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An in vitro and in vivo toxicologic evaluation of a stabilized aloe vera gel supplement drink in mice

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ABSTRACT

Aloe vera gel is increasingly consumed as a beverage dietary supplement. The purpose of this study was to determine potential toxicity of a stabilized aloe vera gel derived from the inner gel fillet and marketed as a drink. The gel juice was assessed through assays of genotoxicity in vivo and acute and subchronic toxicity in B6C3F1 mice. Aloe vera did not increase the SOS DNA repair response in *Escherichia coli* and at 1× and 0.25× it did not increase mutagenesis of Salmonella TA100 resulting in histidine biosynthesis. At 3 and 14 days following acute exposure, male and female mice gavaged with the stabilized aloe gel had daily appearances, total body weight gain, selected organ weights, necropsy and hematology tests similar to control mice gavaged with water. After a 13-week aloe gel feed study, male and female mice evaluated by the same criteria as the acute study plus feed consumption and serum chemistry tests were found to be equivalent to control groups. These data indicate that a commercial stabilized aloe gel consumed as a beverage was not genotoxic or toxic in vivo. These results contrast with those obtained using preparations containing aloe latex phenolic compounds such as anthraquinones.

1. Introduction

Aloe vera human consumption as a beverage has recently increased in popularity with the number of aloe juice introductions almost doubling since 2010 (Beverage Industry, 2012). This increased consumer popularity is reflected by the shift in market availability of aloe juices from specialty outlets to mainstream groceries and drug stores (Zegler, 2011). Consumer interest in aloe beverages stems from the association of aloe juice with a variety of both anecdotal and experimental research-supported health benefits including the prevention or treatment of various tumors (de Melo et al., 2011; Kuo et al., 2002) and arthritis (Cowan, 2010), reduction in symptoms of diabetes (Tanaka et al., 2006) enhancement of immunity (Sawant, 2012) and decreased cholesterol levels (Huseini et al., 2012). These benefits are controversial with some sources pointing out that the putative effects of aloe are unsupported by clinical studies (WHO, 1999); however because consumers are increasingly drinking aloe vera, it is important that marketed products be tested for toxicities following oral consumption.

The aloe plant stores water and other plant nutrients within a clear mucilaginous gel obtained from the parenchymatous cells in the leaves occupying the central area of the leaf cross section (WHO, 1999; Hamman, 2008). The gel is approximately 99% water (Hamman, 2008) and the non-aqueous remainder largely consists of minerals, vitamins, polysaccharides, lipids, phenolic compounds and organic acids. Juice marketed for oral consumption is largely derived from either only the inner leaf fillet gel, which is also called aloe vera gel or fillet gel (Williams et al., 2010) taken by stripping away the outer rind and latex or a whole-leaf juice filtered through activated charcoal to remove a latex material (IASC, 2010). This purification procedure is necessary since the latex, which exists as a separate liquid between the outer rind and inner fillet gel, contains bitter phenolic molecules including anthraquinone C- and O-glycosides, anthrones and some free anthraquinones (Park et al., 1998). The major C-glycoside, aloin A, is the major anthraquinone in aloe and when oxidized, yields aloe-emodin, a free anthraquinone (Park et al., 1998).

Most of aloe vera's anthracene compounds exist as glycosides with fewer free anthraquinones. Because of the polarity conferred by the glycosides, barbaloin, isobarbaloin and related species are

Abbreviations: RBC, red blood cell count; Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; WBC, total white blood cell count; RDW, red blood cell distribution width; MCHC, mean corpuscular hemoglobin concentration; PCV, packed cell volume (PCV); ALT, alanine aminotransferase activity; ALP, alkaline phosphatase activity; CK, creatine kinase; TBIL, total bilirubin; BUN, blood urea nitrogen; PPM, parts per million.

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not absorbed in the upper GI tract when taken orally. In the cecum (rats) or colon (humans) intestinal microflora free anthracenes such as emodin from the sugars (Reviewed in NTP (2011)). However, emodin has low oral bioavailability. Although it is readily absorbed by intestinal cells, it is extensively glucuonidated by these cells before secretion into portal capillaries or excretion back into the intestinal lumen (Liu et al., 2012b).

Chemical components of aloe juice which are responsible for the many putative health benefits are now increasingly being defined. Anthraquinones are associated with well-documented laxative effects (Patel et al., 2012). Emodin has been associated with dose-dependent decreases in mitochondrial membrane potential and apoptosis in rapidly proliferating pancreatic cancer cells in mice (Liu et al., 2012a). The quinone metabolites of emodin also inhibit tumor proliferation (reviewed by Lu et al., 2012). Aloe gel consumption inhibits pro-inflammatory ligands and enzymes in induced rat colitis and these effects can be replicated by oral consumption of aloesin, a glucose-coupled aloe vera chromone (Park et al., 2011). Although phenolics such as emodin are largely removed in aloe beverages, polysaccharides remain a major ingredient and these are immunostimulatory. Acemannan, a galactomannan aloe constituent, triggers multiple points of macrophage activation (Zhang and Tizard, 1996). This saccharide has been reported to reduce experimental and clinical malignancies and experimental infections when taken orally (Im et al., 2010). Veracylglucans, which are malic acid acylated glucosides, have also been shown to possess potent anti-inflammatory effects (Foster et al., 2011).

Several long-term toxicology studies of aloe vera have been reported; however, these reports show an inconsistent safety profile particularly in vivo, likely due to the utilization of different aloe vera preparations such as whole leaf juice or extracts, inner leaf fillet gel juice, or isolated aloe components. In subchronic rat studies, using a certified commercial juice taken from the inner leaf gel, Williams et al. (2010) found no evidence of oral toxicity after 13-weeks. Similarly, a life-span feed study by Ikeno et al. (2002) using dried powder from only the inner leaf fillet feed to Fischer 344 rats found no adverse effects. However, oral consumption of an ethanol extract of whole leaf aloe over 3 months resulted in toxicities to the reproductive system and increased mortality in mice (Shah et al., 1989) and more recently, Pandiri et al. (2011) reported that Fischer 344 rats given non-decolorized whole leaf extract in drinking water over 2-years had a significantly greater incidence of large intestinal tumors with gene mutations similar to those seen in human colorectal cancer. Both of the above studies likely delivered substantially higher anthraquinone levels to test animals than did studies administering inner leaf juice. Anthraquinones such as barbaloin or its aglycone emodin have higher solubility in water miscible organic solvents than in water (Selleckchem.com, 2012), hence ethanol extraction of whole leaf aloe will yield a product selectively enriched in these types of compounds vs. hydrophilic constituents such as polysaccharides. Testing of the free anthraquinones more consistently demonstrates positive toxicity including diarrhea in vivo (Sendelbach, 1989; Patel et al., 2012), weight loss, gall bladder lesions, renal tubule pigmentation and renal tubule hyaline droplets (NTP, 2001), and in vitro mutations in mouse lymphoma (Müller et al., 1996) and Salmonella assays (Westendorf et al., 1990). Clinically, consumption of powder from the aloe leaf has been linked to several cases of hepatitis most likely secondary to a hypersensitivity reaction (Yang et al., 2010). It is clear that the potential for toxicity of aloe vera depends on the derivation methods of the juice and/or plant sections used for juice or powder production.

Aloe literature contains few safety study designs which have evaluated commercial aloe beverages over acute or subacute time periods in vivo. Tanaka et al. (2012) recently reported data including a single oral dose toxicity study in rats gavaged with 150 mg/kg aloe vera gel extract. The test material was inner leaf gel dried to a powder which was then extracted using supercritical carbon dioxide. The study found no mortalities, no abnormalities at necropsy and no differences in body weight gain after 14 days. More often, acute safety studies involving aloe vera have used aloe-derived ingredients. When the non-anthraquinone ingredients are tested, they typically do not demonstrate toxicity in mice or rats. The polysaccharide acemannan for instance, has a 14-day oral no observed effect level of 50,000 ppm (reviewed by Cosmetic Ingredient Review Expert Panel (2007)). The relative lack of acute in vivo studies of current commercial aloe beverages represents a significant gap in aloe vera safety testing.

We believe that to be most relevant, toxicity testing of beverages must utilize commercial products as they are typically prepared for drinking. Most of these products are not whole leaf juices but rather come from the inner gel fillet. In this study, we also aimed to relate our experimental administration levels in test rodents to recommended intake levels in consumers—a step that few aloe safety studies have taken. We feel that appreciating how much in excess of a person-recommended daily dose the animals received facilitates gauging of a practical consumer safety margin from doses found to be non-toxic. We have tested a stabilized aloe gel sold as a beverage through in vitro genotoxicity, in B6C3F1 mice 3-days and 14-days following acute oral administration and in B6C3F1 mice over a 13-week feed period. We based dosing on reasonable excesses over the high-end recommended daily drink quantity for people to assess potential beverage toxicities.

2. Methods and materials

2.1. Genotoxicity assays

Potential mutagenicity and/or DNA damage were assessed in vitro through two bacterial assays. An assay for mutagenesis was used, which is based on the Ames test utilizing *Salmonella typhimurium* strain TA100 but modified for liquid culture and 96-well plate scale (Luria and Delbruck, 1943; Hubbard et al., 1984). The second assay detects potential DNA damage utilizing an *E. coli* strain containing a transgene for beta-galactosidase downstream of the SOS-DNA repair promoter system. Both assays were purchased in a commercial format from EBPI bio-detection products (Mississauga, Ontario, Canada) referred to the as Muta-Chromo Plate and SOS-Chromo Test Assays respectively.

2.1.1. Sample preparation

All aloe juice was obtained from ST&T Toxicology, San Francisco, CA in the commercial-ready form as a stabilized aloe gel. The aloe juice tested has been certified by the International Aloe Scientific Council (IASC) and meets current IASC quality standards for microbiology testing, production, storage, aloe vera content and aloin content. A single lot of juice was used throughout the studies. Non-aqueous content was determined by drying over 18 h using a Savant speedvac plus SC110A (Thermo Scientific, Asheville, NC). The juice was maintained under constant refrigeration until use. Prior to testing, the aloe juice was lyophilized to dryness and determined to be 1.7 ± 0.30% nonaqueous. It was also found to be acidic (pH = 4.3) and since the *S. typhimurium* assay is pH-dependent, the juice for this assay was adjusted to pH = 7.4 using NaOH. To prevent microbial contamination within the stabilized product from being introduced into the *Salmonella* assays, the aloe juice for this assay was sterilized—achieved by filtration (0.22 μm filter); however this process removed pulp from the juice. As an alternative, some juice was sterilized by autoclave to preserve the pulp despite the recognition that heating may result in loss of heat labile compounds (Xiu et al., 2006). Both filtered and autoclaved juices were tested for mutagenesis in *Salmonella*. To test for potential metabolic conversion non-mutagenic xenobiotics into mutagenic species, some samples of aloe juice were tested in the presence of S9 extract, which is an extract of liver from rats treated with a carcinogen such as Aroclor 1254 to induce mixed function oxidase enzymes associated with the metabolism of procarcinogens. These enzymes are not expressed in bacteria (Mortelmans and Zeiger, 2000).

2.1.2. The Muta-Chromo test uses a 96 well plate for each variable tested

In each Muta-Chromo test plate, aloe juice was combined with a reaction mix and − or +S9 extract. The filtered juice was tested at full strength and the pulp-containing preparation was diluted 1:4. A positive control for direct acting mutagenesis (i.e. independent of metabolic conversion) was included in one plate (sodium azide,