Intestinal Handling of Glucose in *Buchholzia coriacea* Treated Male Wistar Rats

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**Abstract**

**Background:** Different studies have unveiled the traditional usefulness and clinical potentials of *Buchholzia coriacea*, a medicinal plant known for its effectiveness in lowering blood glucose. Its role in intestinal glucose uptake was investigated. **Materials and methods:** Thirty male Wistar rats, weighing between 100 - 120 g were used and randomly assigned into three groups of 5 rats each per experiment. Group 1: control (not treated), groups 2 and 3, were treated with 100 mg/kg (BC100) and 200 mg/kg (BC200) of *Buchholzia coriacea* orally respectively for 2 weeks. Fasting blood glucose, luminal and in vitro glucose levels of rats were determined by glucose oxidase method using glucometer stripes plus glucose monitoring system (Fine test glucometer(R)). Luminal electrolytes in the in vitro study were determined by Atomic Absorption Spectrophotometry method. Data were expressed as Mean ± SEM and statistical analysis was by one way ANOVA, and p-values < 0.05 were considered significant. **Results:** There was no significant change in the fasting blood glucose level (mg/dl) of rats in BC100 (78.00 ± 2.16) and BC200 (76.0 ± 3.57) compared with control (79.50 ± 1.70). There was significant increase in glucose uptake (mg/dl/g tissue) in the in vivo experiments in both the ileum and jejunum of BC100 (23.08 ± 0.18; 19.68 ± 0.72) and BC200 (14.50 ± 1.02; 20.55 ± 0.45) compared with control (30.40 ± 1.01; 35.53 ± 1.45), respectively. The glucose uptake at the mucosa end of distal jejunum in the BC100 (292 ± 3.33) and BC200 (209.30 ± 2.67) decreased significantly compared with control (90.00 ± 1.50). There was a significant decrease in K+ concentration and increase in Na+ concentration at the mucosa end in the BC100 and BC200 compared with control. **Conclusion:** The study suggests glucose stowing in the intestinal cells in the in vivo study and inhibition of glucose transport from the in vitro study. The roles of alpha-amylase on the activity of this extract are suggested for future studies.
1. Introduction

Gastrointestinal tract (GIT) continually provides the body with water, electrolytes and nutrients. To accomplish this, it requires movement of food through the gastrointestinal tract, secretion of digestive juices and digestion of food, absorption of water, electrolytes and digestive products. These functions are enhanced by the circulation of blood through the gastrointestinal organs which aid the transportation of absorbed substances and control of all these functions by local, nervous and hormonal systems. The GIT has proven to be a more useful and nutritional tool than mere biochemical classification of carbohydrates, thereby permitting new perceptions of the physiologic effects of carbohydrates [1].

Daily intestinal absorption consists of several hundred grams of carbohydrates. Essentially, all the carbohydrates in the food are absorbed in the form of monosaccharide; only a small fraction is absorbed as disaccharides and almost none as larger carbohydrate compounds. By far, the most abundant of the absorbed monosaccharide is glucose. To realize rapid absorption, the intestine is endowed with legion of co-ordinated cellular mechanisms for controlling the rate of relocation of nutrients such as glucose from the intestinal lumen to the blood [2]. The stimulation of glucose uptake by the body cells is recognized as one of the most important characteristics of insulin [3]. Insulin effect is said to be due to an acceleration of glucose transport through the cell membrane as first proposed by Levine et al., [4].

Evidence that the intestine plays a specialized role in glucose homeostasis has been described at various times and under varied conditions with different models of animals [5] [6] [7]. Hence the role of the intestine in glucose uptake is well established in both normal and diabetic models. Glucose enters eukaryotic cells via two different types of membrane associated carrier proteins, the Na+ coupled glucose transports (SGLT) and glucose transporter facilitators (GLUT) [8]. The outright speed of glucose absorption hinges on the contact time of the small intestine with glucose, the area and length of small intestine exposed, and the number of functional enterocytes and their expression of glucose transporters as well as the presence of luminal sodium ions [9]. However, gut alpha amylase could also affect the rate and amount of glucose that is trans-located into the enterocytes [6].

Currently, medicinal plants are crucial in traditional medical remedies for treating various ailments as two-thirds of the world population rely on them because they are relatively available and affordable compared to other pharmaceutical products [10]. In recent times, during drug discovery and development, medicinal plants have consistently been considered the foremost source of...
pharmaceuticals engaged treatment of many human diseases. This is because of their extraordinary chemical variety and extensive natural importance [11].

Buchholzia coriacea is widely known as wonderful kola, a perennial plant which grows as a tree belonging to the family of capparaceae. The seeds are sheltered in a purple aril which is chewed in some Sub-Saharan African countries and known to have a strident spicy taste. It is a medicinal plant folklorically used in the treatment of feverish conditions in human [12]. The initial work carried out by Delaveau et al., [13], describe the presence of alkaloids L-starchydine and L3-hydrostarchydine as the main constituents but not present in the leaves. Ajaiyeoba et al., [14] reported that Lupeol and B-sitosterol were the most active fractions of the methanolic extract of the stem bark. Research output on different parts of the plant has ascribed several medicinal importances such as; anti-helminthic, anti-plasmodic, anti-microbial, hypoglycemia, anti-diarrhea, anti-spasmodic and analgesic effects to the plant [15]. Owonikoko et al. [16] reported that Buchholzia coriacea seeds in diet hastened gastric ulcer curative rate in acetic acid induced gastric ulcer in rats. Similar report by Salami et al. [17] on Buchholzia coriacea treatment and ischemia-reperfusion gastric ulcer reported better healing rate ascribed to its ability to suppress gastric acid secretion. More notably for this study is the anti-hyperglycemic and the anti-diabetic properties reported for this plant with dearth of knowledge on the role of the intestine in glucose uptake when treated with Buchholzia coriacea.

2. Materials and Methods

2.1. Experimental Animals and Groupings

Thirty Male Wistar rats (100 - 120 g) were purchased from College of Medicine, University of Ibadan Animal House, Ibadan, Nigeria. All rats were housed in wired cages bedded with wood shavings under standard laboratory conditions. They were allowed free access to standard rats pellet (Arand cereals and oil mills Ltd, Oyo, Nigeria) and water was provided ad libitum. The animals were acclimatized for two weeks prior to commencement of the experiments. Subsequently, they were divided into three groups of 10 animals per experiment. The experiments were in two phases, experiments in phase 1 were conducted in vivo while that of phase 2 was an in vitro experiment. All experiments were conducted according to the guidelines and it was approved by the University of Ibadan Animal Care and Use Research Ethics Committee (Assigned number-UI-ACUREC/17/0058), this conforms to the International Guidelines for Handling of Laboratory Animals [18].

2.2. Plant Collection and Extraction

The fresh seeds of Buchholzia coriacea seed were bought from Oje market in Ibadan, they were cut to pieces, air-dried at room temperature. The dried seeds were then pulverized and soaked in ethanol for three days. After 3 days, it was decanted and re-soaked in ethanol dichloromethane n-hexane again for another
3 days. It was finally decanted and filtrate was collected using Whatman No.1 filter paper. The filtrate was refrigerated at 4°C until use.

2.3. Animal Grouping and Treatment

The experimental study was divided into two phases, the in vivo and in vitro studies.

Each phase consisted of 3 groups of 5 animals each viz:
Group 1: Control (distilled water).
Group 2: were given Buchholzia coriacea 100 mg/kg.
Group 3: were given Buchholzia coriacea 200 mg/kg.

2.4. Determination of Glucose Concentration

The in vivo methods:

Determination of fasting blood glucose level

The rats were allowed free access to food and water and housed at relatively constant room temperature. Food was withdrawn from all groups 18 h prior to the start of experiment and blood was obtained from the tail vein of rats for glucose concentration which was determined through a quantitative glucose oxidase method using a glucometer stripe plus glucose monitoring system. The instructions provided by the kit manufacturer were essentially followed.

Determination of intestinal glucose absorption

To evaluate the effect of fasting duration on intestinal absorption of glucose, the method described by Odukanmi et al [7] was used. The rats were allowed free access to food and water and housed in a relatively constant room temperature. Food was withdrawn from the animals prior to the experiment and they were anesthetized with ketamine (75 mg/kg body weight). The trachea was slit opened to allow animals breathe spontaneously. Through a midline laparotomy, the intestinal segments of about 20 cm long were identified for consistency after separating the intestine into 2 major segments. The proximal segment was distal to the ligament of Treitz (Jejunum) while the distal end was just proximal to the ileocecal junction (ileum). The segments were open at both ends then to permit gentle irrigation with Ringers solution. The excess fluid was removed by gently forcing air under low pressure through the segments. The distal end of each segment was then ligated and 4 mL of Ringers solution containing 4.4 g of glucose was infused into each segment through the proximal ends. The proximal open ends were then ligated and the intestinal segments were returned to the abdominal cavity, and were sutured. The temperature of each animal was maintained with a heating lamp. The initial glucose concentration was noted and samples were collected at intervals of 60 minutes from each animal. The glucose concentrations was analyzed through a quantitative assay (Glucose Oxidase) method using Fine test® glucose monitoring system. Each study lasted for 1 hour, at the end of which the two segments were drained of their content and dried with a filter paper. Weights of each intestinal segment were determined.
and amount of glucose absorbed per gram of intestinal segment was evaluated. The difference between the initial glucose concentration and the final glucose concentration was divided by the factor of their respective weight in gram.

**In vitro method**

The everted sac method was used. Tissue preparation and mounting—Rats were anesthetized with ketamine after which laparotomy was conducted and the intestines were isolated from the ligament of Treitz proximally to the caecum distally. The mesentery was carefully removed and the excised intestine was quickly rinsed in ice-cold Ringer solution (glucose free, (mM)—140 NaCl, 5 KCl, 3 CaCl₂, 1 MgCl₂, 20 NaHCO₃, 10 HEPES-Tris, distilled water, pH 7.4). The whole length was separated into 3 equal segments (A-C segments). Each part was subsequently everted according to the previous method described by Odukanmi et al. [6]. The sleeve of tissue was ligated on one end and 1 mL of Ringer solution (Glucose-free) was gently released into the serosa a side of the sac and again ligated from the opened end with the aid of a micropipette that was adequately calibrated. The sac was then introduced into a test tube containing 5 mL Ringer solution (with 10 mM Glucose), and appropriately gassed with carbogen and incubated at 37°C for 1 hour. During the preparatory phase, mounted and un-mounted tissues were kept in ice-cold Ringer, gassed with 5% CO₂ and 95% O₂. After the incubation period, 5µL of samples were taken from serosa and mucosa fluid for determination of glucose concentration. Weight of each segments post experiment was taken with a digital weighing scale and recorded. The absorption rate was determined thus:

$$\text{Luminal or Serosa glucose concentration} = \frac{[(mg/dl)/g\ tissue/h]}.\]

2.5. Statistical Analysis

The data were recorded as mean ± SEM of 5 animals each except otherwise stated and were analyzed using one way ANOVA followed by Newman-Keuls by means of Graphpad prism version 5.0 (Graphpad software, San Diego, CA). Differences were considered significant at p < 0.05.

3. Results

3.1. Effects of *Buchholzia coriacea* on Fasting Blood Glucose Concentration

Figure 1 shows that fasting blood glucose level in 100BC (78.00 ± 2.16 mg/dl) and 200BC (76.50 ± 3.57 mg/dl) treated groups were not significantly different when compared to control (79.50 ± 1.70 mg/dl) following period of treatment.

3.2. Effect of *Buchholzia coriacea* on Jejunal Glucose Uptake from the *in Vivo* Method

Figure 2 shows that jejunal glucose uptake in the 100BC (19.68 ± 0.72 mg/dl/g tissue/h) and 200BC (20.55 ± 0.45 mg/dl/g tissue/h) reduced significantly compared to the control (35.53 ± 1.45 mg/dl/g tissue).
ns = P > 0.05, not significant compared with control.

**Figure 1.** Effect of *Buchholzia coriacea* on blood glucose level after 1 week of treatment.

**Figure 2.** Effect of *Baccholzia coriacea* on jejunal glucose uptake.

### 3.3. Effect of *Buchholzia coriacea* on the Ileal Glucose Uptake from *in Vivo* Method

**Figure 3** shows that there was significant decrease in glucose concentration in the 100BC (23.08 ± 0.18 mg/dl/g tissue) and 200BC (14.50 ± 1.02 mg/dl/g tissue) groups compared with the control (30.40 ± 1.01 mg/dl/g tissue).

### 3.4. Effect of *Buchholzia coriacea* on Mucosa Glucose Uptake in the Everted Sac Method

**Figure 4** describes the glucose uptake at the mucosa end during everted sac procedure. At the proximal jejunum, the mucosa end retained significant increase in glucose concentration in the 100BC (282.7 ± 11.52 mg/dl/g tissue) and 200BC (218.7 ± 4.22 mg/dl/g tissue) treated groups compared to control (100.30 ± 2.17 mg/dl/g tissue). The distal jejunum mucosa glucose increased significantly in the 100BC (292.30 ± 3.33 mg/dl/g tissue) and that of the 200BC (209.30 ± 2.67 mg/dl/g tissue) compared with control (90.00 ± 1.50 mg/dl/g tissue). At the
Figure 3. Effect of Buchholzia coriacea on the ileum glucose uptake.

Figure 4. Mucosa glucose uptake at different jejuno-ileal sites with everted sac method after treatment with Buchholzia coriacea for 1 week.

ileum, the mucosa glucose uptake also reduced as evidenced by a significant increase in the glucose concentration in the 100BC (277.00 ± 15.23 mg/dl/g tissue) and 200BC (224.71 ± 1.33 mg/dl/g tissue) compared to control (102.33 ± 2.19 mg/dl/g tissue).

3.5. Effect of Buchholzia coriacea on Serosa Glucose Uptake with Everted Sac Method

Figure 5 shows significant increase in glucose concentration in the ileum of the 200BC (138.5 ± 0.76 mg/dl/g tissue) and 100BC (103.30 ± 1.20 mg/dl/g tissue) compared with the ileum of the control (46.67 ± 2.40 mg/dl/g tissue). Also, there was significant increase in glucose concentration at the distal jejunum of 100BC (116.80 ± 0.25 mg/dl/g tissue) and 200BC (116.7 ± 2.58 mg/dl/g tissue) compared with control (36.67 ± 2.33 mg/dl/g tissue). Furthermore, there was again a significant increase in glucose concentration at the proximal jejunum of 100BC (92.08 ± 1.58 mg/dl/g tissue) and 200BC (72.33 ± 0.33 mg/dl/g tissue) compared with the control (46.67 ± 1.58 mg/dl/g tissue).
α = P < 0.05: significant increase compared with control.

Figure 5. Serosa glucose uptake at the different jejuno-ileal sites with everted sac method after treatment with Buchholzia coriacea for 1 week.

Table 1. Effect of Buchholzia coriacea on luminal electrolyte concentration during everted sac method.

<table>
<thead>
<tr>
<th></th>
<th>Proximal Jejunum</th>
<th>Distal Jejunum</th>
<th>Ileum</th>
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<tbody>
<tr>
<td></td>
<td>Control 100BC</td>
<td>200BC</td>
<td>Control 100BC</td>
</tr>
<tr>
<td>Sodium (mM)</td>
<td>756 ± 14</td>
<td>694 ± 19*</td>
<td>771 ± 28</td>
</tr>
<tr>
<td>Potassium (mM)</td>
<td>977 ± 36</td>
<td>1657 ± 58*</td>
<td>1010 ± 33</td>
</tr>
</tbody>
</table>

Table 1 shows the effect of Buchholzia coriacea on the sodium and potassium ion concentration of the luminal fluid following the everted sac procedure at the proximal and distal jejunum.

3.6. Effect of Buchholzia coriacea on Luminal Electrolyte Concentration during Everted Sac Method

Table 1 shows the effect of Buchholzia coriacea on luminal electrolyte concentration during everted sac method.

4. Discussion

Plants continue to play an important role in the treatment of diabetes. The increase in demand in industrially developed countries to use alternative approaches to treat diabetes, such as plant-based medicines could be due to the side effects associated with the use of insulin and oral hypoglycemic agents [19], and most probably due to their cost effectiveness. More than 400 plants are being used in different forms to treat diabetes [20]. Buchholzia coriacea was selected on the basis of their use in traditional medicines throughout the world and experimentally determined its role in intestinal glucose uptake in rats.

The fasting blood glucose level from this study indicates no significant difference in the glucose concentration compared with control thereby falling within the normal range. Buchholzia coriacea contains flavonoid and alkaloid which has been reported to have hypoglycemic effects [21]. A study carried out by Lapshak et al. [22] on alloxan induced diabetic rats showed that Buchholzia
Buchholzia coriacea is anti-hyperglycemic. In that study, *Buchholzia coriacea* showed a significant anti-hyperglycemic activity but glucose levels remained within the normal range for normal rats treated with the *Buchholzia coriacea*. Thus, indicating that the aqueous extract did not cause any alteration in glucose levels of normal rats which are in line with the observation from this current study. Other reports affirming *Buchholzia coriacea* anti-hyperglycemic activity include: ethanol and butanol fractions [23] and methanol extract [24] [25]. However, there is no report on its effect on intestinal glucose uptake regulation.

The decrease glucose reported in the *in vivo* study indicates increase in glucose uptake at the ileum and jejunum. Following clinical resection of the jejunum, the ileum plays a major role in glucose uptake. Drozdowski and Thompson, [26] have suggested that up-regulation of intestinal hexoses transporters as a major mechanism of ileum improved sugar uptake. The increase glucose concentration reported in our *in vitro* study indicates inhibition in glucose uptake at the mucosa end though there was evidence of increase glucose concentration in the serosa as well. This finding tends to negate the expectation of lower glucose concentration at the serosa end since there was inhibition of glucose transport from the mucosa side. The only possible source of glucose to the serosa end could be from the enterocytes, and could suggest that the extract enhances release of glucose from the enterocytes to the serosa. This possibility was not accounted for in this study and it constitutes a major limitation to our study.

The glucose lowering effect of the plant extract in the *in vivo* study might be attributed to delayed intestinal glucose absorption or increased glucose utilization by the enterocytes with reference to anaerobic glucose metabolism thereby creating decreased passage of glucose from mucosal end to serosal end of the intestine. The difference is attributable to the glucose retained in the tissue. The results obtained in the present *in vitro* study suggest that ethanol extract of *Buchholzia coriacea* significantly inhibits glucose transport in the everted rat gut, this was followed with questionable increase in glucose concentration at the serosal end. Previous studies by Viviyan et al. [27], recorded that the aqueous seed extract of *Trigonella foenum-graecum* (*T. foenum graecum*) inhibited the glucose absorption in rat everted gut sacs and studies elsewhere recorded that the aqueous extracts of *T. foenum graecum* (seed) and *Eugenia jambolana* (seed) decreases the glucose absorption in rat everted gut sacs [28]. All these plants have quite significant amount of flavonoids, suggesting that the presence of flavonoid could be an important component. This study revealed that the ethanolic extract of *Buchholzia coriacea* contained phytochemical constituents that are potent in inhibiting glucose transport across intestinal membrane which might be due to decreased activity of glucose transporter transmembranal proteins in the small intestine of rat and thereby opening new scope for postprandial glycemic control.

The opposing in the movement of Na+ and K+ ions reported *in vitro* in the mucosa inferred that there was an increase in the transport of the affected elec-
trolytes at the proximal jejunum, both Na+ and K+ decreased at the distal jejunum and ileum sites. The intercellular junctions are more permeable to cations than anions, so that lumen-to-blood concentration differences for Na+ and K+ are generally smaller than those for Cl− and HCO3−. Glucose is transported across membranes by Na+ coupled active carrier system and a family of structurally related Na+ independent glycoprotein transporters, the latter is known as the glucose transporter (GLUT) or the (SLC2A) family, this usually facilitate the movement of glucose across the plasma membrane down its chemical gradient either into or out of the cell [29].

*Buchholzia coriacea* extract was safe up to 200 mg/kg with no mortality in rats. The extract had potential anti-hyperglycemic activities at the 100 mg/kg within the duration of study but exerted its maximum anti-hyperglycemic effect at 200 mg/kg, meaning it was dose-dependent in activity. The probable mechanism by which *Buchholzia coriacea* induces anti-hyperglycemic effect in rats was not fully elucidated. It is possible that the extract mediated its actions either through the prevention of insulin release or early regeneration of β-cells of islets of langarhans, increasing peripheral glucose uptake, slowing down the absorption of sugar from the intestinal gut or by decreasing the release of glucose from the liver [30]. All these measured up our limitations in this study couple with our inability to determine the gene expressions of the SGLT-1 and GLUT transporters. The role played by alpha amylase could also not be ascertained from this study.

5. Conclusion

In conclusion, the study revealed that *Buchholzia coriacea* extract was tolerated by the rats and inhibited glucose uptake in the intestine with mild impression on the electrolyte differences, thereby, suggesting possibility of glucose stowing in the intestinal cells in the *in vivo* study and significant inhibition of glucose transport from the *in vitro* study. The roles of alpha-amyrase on the activity of this extract and elucidation of other possible mechanisms of glucose transport are suggested for future studies.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References


[18] National Institutes of Health (1985) Guide for the Care and Use of Laboratory An-


