1 mL D_2O and then measured in an Avance 300 MHz NMR spectrometer (Bruker). NMR spectroscopy was used to identify and quantify marker molecules in order to characterize the chemical composition of each of the plant materials used in this study.

Cell line and chemicals

Caco-2 cells (American Tissue Culture Society), Dulbecco's Modified Eagle's Medium (DMEM) (Bio Whittaker), 10% fetal bovine serum (Delta Bioproducts), 1% nonessential amino acids (Bio Whittaker), 1% pen/strep fungizone mixture (10 000 U penicillin/mL, 10 000 µg streptomycin/mL, and 25 µg fungizone/mL; Bio Whittaker), Hank's Balanced Salt Solution (HBSS) (Bio Whittaker), trypsin-versene solution (Bio Whittaker), HEPES [*N*-(2-hydroxyethyl) piperazine-*N*-(2-ethanesulfonic acid)] (Bio Whittaker), and insulin (from bovine pancreas; Sigma-Aldrich) were used.

Culturing and seeding out of Caco-2 cells on transwell inserts

The Caco-2 cell line was grown in tissue culture flasks (Corning Costar Corp.) (75 cm^2). The growth media consisted of DMEM supplemented with 10% fetal calf serum, 1% non-essential amino acids, and 1% penicillin G (10 000 IU/mL)/streptomycin sulfate (10 000 µg/mL) solution. The growth medium was changed every 2–3 days and subcultured at 80% confluence by trypsinization. Cells were seeded onto polycarbonate Transwell inserts (Corning Costar Corp.; pore size 0.4 µm, growth area 0.33 cm^2 for transepithelial electrical resistance and 4.7 cm^2 for transport experiments) at a density of 1×10^4 cells/cm^2. The cells were cultured for 21–24 days before the transepithelial electrical resistance experiments or transport experiments were commenced. The culturing conditions were 37 °C in an atmosphere of 90% relative humidity and 5% CO_2.

Transepithelial electrical resistance (TEER) studies

The test solutions consisted of *A. vera* gel and whole leaf extract dissolved in serum-free DMEM at five different concentrations and two pH values (concentrations: 0.1, 0.5, 1.0, 2.5, and 5.0% w/v; pH values: 5.8 and 7.4). Prior to the TEER experiments, the medium in the basolateral chamber was replaced by DMEM buffered at pH 7.4 with 10 mM HEPES. The apical medium was removed and the cell monolayers were incubated with 200 µL of each of the *Aloe vera* test solutions. The TEER was measured at time intervals of 20 min, starting 60 min before application of test solutions and continuing for 120 min after application of the test solutions using a Millicell ERS meter (Millopore) connected to a pair of chopstick electrodes. Only wells with TEER values

above 250 ohm · cm^2 were used, and the TEER values at time zero were used as 100%. The reversibility of the effect of the *A. vera* test solutions on the TEER of the Caco-2 cell monolayers was measured by removing the test solutions from the apical compartment after a period of 120 min and then replacing them with serum-free DMEM, while the TEER was measured for another 120 min. Control experiments were performed under the same conditions but without the test solutions. All experiments were done in triplicate at 37 °C in an atmosphere of 90% relative humidity and 5% CO_2.

Insulin transport studies

Transport of insulin in the apical to basolateral direction was determined in the absence (control) and presence of *A. vera* gel and whole leaf extract test solutions prepared in HBSS at five different concentrations and at two pH values. Prior to the transport experiments, the medium in the basolateral chamber was changed with HBSS buffered at pH 7.4 with 10 mM HEPES. The apical medium was removed and the cells were incubated with 2.5 mL of each of the *Aloe vera* test solutions that contain insulin (170 µg/mL). Samples of 200 µL were taken at 30, 60, 90, 120, 180, and 240 min from the basolateral side. The samples withdrawn from the basolateral side were replaced with an equal volume of HBSS containing 10 mM HEPES. All experiments were done in triplicate in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C.

The insulin present in the samples was determined by means of reverse-phase high-performance liquid chromatography (RP-HPLC). The analysis was carried out on an Agilent 1100 series HPLC equipped with a gradient pump, an autosampler, a UV detector, and Chemstation Rev. A.08.03 data acquisition software (Agilent Technologies). A Jupiter C_{18} column (250 mm × 4.6 mm, 5-µm spherical particles, 300-Å pore size, 13.3% carbon load, endcapped) (Phenomenex) was used. The mobile phase consisted of acetonitrile (A) and water with 0.1% w/v of orthophosphoric acid (B). The separations were performed by gradient elution at a flow rate of 1.0 mL/min. The gradient used was as follows: for 0–6 minutes, a linear gradient from 80% to 40% B; for 6–8 minutes, 40% B was maintained; for 8–8.2 minutes, a linear gradient from 40% to 80% B, which was then maintained at 80% B until 12 min. A detection wavelength of 210 nm was used. Quantification was done by peak area measurements in comparison with the standard solutions. Prior to chromatographic analysis of insulin in the transport samples, the RP-HPLC method was validated in terms of linearity, accuracy, precision, sensitivity (limit of detection and limit of quantification), specificity, and peak asymmetry.

Data analysis

The insulin concentrations in the transport samples were corrected for dilution and plotted as percentage cumulative insulin transport as a function of time. Apparent permeability coefficients (P_{app}) were calculated according to the following equation [21]:

$$P_{app} = \left(\frac{dc}{dt}\right)\left(\frac{1}{A \times 60 \times C_0}\right) \quad (1)$$

where dc/dt is the amount of insulin transported within a given time period (mg/min), A is the surface area of the insert (cm^2), and C_0 is the initial drug concentration (mg/ml).

Permeation-enhancement ratios (R) were calculated from the P_{app} values with the following equation [21]:

$$R = \frac{P_{app}\,test}{P_{app}\,control} \quad (2)$$

where R is the permeation-enhancement ratio, P_{app} test is the apparent permeability coefficient for the test solution, and P_{app} control is the apparent permeability coefficient (cm/s) for the control solution.

The results were statistically analyzed by means of one-way repeated analysis of variance (ANOVA) to determine differences between TEER values, apparent permeability coefficients (P_{app}), and the permeation-enhancement ratios (R) of the different test solutions. Differences were considered significant if $p \le 0.05$.

Results and Discussion

The ^{1}H-NMR spectra of the *A. vera* gel and whole leaf materials are shown in **Fig. 1**, while the quantities of the chemical markers as determined by NMR spectroscopy are depicted in **Table 1**.

Aloverose (partly acetylated polymannose), glucose, and malic acid are used as the major markers for *A. vera* gel, while *iso*-citric acid is used as an additional marker for whole leaf extract. Because NMR distinguishes selectively between aloverose and other polysaccharides, it is clear from the results that the *A. vera* gel material used in this study contained all the necessary markers, especially aloverose, to identify it as a 200 : 1 powder of *A. vera* origin. The *A. vera* whole leaf extract material used in this study contains a significant quantity of the whole leaf marker and was identified as a 100 : 1 powder of *A. vera* origin, probably produced from whole leaf juice.

The effect of the *A. vera* materials at five different concentrations on the TEER of Caco-2 cell monolayers is presented in **Fig. 2** and **Fig. 3**.

A reduction in the TEER of Caco-2 cell monolayers is often used as an indication of the opening of tight junctions between adjacent epithelial cells, which indicates an enhancement of the paracellular permeability of the epithelial cell layer [22,23]. Both the *A. vera* gel and whole leaf extract were able to reduce the TEER of the Caco-2 cell monolayers significantly ($p \le 0.05$) compared with the control. The TEER reduction occurred in an apparently concentration-dependent way and was also slightly influenced by the pH of the environment. After removal of the *A. vera* test solutions from the apical side of the cell monolayers, the TEER recovered relatively fast over the first 20 min and eventually returned to the original value. A total recovery of the cell monolayer integrity in terms of TEER was obtained at 120 min after removal of the test solutions from the cell surface, indicating a fully reversible effect of the plant materials on the tight junctions of the epithelial cells, which also may be an indication that no structural damage occurred to the tight junctions between adjacent cells. *N*-trimethyl chitosan chloride (TMC, 2.5% w/v as positive control) reduced the TEER to a similar extent compared with the *A. vera* materials, except at pH 7.4, where its effects were higher than that of the *A. vera* whole leaf extract, but its effect was less reversible and the TEER values did not return to their original values.

The *A. vera* gel reduced the TEER to a higher extent compared with the *A. vera* whole leaf extract; for example, a concentration of 5% w/v gel reduced the TEER to 46.31 ± 3.65% of the initial val-

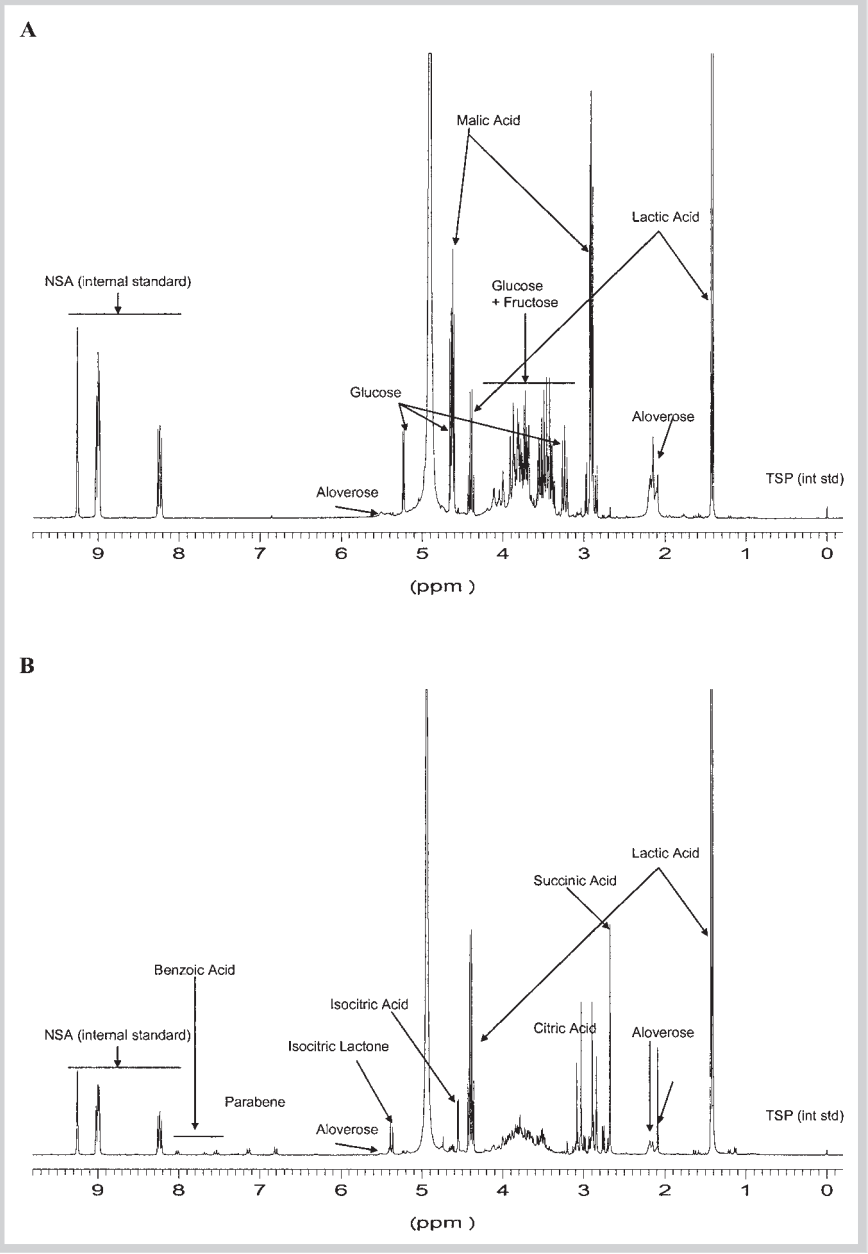

Fig. 1 ^{1}H-NMR spectra of (**A**) *A. vera* gel and (**B**) *A. vera* whole leaf extract, labeled with the main chemical constituents and markers for each plant material.

Table 1 Chemical composition of the *A. vera* gel and whole leaf materials as determined by ^{1}H-NMR.

Chemical	*A. vera* gel		*A. vera* whole leaf extract	
	Content (%)	Content (mg/L)	Content (%)	Content (mg/L)
Aloverose	12.7	892.1	5.5	383.3
Glucose	16.7	1171.2	detected	
Malic acid	20.0	1403.4	1.2	87.2
Lactic acid	5.1	359.2	21.5	1506.8
Citric acid	not detected		16.9	1181.0
WLM	detected		11.4	794.9
Maltodextrin	not detected		not detected	
Acetic acid	not detected		0.6	39.0
Succinic acid	trace		detected	
Fumaric acid	not detected		not detected	
Formic acid	not detected		not detected	
Sodium benzoate	not detected		0.3	22.7
Potassium sorbate	not detected		not detected	

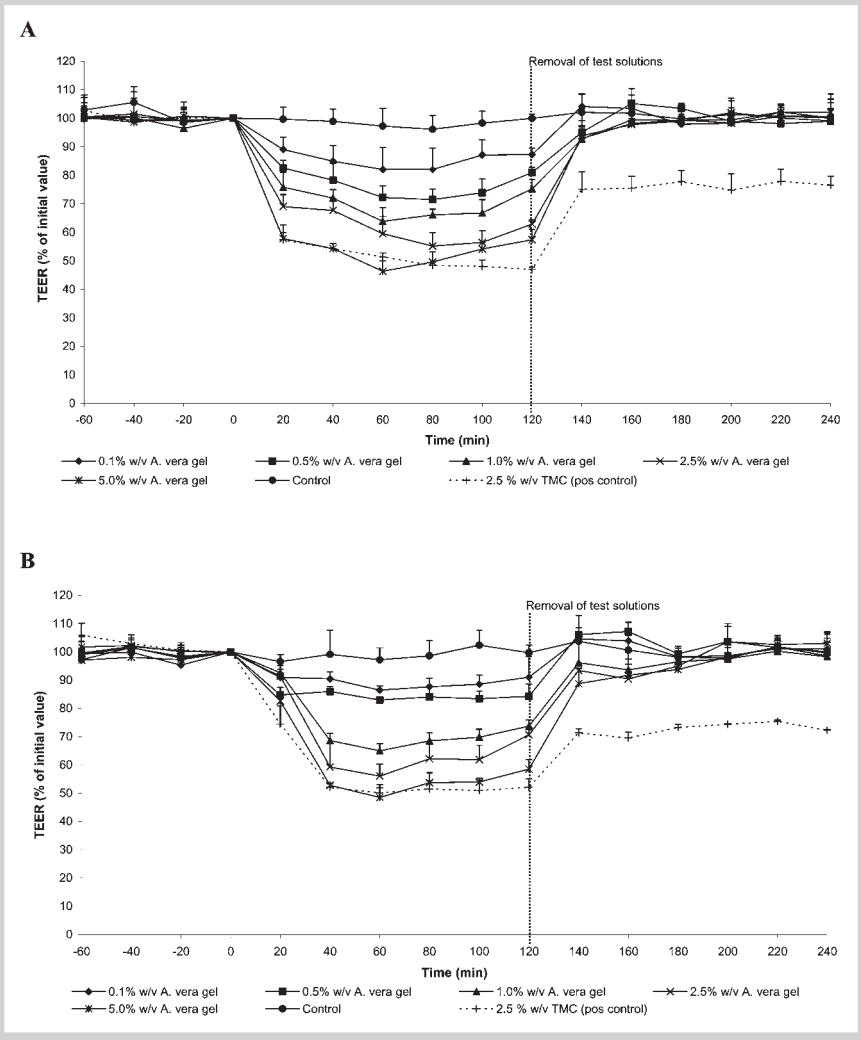

Fig. 2 TEER of Caco-2 cell monolayers plotted as a function of time after incubation with (**A**) *A. vera* gel and (**B**) *A. vera* whole leaf extract at pH 5.8.

ue at pH 5.8 and to 48.51 ± 3.43% at pH 7.4, while a concentration of 5% w/v whole leaf extract reduced the TEER only to 56.83 ± 1.44% of the initial value at pH 5.8 and to 65.43 ± 2.62% at pH 7.4. The whole leaf extract was also more sensitive to the environmental pH value than was the gel. Because this difference in the TEER reduction effect is likely to be linked to the chemical composition of the two plant materials, such as the higher acetylated mannan concentration in the gel than in the whole leaf extract (as indicated in **Table 1**), further investigations including comparative studies between the isolated chemical components of each plant material are needed.

Before the insulin samples from the transport studies were analyzed by means of RP-HPLC, the following results were obtained from the validation of the method. A regression value (R^2 value) of 0.9999 was obtained for the calibration curve, which indicates a linear relationship between the insulin concentration and the instrument response (peak area) over the tested concentration range of 2–200 µg/mL. The mean insulin recovery from spiked samples ranged from 98.99% to 100.77%, and the percentage relative standard deviation (%RSD) was less than 2%; therefore, the accuracy of the analytical method was considered acceptable. The mean insulin recovery between different days ranged from 101.55% to 103.47%, and the %RSD was less than 6%, while it ranged from 97.85% to 103.21% with a %RSD less than 1% for inter-day precision measurements. The limit of detection was determined as 0.1 µg/mL insulin, and the limit of quantitation was determined as 0.5 µg/mL insulin (%RSD < 15%). Insulin was determined in the presence of the possible excipients, and no interference with the insulin peak could be observed. The tailing factor for the insulin peak was calculated as 1.5, which is considered acceptable as it does not exceed a value of 2.

The effect of the *A. vera* gel and whole leaf extract at five different concentrations on the cumulative transport of insulin across Caco-2 cell monolayers is shown in **Fig. 4** and **Fig. 5**. The calculated apparent permeability coefficient values (P_{app}) and transport-enhancement ratios (R) for insulin transport in the presence of *A. vera* gel are depicted in **Table 2**, and those in the presence of *A. vera* whole leaf extract are shown in **Table 3**.

Insulin is poorly absorbed via the intestinal mucosa because of extensive proteolytic degradation by intestinal enzymes and insufficient membrane permeability due to its high molecular weight and hydrophilicity [24]. In accordance with the TEER results, both *A. vera* gel and whole leaf extract materials were able to significantly ($p \leq 0.05$) enhance the transport of insulin across Caco-2 cell monolayers in an apparently concentration-dependent way. The influence of pH on the transport-enhancement effect was, however, more pronounced compared with that observed in the TEER results, with a higher transport-enhancement effect at pH 5.8 than at pH 7.4. This phenomenon can possibly be explained by the charge of the phytoconstituents, which may in-

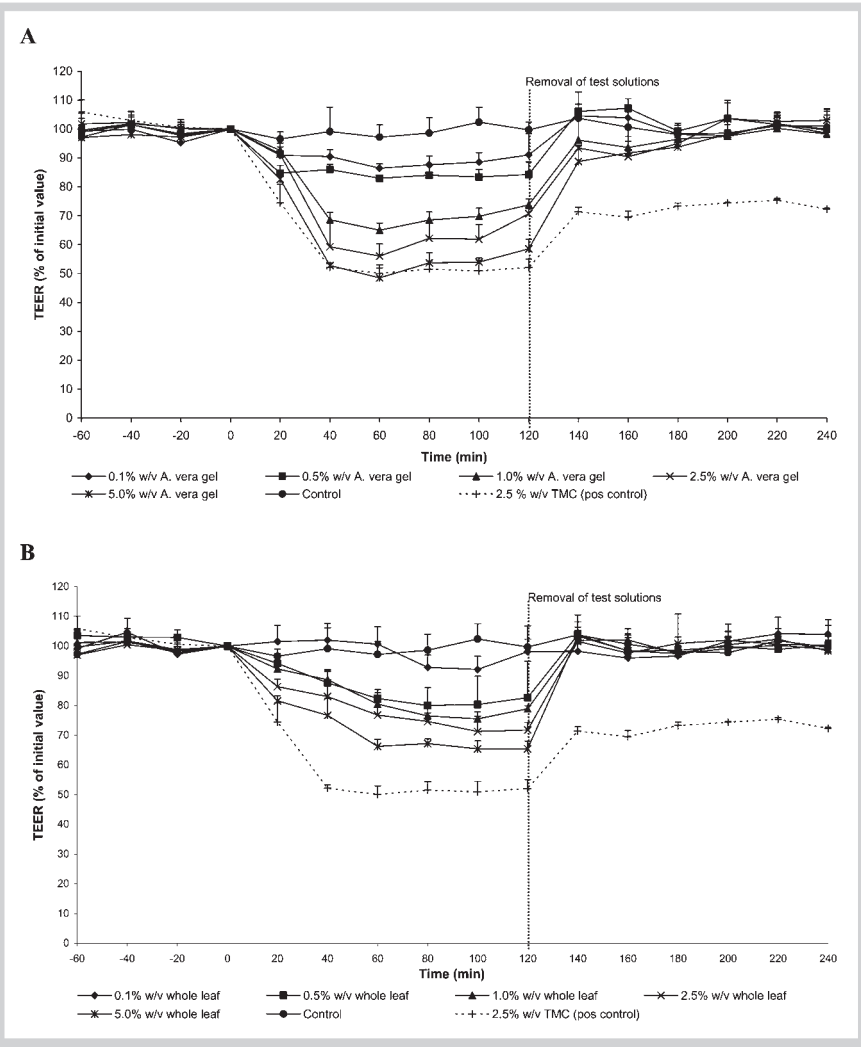

Fig. 3 TEER of Caco-2 cell monolayers plotted as a function of time after incubation with (**A**) *A. vera* gel and (**B**) *A. vera* whole leaf extract at pH 7.4.

crease with protonation at acidic pH values as in the case of chitosan, which needs to be positively charged (when protonated at a low pH) in order to exhibit an absorption-enhancement effect.

The calculated transport-enhancement ratios (R values in **Table 2**) show that in the presence of *A. vera* gel the insulin transport was between 1.92-fold (at a concentration of 0.5% w/v) and 2.54-fold (at a concentration of 5.0% w/v) higher than that of the control at pH 5.8, while at a pH of 7.4, it was between 1.86-fold (at 0.5% w/v) and 2.48-fold (at 5.0% w/v) higher than that of the control. The R values (**Table 3**) indicate that in the presence of *A. vera* whole leaf extract, insulin transport was between 2.02-fold (at 0.5% w/v) and 3.05-fold (at 5.0% w/v) higher than the control at pH 5.8, while at a pH of 7.4, it was between 1.75-fold (at 0.5% w/v) and 2.16-fold (at 5.0% w/v) higher compared with the control. In contrast to the TEER results, the whole leaf extract was more effective in insulin transport enhancement compared with the gel at pH 5.8 at certain concentrations. However, in accordance with the TEER results, the gel was more effective in insulin transport enhancement at all concentrations compared to the whole leaf extract at pH 7.4.

The insulin transport results from this study indicate that *A. vera* gel and whole leaf extract are potential intestinal absorption-enhancing agents for poorly absorbable drugs such as insulin. These results are in accordance with a previous study where *A. vera* gel and whole leaf extract showed the ability to enhance the bioavailability of vitamins C and E in humans [16]. It can be deduced from this *in vitro* study that the transport-enhancement effect of the *A. vera* leaf materials was probably achieved by means of opening of the tight junctions to allow for increased paracellular movement of the insulin molecules. Evidence for paracellular transport enhancement was obtained by the ability of the plant materials to significantly reduce the TEER of the epithelial cell monolayers as well as by a significant increase in the transport of a macromolecular, hydrophilic compound for which transcellular transport is normally excluded. Such a macromolecule could only be transported across the epithelial cell monolayers when the tight junctions are opened to allow for movement via the paracellular pathway, which is in accordance with previous results where FITC-dextran with a molecular weight of up to 20 000 was shown to move through the paracellular route of Caco-2 cell monolayers when incubated with an absorption enhancer such as *N*-trimethyl chitosan chloride by means of confocal laser scanning microscopy [25].

However, these results also indicate a potential drug–herb interaction when *A. vera* products are taken simultaneously with drugs whose absorption is not intended to be enhanced or changed in any way. An uncontrolled increase in the bioavailability of these drugs may result in adverse side effects, and toxic levels of the drug may be reached.

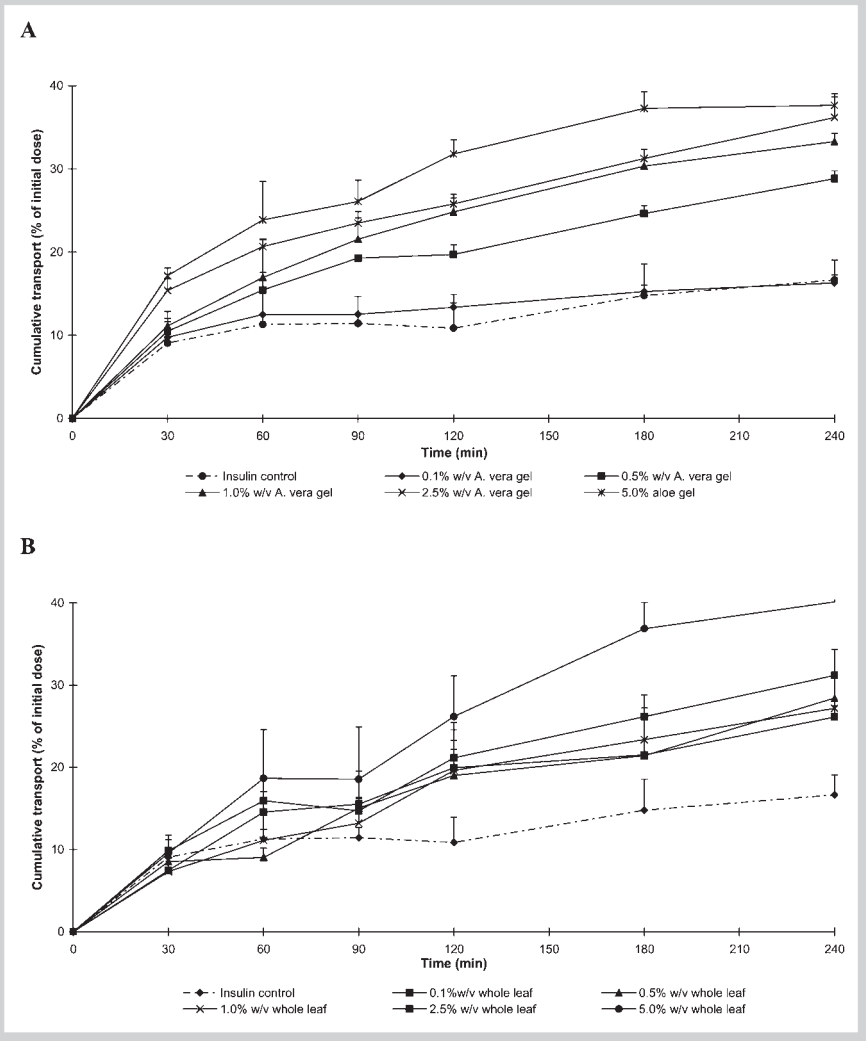

Fig. 4 Cumulative insulin transport across Caco-2 cell monolayers as a function of time in the presence of (**A**) *A. vera* gel and (**B**) *A. vera* whole leaf extract at pH 5.8.

Table 2 Apparent permeability coefficient values (P_{app}) and transport-enhancement ratios (R) for insulin transport in the presence of *Aloe vera* gel.

Aloe vera gel concentration (% w/v)	$P_{app} \times 10^{-6}$ (cm/s) at pH 5.8	R	$P_{app} \times 10^{-6}$ (cm/s) at pH 7.4	R
Control	1.94 ± 0.43	1.00	1.66 ± 0.22	1.00
0.1	1.84 ± 0.38	0.95	2.74 ± 0.34*	1.65
0.5	3.73 ± 0.13*	1.92	3.09 ± 0.37*	1.86
1.0	4.56 ± 0.30*	2.35	3.36 ± 0.20*	2.02
2.5	4.55 ± 0.29*	2.35	3.84 ± 0.12*	2.31
5.0	4.93 ± 0.38*	2.54	4.11 ± 0.32*	2.48

Note: Each value represents the mean ± SD of 3 experiments. * $p \leq 0.05$ compared with control.

Table 3 Apparent permeability coefficient values (P_{app}) and transport-enhancement ratios (R) for insulin transport in the presence of *Aloe vera* whole leaf extract.

Whole leaf extract concentration (% w/v)	$P_{app} \times 10^{-6}$ (cm/s) at pH 5.8	R	$P_{app} \times 10^{-6}$ (cm/s) at pH 7.4	R
Control	1.91 ± 0.35	1.00	1.66 ± 0.22	1.00
0.1	3.48 ± 0.37*	1.82	2.01 ± 0.15	1.21
0.5	3.86 ± 0.40*	2.02	2.91 ± 0.30*	1.75
1.0	3.87 ± 0.29*	2.03	3.44 ± 0.25*	2.07
2.5	4.18 ± 0.50*	2.19	3.53 ± 0.34*	2.13
5.0	5.83 ± 0.13*	3.05	3.59 ± 0.40*	2.16

Note: Each value represents the mean ± SD of 3 experiments. * $p \leq 0.05$ compared with control.

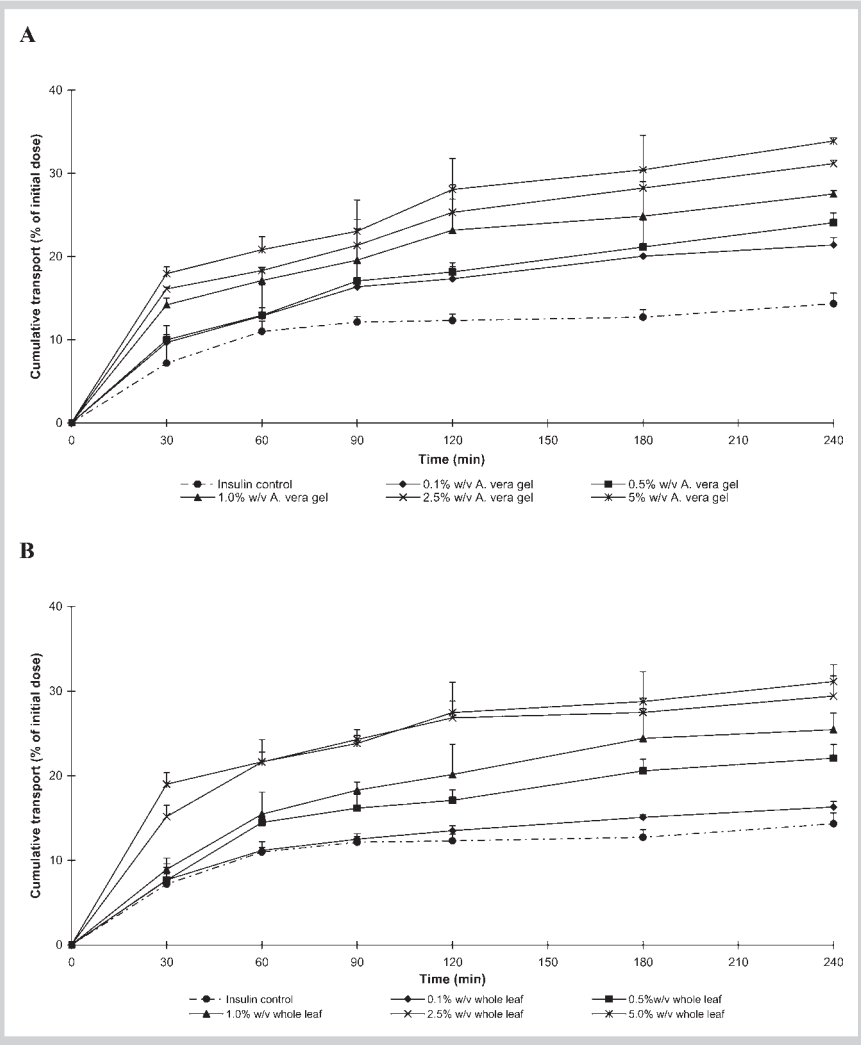

Fig. 5 Cumulative insulin transport across Caco-2 cell monolayers as a function of time in the presence of (**A**) *A. vera* gel and (**B**) *A. vera* whole leaf extract at pH 7.4.

Acknowledgements

The financial contribution of Tshwane University of Technology and the National Research Foundation of South Africa towards this research project is acknowledged.

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