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

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A R T I C L E   I N F O

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A B S T R A C T

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.

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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) and arthritis (Cowan, 2010), reduction in the 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 purification procedure is necessary since the latex, which exists 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
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 glucuronidated 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 aloeosin, a glucose-coupled aloe vera chromosome (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). Veracylglycans, which are mallow 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 Ileno 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 (Iuria 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 EBP 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 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 5C110A (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 ± 0.3 monosaccharides. 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 Acoral 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 −95 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, 364 I. Sehgal et al./Food and Chemical Toxicology 55 (2013) 363–370
Toady 3 and 14 day time periods of concentrated AVOA juice administered by gavage

2.2.1. Preparation and administration
To administer sufficient juice to mice for this study, juice was concentrated 10-fold by lyophilization in a VirTis Benchtop lyophilizer (Warminster, PA). At concentrations greater than 10-fold, the juice was found to be too viscous to exit a gavage needle. The concentrated juice was administered by gavage with a 20 gauge curved stainless steel gavage needle (Parker & Sons, New Hyde Park, NY) to male and female B6C3F1 hybrid mice twice over a 24-h period.

2.2.2. Animals in gavage studies
The particular mouse chosen for these studies is a cross between the C57BL/6NHis inbred female and the C3H/HeNHis inbred male and is used commonly in toxicology and carcinogenesis studies including those by the NTP in their Technical Reports series. All mice were purchased from Harlan Laboratories, Indianapolis, IN, at between 4 and 6 weeks of age. After a two week period of quarantine, the mice were acclimated to control diet and numbered for identification by ear tag. Reports series. All mice were purchased from Harlan Laboratories, (Indianapolis, to toxicology and carcinogenesis studies including those by the NTP in their Technical

2.3. Quantity of stabilized juice administered
The dose administered to mice averaged 8.7 times that expected to be generally consumed orally by a person for male mice and 10.3 times for male mice. To arrive at this dosing average, we used the aloe juice producer recommended daily consumption level of one 8 oz (236.56 mL) drink per day. We assumed a typical human to weight 70 kg and scaled a human to mouse using body surface area (Mosteller, 1987) since surface area ratio requires a greater burden of test substance administration to the mouse than a straight weight ratio. For the animal’s safety, and per IACUC protocol, mice were gavaged at 1.0% of their body weight and were gavaged twice in 1 day 6 hours apart with 10-fold concentrated juice. Conversion of this dose to the more traditional mg of non-aqueous aloe/kg body weight would yield an equivalency of approximately 3400 mg/kg.

2.3. Toxicity over a 13 week time period of concentrated AVOA juice administered through feed

2.3.1. Preparation and administration of feed
Mice were prepared as in gavage studies except that nine mice housed in three boxes per group were used in feed studies. To administer sufficient juice to mice for the study, juice was concentrated 2-fold by lyophilizing and compounded into Teklad Global 16% Protein Rodent Diet 2016 chow in place of normally added 12% water (Harlan Laboratories, Madison, WI). Test groups of males and females were fed either aloe feed or control feed ad libidum. Feed consumption was measured three times per week by weighing residual feed in vertical glass feed jars, then re- placed with fresh feed. For graphical presentation (Fig. 2), daily average feed consumption is depicted at 1 week intervals. After the 13-week feed period, blood samples were collected from the right ventricle for hematology and clinical chemistry analyses and mice were humanely sacrificed by asphyxiation using CO2 gas. This method is acceptable according to the “AVMA Guidelines on Euthanasia, 2007”.

2.3.2. Amount of juice consumed in feed studies
Aloe juice was added to make up 12% of feed composition before drying. Female aloe mouse feed consumption ranged from 3.0 to 4.5 g average per day after week 1 while males consumed 2.9–4.5 g average per day. Based on body surface area ratios between mice and a person (as calculated in Section 2.2.3), we calculated the mice consumed between 1.54 and 2.10 (female) and 1.25–1.77 (male) times the recommended high daily aloe juice for a person.

2.4. Parameters evaluated in mouse studies
For gavage studies of 3 or 14 day periods, we recorded attitude and alertness of the mice, mortalities, and/or adverse signs daily. In the 13-week feed studies, mice were observed as for 3/14 day mice at least three times weekly. Gross necropsies were performed on all animals and data collected included final body weight, as well as kidney (left), heart, lung, liver and testes (males) weights. Hematology was assessed on blood samples collected in potassium EDTA containers from 14-day acute gavage mice and on feed study mice. This hematology included erythrocyte count (RBC), hemoglobin (Hb), hematocrit (HCT), red blood cell distribution width (RDW), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, mean platelet volume (MPV), and total white blood cells (WBC’s). Additional complete blood count data were acquired for 13-week feed study mice and included packed cell volume (PCV), total white blood cell count (WBC’s), neutrophils, lymphocytes, monocytes, and eosinophils. Clinical chemistry was assessed on plasma separated blood collected from feed-study mice and included alanine aminotransferase activity (ALT), alkaline phosphatase activity (ALP), creatine kinase (CK), total bilirubin (TBIL), blood urea nitrogen (BUN) and creatinine.

Livers from the 13-week feed mouse groups (male and female) were independently evaluated by at least 2 reviewers, one of whom is a boarded-veterinary pathologist. Finding of pathologic anomalies would trigger further evaluation of all organs preserved from the mice. Body weight changes and weights of the liver and kidney and all hematologic and chemistry parameters were statistically analyzed within each sex by Student’s T-test, with significance as p < 0.05. Feed consumption over the 13-week period in control and aloe-mice was assessed by Two-way repeated measures ANOVA with Bonferroni posttests. GraphPad Prism 5 was used to perform statistical analyses.

3. Results

3.1. Genotoxicity assays

3.1.1. Mutagenesis in S. typhimurium
Sterile filtered aloe vera juice was tested at full strength (drink) concentration and was not mutagenic to test bacteria in the absence or presence of S9 extract activation (Table 1). These aloe juices showed less positive wells than blank controls suggesting a potential inhibition of test bacteria proliferation at full strength in this assay. Autoclaved juice, which contained pulp, was difficult to dispense into the 96-well plates and was therefore diluted 1:4. This diluted pulp-juice in the absence and presence of S9 did show a background level of positive wells but these well numbers were not greater than blank controls and this condition was therefore scored non-mutagenic.

3.1.2. DNA damage repair assay
Aloe juice did not damage DNA in test bacteria in the absence of S9 extract at any tested concentration over 3 logarithms; there was no dose related increase in SOS transgene induction and no significant change over a water-only control (Fig. 1). The juice also did not induce DNA damage repair in the presence of S9 extract at levels of 5 × or less. At concentration of 10 ×, and in the presence of S9 extract, the aloe juice was either cytotoxic or bacteriostatic to the E. coli bacteria. The lower quantity of bacteria produced a higher ratio of SOS-induction to bacteria. The increased optical density (OD) values obtained at 10x concentration therefore were interpreted as being greater than blank controls and this condition was therefore scored non-mutagenic.
effect on bacterial growth and not as indicative of increased DNA damage.

3.2. Toxicity at 3- and 14-days following gavage

3.2.1. Male mice
We found no mortalities in any male mice gavaged with high levels of AVOA aloe juice. Mice were found to be bright and alert, equivalent to control mice. Males in both 3 and 14-day groups showed body weights and weights of the kidney and liver similar to control mice. Necropsy was unremarkable. Hematologic values for male mice 14 days post-aloe were similar to control group mice. These data are summarized in Tables 2 and 3.

3.2.2. Female mice
Female mice in both 3 and 14-day groups had body weights similar to controls. Liver and kidney weights as a percentage of final body weight of all aloe-females were not significantly different than controls. One female mouse from the 14-day post-aloe juice group was removed from the study because of injuries sustained after being attacked by cage mates. The hematologic values for females 14 days post-aloe juice were not different than controls with one exception—the treated group showed a statistical difference in the mean corpuscular volume (MCV) vs. the control. The MCV difference most likely arose because the members in each group had very similar scores to other group members and this lead to a small sample deviation. We concluded these differences were statistical rather than biological as the treated- and control-group means were less than 4% apart for MCV. These data are summarized in Tables 4 and 5.

3.2.3. Histology of livers
Microscopic study of livers from each group of mice revealed generally normal appearing tissue. Some regions in both aloe and control females showed multifocal areas of minimal extramedullary hematopoiesis (EMH, data not shown), a common finding in mice of this age.

3.3. Results 13-week feed study

3.3.1. Males at 13-weeks
We found no mortalities in any mice. Mice were observed to be bright and alert. Feed consumption over the 13 week course of study was not different between groups (Fig. 2A). Gross necropsy was unremarkable in all male mice. Male aloe-supplemented mice showed body and organ weights similar to control mice (Table 6). Of hematologic parameters evaluated, neutrophil percent and lymphocyte percent of total white cells were found to be statistically different from control males (Table 7). Neutrophils were higher and lymphocytes were less than control males; however all blood cell quantity values for aloe-mice were within normally observed breed ranges¹. We concluded that mild white cells differences were due to comparison with a control group that expressed a relatively low total WBC and low neutrophil number yielding a low neutrophil% and high lymphocyte%. A panel of selected clinical chemistry

tests (Table 8) run on plasma collected at necropsy showed no significant differences between aloe-fed and control males.  

3.3.2. Females at 13-weeks  
Female mice in after 13 weeks of aloe feed had body weight gains similar to female controls. Mice were found to be bright and alert. As observed with males, feed consumption over the 13 week course of study was not different between groups (Fig. 2B). Necropsy was unremarkable in all mice. Organ wet weights of females (Table 6) were not significantly different than controls with the exception of lung weights; however, these lung weights of females (Table 6) were not significantly different than controls; however, the mouse showed no other subjective/clinical abnormalities (described in Section 3.3.3), we could find no indication of toxicity in these two lower-than-control values.

3.3.3. Histology of livers  
Microscopic study of sample livers from each group revealed a generally normal appearing tissue. Some regions in both aloe and control females showed multifocal areas of minimal extramedullary hematopoiesis (EMH).

4. Discussion  
As the popularity of aloe vera beverages continues to grow, some concerns have been raised about potential toxic effects arising from oral intake—effects that are not anticipated when aloe is used topically as a cosmetic or skin balm. One such concern raised in studies involving whole leaf juice is carcinogenicity (NTP, 2011). While the short term nature of our current animal studies does not lend itself to assessment of tumor formation, we did determine potential genotoxicity of the stabilized juice in vitro using two different methodologies. Genotoxicity assays aim to discern the mutation potential of chemicals (Rao et al., 2004), i.e., the ability to alter cellular DNA which can in turn contribute to carcinogenicity. In vitro assays are both more rapid and less costly than animal life-time studies and can be selected with one of several endpoints. In our genotoxicity tests with a stabilized juice from the inner gel, we used assays for DNA damage/repair and fixed gene mutations. We found the juice was not mutagenic to test bacterial in the absence or presence of S9 extract metabolic activation. The juice also did not damage DNA in test bacteria in the presence or absence of S9 extract. These negative toxicity results with the stabilized gel product are consistent with those of Williams et al. (2010) who tested an inner leaf gel-derived aloe powder against four S. typhimurium tester strains in Ames mutation assays. Isolated anthraquinone components of aloe juice have tested positive in genotoxicity assays (Nesslany et al., 2009; NTP, 2001; Muller et al., 1996); however, aloe juices derived from the inner leaf gel contain low to undetectable levels of these anthraquinones (<10 ppm to achieve IASC certification2). Our data provide further evidence that aloe juices devoid of significant anthraquinone levels are not genotoxic in vitro.

We recognize that caution must be exercised in extrapolating these results to in vivo consumption. In vitro genotoxicity assays are obviously limited not only by the brief nature of exposure and homogeneous nature of the target, but also the ability of single species test bacteria to mimic the spectra of intestinal flora’s effect on chemicals in the juice. This would become relevant for intestinal metabolism of aloe vera juices containing the latex since anthracene glycosides such as aloins are hydrolyzed by intestinal Eubacterium and Bifidobacterium species freeing anthrones and anthraquinones (NTP, 2011) for membrane permeation and absorption. Optimal answers to carcinogenicity will require lifetime studies. In that regard, we are currently conducting a 2-year feed study of the stabilized aloe juice and have found no significant differences in tumor formation or other signs of toxicity between control and aloe-feed mice as of 18 months (unpublished results).

In this study, we also determined potential for acute toxicities after orally administering a large quantity of concentrated juice to mice. At both 3 and 14 day time periods after gavage, all mice remained bright, alert, and active. We noted no loose stools or evidence of wet feces in the cage or on the perineal regions. Gross necropsy appearance and selected organ weights were generally equal to control mice gavaged with water. Necropsy, absorption, and hematologic studies also indicated few differences between aloe and untreated groups. In some instances, individual mice had a measured value which fell outside of the controls; however, the mouse showed no other subjective/
objective parameters suggesting toxicity. Indeed, given the large number of individual mice and the multiple parameters quantitated for each mouse, it was anticipated that there would be instances of an individual mouse having one or two values outside of a $p < 0.05$ significance. We could link no adverse effects to high intake of the aloe juice extract over a <24 h period. We believe these acute study data using a stabilized aloe gel beverage fills an important gap in the field of oral aloe safety assessment.

Toxicity, where reported with aloe consumption, more often follows longer term administration or consumption. In this study, we evaluated mice given aloe juice dried into feed over a subchronic time period (13 weeks). Our subchronic studies were performed in mice rather than in rats in order to remain consistent with 3 and 14 day studies. Throughout the study period, mice remained alert and possessed an active attitude. Average feed consumption, body and organ weights matched the control group and with few exceptions, both hematologic and clinical chemistry tests were equivalent in both males and females. Our results with the stabilized aloe gel parallel those reported by Williams et al. (2010), Ikeno et al. (2002) and Tanaka et al. (2012) who found no

<table>
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<th>Table 4</th>
<th>Necropsy assessment of female mice gavaged.</th>
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<tbody>
<tr>
<td></td>
<td>Female control (group mean)</td>
</tr>
<tr>
<td><strong>Mortalities</strong></td>
<td>0/6</td>
</tr>
<tr>
<td>Abnormal symptoms</td>
<td>0/6</td>
</tr>
<tr>
<td>Necropsy abnormalities</td>
<td>0/6</td>
</tr>
<tr>
<td><strong>Weight change% init. b. wt.</strong></td>
<td>3 days: 1.67 ± 2.97</td>
</tr>
<tr>
<td>Kidney (% fin. b. wt.)</td>
<td>0.80 ± 0.07</td>
</tr>
<tr>
<td>Liver% fin. b. wt.</td>
<td>5.10 ± 0.87</td>
</tr>
</tbody>
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* Daily observation.
\[a\] Initial body weight.
\[b\] Mean ± sd.

<table>
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<tr>
<th>Table 5</th>
<th>Female gavage hematology.</th>
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<tbody>
<tr>
<td>Hematology test</td>
<td>Female control</td>
</tr>
<tr>
<td>RBC (10^6/μL)</td>
<td>9.33 ± 0.340</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>14.14 ± 0.590</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>47.66 ± 2.25</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>12.04 ± 0.380</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>51.06 ± 1.05</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>15.14 ± 0.230</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>136.10 ± 0.07</td>
</tr>
<tr>
<td>Platelets (10^3/μL)</td>
<td>1161.50 ± 157.75</td>
</tr>
<tr>
<td>WBC's (10^3/μL)</td>
<td>1.54 ± 1.03</td>
</tr>
</tbody>
</table>

* Mean ± sd.

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<tr>
<th>Table 6</th>
<th>13-Week feed toxicology means and standard deviations for changes in mouse body and organ weights as percent of total body weight at time of necropsy.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight changes</td>
<td>Control male</td>
</tr>
<tr>
<td><strong>Weight change% init. b. wt.</strong></td>
<td>14.6 ± 7.7</td>
</tr>
<tr>
<td>Kidney (left)% fin. b. wt.</td>
<td>0.68 ± 0.11</td>
</tr>
<tr>
<td>Liver% fin. b. wt.</td>
<td>4.62 ± 0.21</td>
</tr>
<tr>
<td>Lungs% fin. b. wt.</td>
<td>1.04 ± 0.20</td>
</tr>
<tr>
<td>Heart% fin. b. wt.</td>
<td>0.54 ± 0.07</td>
</tr>
<tr>
<td>Testicle (right)% fin. b. wt.</td>
<td>0.33 ± 0.03</td>
</tr>
</tbody>
</table>

* Mean ± sd.

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<tr>
<th>Table 7</th>
<th>13-Week feed toxicology study means and standard deviations for hematology at time of necropsy.</th>
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<tbody>
<tr>
<td>Hematology test</td>
<td>Control male</td>
</tr>
<tr>
<td>RBC (10^6/μL)</td>
<td>9.08 ± 0.31</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.6 ± 0.43</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>43.2 ± 1.25</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>12.8 ± 0.29</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>47.5 ± 0.78</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>15.0 ± 0.25</td>
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<tr>
<td>MCHC (g/dL)</td>
<td>31.5 ± 0.45</td>
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<tr>
<td>Platelets (10^3/μL)</td>
<td>239.6 ± 26.5</td>
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<tr>
<td>WBC's (10^3/μL)</td>
<td>1076.9 ± 61.5</td>
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<tr>
<td>MPV (fL)</td>
<td>6.60 ± 0.12</td>
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<tr>
<td>PCV (%)</td>
<td>40.75 ± 1.49</td>
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<tr>
<td>WBC's (10^3/μL)</td>
<td>3.19 ± 0.49</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>29.6 ± 7.1</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>66.2 ± 7.8</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>3.6 ± 2.8</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>2.5 ± 1.2</td>
</tr>
</tbody>
</table>

* Mean ± sd.

\[a\] Significant at $p < 0.05$.
evidence of oral toxicity after longer term exposures to products that did not contain the aloe latex. These data again demonstrate the importance of evaluating beverage products as they are sold and point to the fallacy of drawing comparisons with effects of unfiltered whole-leaf juices or other aloe products such as latex-containing capsules (Yang et al., 2010).

Producer recommended levels of aloe juice for consumers ranges from a few teaspoons to one cup. To place our dosing levels in the context of anticipated customer drink levels, we scaled the dose given to mice to a human making assumptions regarding a “typical” person and using body surface area. Using the conversion, we approximate that the acute dosing up to 10-fold greater than a one-cup level and a more modest 1.2–1.1-fold greater in the sub-chronic study. Given the relative expense of undiluted aloe beverages, consumers may not drink one-cup per day and therefore we feel these excesses are reasonable. Individuals with idiosyncratic reactions to acute or chronic aloe drinks may occur and the levels we tested in this study may not predict reaction in those individuals. Allergic reactions, for instance have been associated with aloe glycoproteins (Reynolds and Dweck, 1999) in people yet are not reported in in vivo safety studies involving aloe juices or individual ingredients.

In summary, we examined a commercially available stabilized aloe vera beverage prepared from the inner gel fillet through genotoxicity, acute and subchronic assays. The assays performed demonstrated no evidence that this stabilized aloe vera gel is toxic at levels that consumers will reasonably drink; however, our conclusions herein do not rule out potential toxic and/or adverse effects which may occur with repeated consumption of stabilized aloe vera gel over a prolonged time period.

Conflict of Interest

Financial sponsorship was provided through collaborative agreement with ST&T consultants. ST&T consultants has received support and aloe juice from the manufacturer of a stabilized aloe vera gel in order to conduct safety studies including those presented here. Co-authors (M.S., W.W.) who consult for ST&T participated in the study’s design; however, all data collection, analysis and report writing were done exclusively by co-authors at Louisiana State University’s SVM. The decision to submit this article was collectively agreed upon by all co-authors. No co-authors have any financial ownership with aloe juice manufacturers.

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