



ASSESSMENT OF THE EFFECT OF BROMELAIN ON GLUCOSE UPTAKE USING EVERTED GUT SAC TECHNIQUE

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ARTICLE INFO

Received:

24 Apr 2023

Received in revised form:

10 Sep 2023

Accepted:

17 Sep 2023

Available online:

28 Oct 2023

Keywords: Bromelain, Gut sac, Glucose, Chick ileum, Kinetics

ABSTRACT

The current work mainly focused on the determination of the bromelain effect on glucose uptake through the everted gut sac technique. Everted gut sacs are majorly used in evaluating the uptake mechanisms and absorption potential. Bromelain is a natural phytochemical obtained from pineapple. It can alter various body mechanisms. Everted gut sacs were prepared from chick ileum. After thorough cleaning, the gut sacs were filled with Krebs-Henseleit bicarbonate buffer (KHB). Glucose at varying concentrations was added into mucosal compartment fluid. Bromelain was also added to the mucosal compartment with simulated gastric fluid. At time intervals of 5, 10, 15, 20, 30, 45, and 60 min, 0.5ml of samples were collected from the mucosal fluid for glucose level estimation. A kinetic study was also conducted to understand the transport/inhibition of glucose across the intestinal membrane. Incubation of the chick-everted gut sacs with Bromelain at concentrations of 10 and 20µg/mL increased the transport of glucose. The glucose transport was significant at various concentrations i.e., 5.5, 6.5, 7.5, and 8.5 mM/L in the incubation medium. The pattern of glucose uptake *ex-vivo* was analyzed using Michaelis-Menten and Lineweaver-Burk Plots. When compared to control, K_m has decreased, but V_{max} remained unchanged in the presence of Bromelain at a dose of 10µg/ml. Whereas in the case of Bromelain given at a dose of 20µg/mL, the V_{max} has increased and K_m remained constant when compared with control. Thus, the current research indicated that Bromelain at various doses increased glucose uptake.

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To Cite This Article: Doppalapudi S, Suryadevara V, Ramineni SR, Chadalavada AK. Assessment of the Effect of Bromelain on Glucose Uptake Using Everted Gut Sac Technique. *Pharmacophore*. 2023;14(5):33-9. <https://doi.org/10.51847/Rrhik6LrZc>

Introduction

Many factors affect the systemic availability of drug molecules through the oral route. Intestinal absorption is considered a key factor for the bioavailability of oral dosage forms [1, 2]. Several factors can affect the transfer process of drug compounds across the intestinal epithelial mucosa like, physicochemical properties of drugs, formulation aspects, physiological properties of the gastrointestinal tract (GIT), and other co-administered agents. The factors related to drugs like solubility, ion charge, penetration capacity, particle size, particle shape, salt and isomeric forms, polymorphism, and stability have been subjected to wide research and modified using material science and product technology [3, 4]. However, systemic factors such as membrane transporter, intestinal enzymes, membrane permeability, area or site of absorption, mechanism of absorption, etc. are difficult to manipulate [5, 6]. In the last two to three decades drug interactions, metabolic pathways, and absorption mechanisms have been extensively investigated through *in-vivo* as well as *in-vitro* models [7, 8]. Drug absorption, metabolism, and drug interaction are relatively complex processes. Along with the passive absorption process, the absorption through the intestine is favored by several carrier molecules or pumps existing in the intestinal epithelium [9, 10]. The influx pump moves drug substances from the intestinal mucosal area to the serosal side. Another pump, the efflux pump, works in the reverse direction and moves drugs from the serosal area to the mucosal side. Various kinds of influx and efflux transporters have been found in

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the intestinal area. In general, when the efflux transporters get interfered with during the drug absorption phase, it decreases bioavailability [11, 12]. Several methods have been used to assess drug absorption, which include physicochemical models, in silico computational models, in situ models, *in vitro* models, and *in vivo* animal models [13, 14]. The techniques employed for the determination of intestinal absorption and permeability of drug molecules should be ideally reliable, cost-effective, fast, and highly predictive [15, 16]. Several *in-vitro* techniques have been utilized to investigate intestinal transport, including the everted gut sac technique, using a chamber, isolated epithelial cells, and brush-border and basolateral membranes isolated from enterocytes. The functioning of carrier-mediated transport in the process of drug absorption and drug interaction could be evaluated with the help of the everted sac model [17]. Food and drugs, ions, and other transport modulators act as external factors that affect carrier-mediated transport [18]. The major positive aspects of the gut sac model are wider surface area availability for the process of absorption and mucus layer presence [19]. The intestinal epithelium has many functions, including serving as a barrier against invasion from the environment, digestion and absorption of nutrients, and water and electrolyte/ solute transport.

Compounds extracted from plant sources are widely used in commercial medicine preparations these days for treating heart, and respiratory diseases and many other health issues. Most healthcare personnel are focused on the positive and significant negative outcomes of the usage of herbal medicines to treat diseases [20]. Apart from regular therapeutic uses, they also affect the absorption of other drugs. Bromelain is a natural agent derived from pineapple. It is considered a protein-digestive enzyme that is used along with other medications to reduce inflammation, osteoarthritis, and muscle pain. It is also employed as a digestive aid. It shows a protective effect in cardiac and diabetic patients [21]. It ought to exhibit ameliorative effects in diabetic rats [22]. The main objective of the present research was to determine the effect of bromelain on glucose uptake through the everted gut sac technique. As the effect of Bromelain through the everted gut sac technique on glucose uptake has not been studied earlier, this novel research could help in better understanding the underlying mechanism.

Materials and Methods

Procurement of Materials

Bromelain was commercially procured from Sisco Research Laboratories (New Delhi, India). D-glucose and all other chemicals required for the research were commercially procured from Fischer Scientific Pvt. Ltd. (Mumbai, India).

Procurement and Preparation of Everted Gut Sacs

Freshly isolated chick ileum was procured from a nearby slaughterhouse and quickly moved to the laboratory. Manually, the total small intestine part was removed immediately by cutting across the duodenum upper end and the ileum lower end with the stripping of the mesentery. The small intestine was then washed out with normal saline solution (0.9% w/v sodium chloride) using a syringe. The collected intestine was cut into small pieces each of 5 ± 1 cm length. The intestinal segments were everted according to the method described in past studies [23]. After being everted, the segments of guts were blotted with a piece of Whatman filter paper no. 40 and weighed. One gram of glass weight was passed through every everted gut segment to empty the gut sac without any remnants. This is mandatorily done to avoid any peristaltic muscular contractions, which could potentially change the shape and internal volume of the sac. Then, the everted intestinal gut sacs were filled with 0.5 mL of Krebs-Henseleit bicarbonate buffer (KHB).

Evaluation of Intestinal Glucose Uptake

D-glucose (2g/L) was added to the medium just before the start of the experiment. The pH was maintained at 7.4. The gut sac was inserted with a blunt end syringe and from that, the needle was taken off with utmost care to avoid any rupture to the sac. The upper end of the sac was tied tightly using a thread. The compartment with buffer in the sac was considered a serosal fluid compartment. This filled sac was lodged into a 35 mL KHB bath (mucosal fluid compartment) and mounted upright. This gut sac bath was placed in a carbon dioxide incubator adjusted at 5% CO₂ and 37°C.

For studying the effect of Bromelain on the uptake of glucose (substrates), glucose at varying concentrations like 5.5, 6.5, 7.5, and 8.5mM was added into mucosal compartment fluid. These doses of glucose were selected to maintain the sensitivity of the study [24]. Bromelain was also added in the same compartment in simulated gastric fluid at doses of 10 and 20µg/mL. These doses of Bromelain were selected based on past research [25]. Then, at time intervals of 5, 10, 15, 20, 30, 45, and 60 min, samples were collected from the mucosal fluid for analysis of glucose levels. 0.5ml of the sample has been collected and the same amount of glucose solution has been replaced to maintain the sink conditions. After 60 minutes of the incubation period, the sacs were taken out from the gut sac bath, blotted using filter paper, and the weights were noted. The serosal fluid was collected into a test tube by making a small cut. The emptied sac was shaken gently to remove the adhered fluid and the tissue was weighed. The final serosal volume was determined by subtracting (after incubation) the weight of the empty sac from that of the filled sac. The gut fluid uptake was determined by measuring an increase in the volume of fluid in the gut wall [26].

Glucose concentrations in both compartments were measured with a commercially available glucose estimation kit (Beacon Diagnostics Pvt. Ltd., Navsari, Gujarat, India) using a semi-autoanalyzer (VChem Next, Vector Biotek Pvt. Ltd., Navsari, Gujarat, India). The amount of D-glucose transported from the mucosal compartment was characterized as 'uptake', while the

serosal gain of the substances is treated as 'release'. Uptake and release of glucose were expressed as mM/g tissue wet weight. A similar procedure was followed for control, i.e., without the addition of Bromelain (**Tables 1-3 and Figures 1-3**).

Kinetic Study on Glucose Uptake

A kinetic study was performed to know the process of glucose transport or inhibition across the intestinal membrane. According to enzyme kinetics, the quantity of glucose transported per hour was in correlation to the velocity of transfer. In other words, it is the concentration difference of the glucose between compartments at the beginning and end of an experiment [27]. The Michaelis-Menten constant (K_m), is the affinity of the transferring enzyme (glucose transporter) for the substrate (glucose). The maximal velocity (V_{max}), is the rate of transfer reaction in the presence as well as in the absence of the Bromelain, which was determined using Michaelis-Menten and the Lineweaver-Burk Plots in Microsoft Excel. A comparison of the difference between the control and experimental groups was done using an unpaired t-test (**Table 4**).

Statistical Analysis

The results were expressed in mean \pm standard error of the mean. Statistical analysis was done with Graph Pad Prism Software (Version 5.0).

Results and Discussion

Estimation of blood glucose level by *in vitro* mode is gaining significance in the determination of antidiabetic activity of plant component-based research [28]. The research on Bromelain as a health supplement is in light in various ways. It affects fat content and improves digestion. However, very little research was done on its usage in the case of diabetes. Control of blood glucose is a challenging thing in the case of diabetes patients. So, the current research was focused on evaluating the effect of Bromelain on glucose uptake which could be beneficial in the case of patients suffering from diabetes. The results obtained from the present research showed that Bromelain increases the uptake of glucose which potentially reduces glucose levels in the body.

Evaluation of Intestinal Glucose Uptake

Everted chick gut sacs were subjected to a study of glucose uptake. In the control group, only glucose was administered without any Bromelain. In this case, at the end of 60 min, only 31.45 μ mol of glucose has been uptaken even at higher concentrations of glucose in the medium i.e., 8.5mM. This shows normal uptake of glucose without any other factorial influence. A linear increase in glucose uptake was observed with respect to an increase in time and glucose concentrations (**Table 1 and Figure 1**).

Table 1. Glucose Uptake in Control Gut Sac

S. No	Time (min)	Glucose uptake (μ mol/g tissue) at various Glucose Concentrations (mM)*			
		5.5	6.5	7.5	8.5
1	5	0.45 \pm 0.03	1.02 \pm 0.02	2.47 \pm 0.06	3.26 \pm 0.09
2	10	2.47 \pm 0.11	4.06 \pm 0.08	6.44 \pm 0.12	8.23 \pm 0.15
3	15	4.51 \pm 0.09	7.85 \pm 0.20	10.57 \pm 0.29	14.12 \pm 0.31
4	20	6.08 \pm 0.33	10.23 \pm 0.25	16.03 \pm 0.21	19.31 \pm 0.11
5	30	7.23 \pm 0.16	16.95 \pm 0.28	18.19 \pm 0.31	24.43 \pm 0.47
6	45	12.35 \pm 0.14	20.70 \pm 0.09	21.03 \pm 0.10	30.12 \pm 0.16
7	60	17.53 \pm 0.31	21.02 \pm 0.08	29.58 \pm 0.25	34.15 \pm 0.28

Note: *Mean \pm SEM (Standard Error of Mean) values for three samples

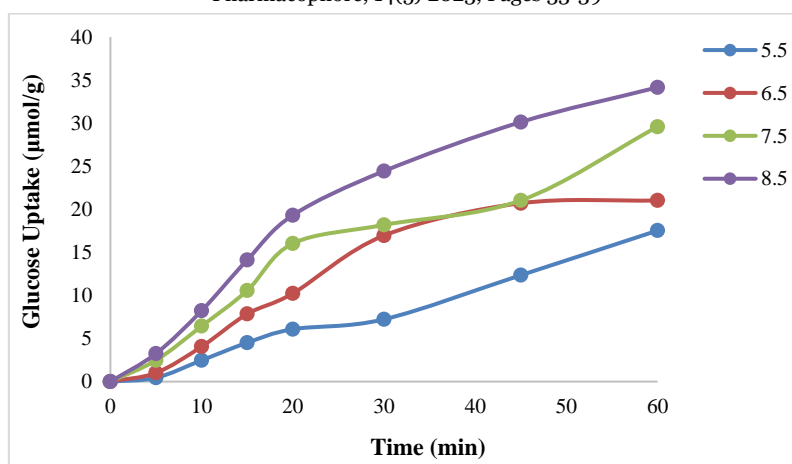


Figure 1. Glucose Uptake in Control Gut Sac

Incubation of the chick-everted gut sacs with Bromelain at concentrations of 10 and 20 µg/mL resulted in the increase of transport of glucose from mucosal to serosal fluid. The glucose transport was significant at varied concentrations i.e., 5.5, 6.5, 7.5, and 8.5 mM/L in the incubation medium. When Bromelain was administered at a dose of 10 µg/mL, the amount of glucose uptake through gut sacs was increased. This increase is drastic and a 1.36-fold increase in glucose uptake was observed with 5.5mM. 2.26 and 2.84-fold increase in glucose uptake was observed with 6.5 and 7.5mM glucose concentrations. This uptake was 2.71-fold with 8.5mM glucose concentration which is a bit low compared to 7.5mM (Table 2 and Figure 2).

Table 2. Effect of Bromelain on Glucose Uptake in Gut Sac at 10 µg/mL dose

S. No	Time (min)	Glucose uptake (µmol/g tissue) at various Glucose Concentrations (mM) *			
		5.5	6.5	7.5	8.5
1	5	1.04 ± 0.01	2.22 ± 0.03	3.71 ± 0.02	5.90 ± 0.06
2	10	3.58 ± 0.04	6.24 ± 0.07	10.51 ± 0.04	14.02 ± 0.08
3	15	5.73 ± 0.11	14.63 ± 0.14	24.35 ± 0.19	28.10 ± 0.17
4	20	7.85 ± 0.16	24.81 ± 0.18	43.49 ± 0.27	45.22 ± 0.29
5	30	13.77 ± 0.09	34.81 ± 0.21	59.86 ± 0.44	66.10 ± 0.47
6	45	19.07 ± 0.18	42.02 ± 0.40	73.61 ± 0.73	83.09 ± 0.68
7	60	23.91 ± 0.22	47.58 ± 0.35	84.27 ± 0.91	92.86 ± 0.84

Note: *Mean ± SEM (Standard Error of Mean) values for three samples

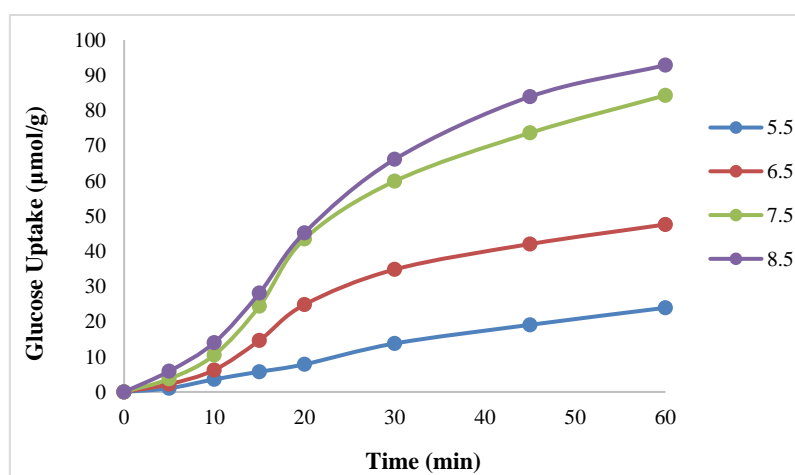


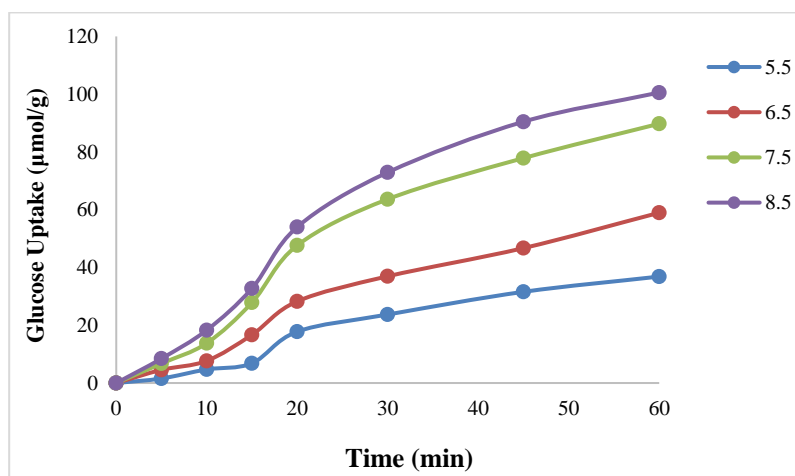
Figure 2. Effect of Bromelain on Glucose Uptake in Gut Sac at 10 µg/mL dose

When Bromelain was administered at a dose of 20 µg/mL, the amount of glucose uptake through gut sacs was increased. This increase is more when compared to Bromelain administered at 10 µg/mL. A 2.10-fold increase in glucose uptake was observed with 5.5mM. 2.80 and 3.04-fold increase in glucose uptake was observed with 6.5 and 7.5mM glucose concentrations. This uptake was 2.94-fold with 8.5mM glucose concentration which is a bit low compared to 7.5mM (Table 3 and Figure 3).

Table 3. Effect of Bromelain on Glucose Uptake in Gut Sac at 20 μ g/mL dose

S. No	Time (min)	Glucose uptake (μ mol/g tissue) at various Glucose Concentrations (mM)*			
		5.5	6.5	7.5	8.5
1	5	1.57 \pm 0.03	4.56 \pm 0.03	6.76 \pm 0.04	8.47 \pm 0.05
2	10	4.71 \pm 0.02	7.68 \pm 0.05	13.80 \pm 0.09	18.30 \pm 0.12
3	15	6.85 \pm 0.04	16.71 \pm 0.11	27.86 \pm 0.16	32.77 \pm 0.18
4	20	17.79 \pm 0.08	28.28 \pm 0.20	47.66 \pm 0.33	54.02 \pm 0.48
5	30	23.71 \pm 0.15	36.95 \pm 0.29	63.65 \pm 0.57	72.94 \pm 0.69
6	45	31.57 \pm 0.19	46.71 \pm 0.36	77.86 \pm 0.81	90.47 \pm 0.93
7	60	36.85 \pm 0.27	58.95 \pm 0.55	89.77 \pm 0.90	100.56 \pm 0.95

Note: *Mean \pm SEM (Standard Error of Mean) values for three samples

**Figure 3.** Effect of Bromelain on Glucose Uptake in Gut Sac at 20 μ g/mL dose

This effect of Bromelain on glucose uptake has been studied only once in rats with diabetes [29]. Although this study was the first one to evaluate the Bromelain effect on glucose uptake using *ex-vivo* animal tissue, it has not exclusively focused on glucose uptake. So, the current study was done and successively reported the glucose uptake effect of Bromelain.

Kinetic Study on Glucose Uptake

The pattern of glucose uptake *ex vivo* in various experimental protocols was analyzed using Michaelis-Menten and Lineweaver-Burk Plots (**Table 4**). When compared to control, K_m has decreased, but V_{max} remained unchanged in the presence of Bromelain at a dose of 10 μ g/mL. This is an unusual phenomenon that is out of the regular enzyme mechanisms. Here the enzyme was activated definitely which is indicated by an increase in glucose uptake. Past studies showed similar conditions when certain enzymes are used. Whereas in the case of Bromelain given at a dose of 20 μ g/mL, the V_{max} has increased and K_m remained constant when compared with control. This is also an unusual phenomenon that is observed out of the enzyme kinetics. In both cases, the enzyme was activated. But there is no enzyme inhibition. This might be attributed to the novel concept of crowding as indicated in past research [30]. The common principle is that as the enzyme concentration increases, the velocity of the reaction increases. However, the difference between the levels of glucose uptake when Bromelain was administered at 10 and 20 μ g/mL is not that high. This indicated that the efficacy of Bromelain was lowered at higher doses.

Table 4. Effect of Bromelain on Kinetic Parameters of Glucose Transport

S. No	Treatment	V_{max} (mM/h)	K_m (mM)
1	Control	0.027	24.03
2	Bromelain (10 μ g/mL)	0.024	11.36
3	Bromelain (20 μ g/mL)	0.096	24.39

Some enzyme effectors alter only one of the two Michaelis-Menten parameters like V_{max} and K_m . This condition is due to a phenomenon called crowding. Crowding indicates the surrounding solvent effect on the kinetic parameters of enzymes. In *ex-vivo* conditions, the crowding agents could be the physiological salt solutions used in the laboratory. This could be extrapolated to *in-vivo* conditions. Past studies strongly indicated the concept of crowding in the case of some enzyme activities [31]. However, apart from the crowding, Bromelain showed increased glucose uptake.

Conclusion

The effect of Bromelain on glucose uptake was studied using the everted gut sac technique on chick ileum. From this research, it was observed that Bromelain at various doses has increased the uptake of glucose through the entire gut sac. The enzyme kinetics has supported the same effect. This leads to reduced blood glucose levels, which would be a benefactor in diabetics.

Acknowledgments: The authors are thankful to the management of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences for their sheer support throughout the work.

Conflict of interest: None

Financial support: None

Ethics statement: None

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