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Garcinia buchananii bark extract is an effective anti-diarrheal remedy for lactose-induced diarrhea ☆

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ABSTRACT

Ethnopharmacological relevance: The extract from the stem bark of *Garcinia buchananii* trees is used as an anti-diarrhea remedy in sub-Saharan Africa. We tested the hypothesis that *G. buchananii* bark extract and its anti-motility fractions are effective treatments against lactose-induced diarrhea.

Materials and methods: A high-lactose (35%) diet was used to induce diarrhea in Wistar rats, which were then treated with either *G. buchananii* bark extract (0.1, 0.5, 1.0 and 5.0 g bark powder), and its anti-motility fractions isolated using preparative thin layer chromatography; termed PTLC1 (15 mg) and PTLC5 (3.8 mg) or loperamide (8.4 mg). Drug preparations were dissolved in 1 L except PTCL1 and PTLC5 that were dissolved in 100 mL tap water. Numerous parameters were measured in each condition including consistency, fluid and mucus content of feces, body weight, water and food consumption, urine production and bloating.

Results: Diarrheic rats produced watery or loose, mucuoid, sticky, feces. Fluids constituted 86% of stool mass compared with only 42% for control rats fed standard chow. Compared with controls, diarrheic rats produced more urine, lost weight and had bloated ceca and colons. All doses of the extract, its anti-motility fractions and loperamide individually stopped diarrhea within 6–24 h of administration, whilst significantly reducing mucus and fecal fluid content, urine production and intestinal bloating. Rats treated with 0.1 g extract, PTLC1 and PTLC5 gained weight, whilst PTLC5 also increased water intake.

Conclusions: *Garcinia buchananii* extract and its anti-motility fractions are effective remedies against lactose-induced diarrhea. The extract contains compounds that reverse weight loss, promote food and water intake, supporting the notion that characterization of the compounds could lead to new therapies against diarrheal diseases.

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1. Introduction

Diarrheal diseases kill more children, especially those under five years of age than AIDS, malaria, and measles combined (UNICEF/WHO, 2009). Each year, 2.5 billion cases of acute infectious diarrhea occur in children below five years of age alone, and this accounts for over 1.5 million child deaths in low and middle-income countries, mainly in Africa and South Asia (Thapar and Sanderson, 2004; UNICEF/WHO, 2009). Furthermore, infectious diarrheal diseases are a significant cause of morbidity and mortality in HIV/AIDS patients, people displaced by disasters and wars and the elderly, (Nwachukwu and Okebe, 2008; Thielman and Guerrant, 1996) and a significant health care burden and loss of productivity around the world (Ocfemia and Taylor, 2004; UNICEF/WHO, 2009).

During the past four decades, concerted efforts have been made to combat the high morbidity and mortality rates associated with diarrheal diseases through improved sanitation and treatments using oral rehydration solution (ORS), anti-secretory and anti-motility agents, vaccinations, zinc supplements, antibiotics and other regimens (Guerrant et al., 2003; Kelly, 2011; UNICEF/WHO, 2009). These measures have succeeded in curbing the high mortality rates associated with diarrheal diseases (Guerrant et al., 2003; Kelly,

Abbreviations: ext., Extract; *G. buchananii*, *Garcinia buchananii*; g/L, Gram/Liter; HCA, (−) – Hydroxycitric acid; HLD, High lactose diet; h, Hours; 5-HT, 5-hydroxytryptamine (serotonin); LD, Lactose-induced diarrhea; LP, Loperamide; mg/L, Milligram/liter; OD, Osmotic diarrhea; ORS, Oral rehydration solution; PTLC, Preparative thin layer chromatography; SD, Standard chow diet; UNICEF, United Nations Children's Fund; vs., Versus; WHO, World Health Organization

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2011; UNICEF/WHO, 2009). However, millions of people are still dying from diarrheal diseases, suggesting a critical need for novel and affordable anti-diarrheal drugs.

The ultimate goal in diarrhea treatment is to prevent or reverse dehydration, gastrointestinal hyper-motility and fecal urgency, shorten the duration of the illness, reduce the pain or stress, and in some cases treat the infection and prevent nutritional complications (Brown, 2003; Field, 2003; Kelly, 2011; UNICEF/WHO, 2009). Key drawbacks to current treatment strategies are that they do not necessarily reduce the duration of the illness; as well as the fact that in developing countries 39% of the population have no access at all to modern anti-diarrhea therapies (UNICEF/WHO, 2009). It is therefore believed that over 80% of the population in developing countries depend on phytotherapy to treat diarrheal illnesses (Groombridge and Jenkins, 2002).

Garcinia buchananii (*G. buchananii*), Authority Baker, Family Clusiaceae (Brown, 1894), a plant native to Eastern, Central and Southern Africa is used by the indigenous population to treat dysentery, abdominal pain, and a range of infectious diseases (Balemba et al., 2010; Chinsembu and Hedimbi, 2010; Kisangau et al., 2007). In native communities, patients can treat themselves by either chewing the dried stem and root barks, or grinding the bark into powder, which is then added to water or beverages for drinking (Balemba et al., 2010; Chinsembu and Hedimbi, 2010). Recently, we showed that the aqueous extract from the stem bark of *G. buchananii* trees is a non-opiate preparation, which reduces peristalsis by inhibiting neurotransmission (Balemba et al., 2010) and 5-HT_3 and 5-HT_4 receptors (Boakye et al., 2012). Furthermore, the extract has anti-inflammatory, and anti-nociception effects (Castro et al., 2011). The compounds having anti-motility properties appear to be flavonoids, or a combination of flavonoids with alkaloids or steroids (Boakye et al., 2012). Clearly, research aimed at defining the bioactive components and mechanisms of action as well as indigenous uses suggest that *G. buchananii* could be an effective anti-diarrhea medication and also a source of novel non-opiate anti-diarrheal compounds. Currently, the only drugs available that rapidly shorten the duration of diarrhea and alleviate pain are opiates (Ruppin, 1987; Riddle et al., 2008). These drugs cause constipation, drowsiness and are addictive. Consequently they are not recommended for children (Kelly, 2011; Riddle et al., 2008). This indicates the unmet need for new non-opiate anti-motility compounds and the need for the formal testing of the efficacy of *G. buchananii* bark extract and its derivatives as treatments against diarrheal diseases. This also requires the use of diarrheal models.

Ingesting large quantities of lactose (45%–87%) causes osmotic diarrhea through increased secretion in the small and large intestine of animals (Bueno et al., 1994; Lawrence et al., 1956; Liuzzi et al., 1998) and lactose causes diarrhea in humans with lactose-intolerance accounting for over 50% of the world population (Haemmerli et al., 1965; Lomer et al., 2008). A novel hypothesis suggests that diarrhea, flatulence, nausea, pain and other symptoms of lactose-intolerance arise from the effects of toxic bacterial metabolites such as alcohols, acids, ketones and methylgyoxal on gut effector tissues including the epithelium, muscle and nervous tissue (Campbell et al., 2010). It has been shown that a high-lactose diet induces severe and persistent diarrhea, intestinal damage and malnutrition in experimental animals (Arciniegas et al., 2000; Bueno et al., 1994; Fijlstra et al., 2010; Liuzzi et al., 1998; Norton et al., 2001). These effects of lactose-induced diarrhea are to some extent, similar to changes seen in children suffering from gastroenteritis or chronic diarrhea (Bueno et al., 1994). Interestingly, diarrhea due to lactose intolerance is a common complication of infectious diarrhea in children with malnutrition (Brown, 2003; Moore et al., 2010; Nyeko et al., 2010).

The aims of this study were to investigate the effectiveness of *G. buchananii stem* bark extract in treating lactose-induced diarrhea in rats, determine the effective dose, and test the effectiveness of its anti-motility fractions PTLC1 and PTLC5 as anti-diarrheal agents.

2. Materials and methods

2.1. Inducing diarrhea in rats using a high-lactose diet

The study was conducted in accordance with the regulations of the University of Idaho Institutional Animal Care and Use Committee (IACUC). Sixty two, 10 week old Wistar rats (389.2 +/ −6.3 g) were obtained from Harlan Animal Research Laboratory (Hayward CA, USA). Rats were individually caged (23–24 °C; 12:12 h light-dark cycle) and quarantined for one week. Rats were fed a standard chow diet ad libitum (Animal Specialties, Hubbard, OR, USA) and had free access to water for four days prior to the inducement of diarrhea. A high-lactose diet (HLD) containing 35% lactose in place of starch (3.004 kcal; Purina Mills; Richmond, Indiana, USA) was fed to 52 rats. Diarrhea was induced within 24–48 h after consuming the diet. Rats were monitored for changes in consistency of pellets and stool mass, fecal fluid and urine production (mass; g and volume; mL). Rats were considered diarrheic if they produced watery stools, soft, yellowish stools compared to normal, pliable, soft, well-formed pellets as previously described by other researchers (Arciniegas et al., 2000; de Groot et al., 1995; Lawrence et al., 1956).

Four days after introducing rats to a HLD, diarrheic rats were treated using varying doses of *G. buchananii* extract, its anti-motility fractions, PTLC1 and PTLC5 (Boakye et al., 2012) and loperamide for standard comparison. Rats were maintained on a HLD during the entire treatment period.

2.2. Preparation of G. buchananii bark extract, PTLC1 and PTLC5 fractions

Garcinia buchananii bark powder was prepared from stem barks collected from trees in their natural habitat in Karagwe, Tanzania, as described previously by Balemba et al. (2010). A sample can be found at the University of Idaho Stillinger herbarium (voucher 159,918). 0.1 g, 0.5 g, 1.0 g and 5.0 g *G. buchananii* bark powder were each suspended in 1 L of tap water, stirred for 30 min and filtered. The filtrate was then immediately used to treat rats against lactose-induced diarrhea.

The anti-motility fractions were obtained from aqueous *G. buchananii* bark extract using a preparative thin layer chromatoghraphy (PTLC) separation method as described previously (Boakye et al., 2012).

2.3. Treating diarrheic rats with G. buchananii extract, PTLC1 and PTLC5 fractions

Twenty nine rats on a HLD were randomly assigned and treated with varying doses of *G. buchananii* extract. In total, nine rats were treated with 0.1 g, seven rats with 0.5 g, six rats with 1.0 g, and seven rats with 5.0 g *G. buchananii* bark extract. A total of eight rats were treated with PTLC1 or PTLC5. Four rats per group were treated with either PTLC1 or PTLC5 at a dose of 15 mg and 3.8 mg in 100 mL tap water, respectively.

For control treatments, seven rats were treated with loperamide (8.4 mg/L tap water), eight rats were left untreated (HLD control) and ten rats received control standard chow diets (SD). Rats were treated for a total of four days, receiving freshly made drugs every two days.