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# Absence of in vivo genotoxicity of a purified *Aloe vera* whole leaf juice concentrate when tested in rats using the comet assay

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## ABSTRACT

Hydroxyanthracene derivatives (HADs) are a class of compounds naturally present in edible plants, including *Aloe vera*. HAD compounds such as aloin A, B and aloe-emodin found in the latex layer of *Aloe vera* leaf have been shown to be genotoxic in bacteria and mammalian cell assays, possibly attributing to colonic carcinogenicity observed in a 2-year rodent cancer bioassay of an orally administered *Aloe vera* whole leaf extract. In commerce, HADs in *Aloe vera* leaves are typically removed through an activated charcoal filtration process, also known as decolorization, during the production process of *Aloe vera* ingredients for use in foods and supplements. However, limited evidence is available regarding the *in vivo* genotoxicity potential of highly purified decolorized *Aloe vera* ingredients. The present study evaluated *in vivo* genotoxicity potential of an orally administered purified *Aloe vera* whole leaf juice concentrate containing negligible levels of HADs (test article [TA], containing 0.3 ppm of aloins and non-detectable aloe-emodin [LOQ = 0.02 ppm]). Methods: A GLP-compliant *in vivo* comet assay (OECD 489) was performed to test DNA damage in the colon and kidney following oral gavage administration of TA at 500, 1,000 and 2,000 mg/kg bw/day in male F344 rats for 2 consecutive days. Vehicle control (purified water), and ethyl methanesulfonate (EMS) were used as negative and positive controls, respectively. Results: Rats administered TA exhibited no significant differences in final body weight compared to concurrent control animals. No other abnormal clinical observations associated with TA exposure were observed. For both kidney and colon, no dose level exhibited a statistically significant increase in DNA damage compared to the concurrent vehicle control group, and there was not a dose-related response. The positive control, EMS, resulted in statistically significant increases in DNA damage in the kidney and colon of the animals. Conclusion: the comet assay revealed that the purified *Aloe vera* whole leaf juice concentrate with de minimis HADs did not induce DNA damage in colon and kidney tissues under the experimental conditions described.

## INTRODUCTION

- HADs are a class of compounds naturally present in edible plants, including *Aloe vera*. HAD compounds such as aloin A, B and aloe-emodin found in the aloe leaf latex layer have been shown to be genotoxic in bacteria and mammalian cell assays, possibly contributing to colonic carcinogenicity linked to an orally administered aqueous extract of *Aloe vera* whole leaves, which contained 6,400 ppm aloin A and 71 ppm aloe-emodin.
- Many commercial aloe food and supplement products are made with aloe ingredients in which HADs are removed through an activated charcoal filtration process, also known as decolorization. These purified aloe ingredients are not known to have any laxative properties linked to HADs due to their insignificant levels.
- Genotoxicity studies of *Aloe vera* materials with < 10 ppm aloins have mostly reported negative outcomes in prokaryotic and eukaryotic test systems. However, a decolorized whole leaf extract with 63 ppm aloins was found mutagenic at doses of 6 to 8 mg/mL in a mouse lymphoma TK assay study in 2014 (**Table 1**).
- In view of contradictory genotoxicity results of decolorized *Aloe vera* preparations, further study was warranted in accordance with internationally recognized genotoxicity testing approaches (e.g., the OECD and EFSA guidelines).
- The objective of this study was to evaluate genotoxicity potential of a decolorized/purified *Aloe vera* whole leaf juice concentrate with trace HADs (thereafter referred to as test article [TA]), in the *in vivo* comet assay to confirm whether the genotoxic response observed *in vitro* is expressed *in vivo*.

**Table 1. Reported Genetic and Related Effects of *Aloe vera* Inner Leaf, Gel and Decolorized Whole Leaf Materials (Adapted from IARC *Aloe vera* Monograph, Table 4.1, 2017)**

| Test system | Results | | Dose (LED or HID) | *Aloe vera* preparation | Reference | Study Quality |
|---|---|---|---|---|---|---|
| | Without S9 | With S9 | | | | |
| *Salmonella typhimurium*, TA100, reverse mutation | negative | negative | NR | Stabilized gel; aloin A and B, ≤ 10 ppm; in some instances, material was sterilized by filtration or autoclaving | Sehgal et al. (2013a) | + |
| *Salmonella typhimurium*, TA98, TA100, TA1535, TA 1537, reverse mutation | negative | negative | 10 mg/plate | Qmatrix® inner leaf fillet; aloins < 10 ppm | Williams et al. (2010) | + |
| *Salmonella typhimurium*, TA98, TA100, reverse mutation | negative | negative | 6 mg/plate | Decolorized whole leaf extract | Boudreau et al. (2013) | ++ |
| *Salmonella typhimurium*, TA97, TA98, TA100, TA1535, reverse mutation | negative | negative | 10 mg/plate | Gel | Boudreau et al. (2013) | |
| *Salmonella typhimurium*, TA98, TA100, reverse mutation | negative | negative | 21 × initial concentration | Decolorized whole leaf extract; aloin A & B at ~1 ppm, material sterilized by filtration | Sehgal et al. (2013b) | - |
| *Escherichia coli*, WP2 *uvrA*/pKM101 | negative | negative | 6 mg/plate | Decolorized whole leaf extract | Boudreau et al. (2013) | ++ |
| *Escherichia coli*, WP2 *uvrA*/pKM101 | negative | negative | 3 mg/plate | Gel | Boudreau et al. (2013) | |
| *Escherichia coli*, SOS DNA damage repair assay | negative | negative | 10 × initial concentration | Stabilized gel; aloin A and B ≤ 10 ppm | Sehgal et al. (2013a) | + |
| *Escherichia coli*, SOS DNA damage repair assay | negative | negative | 21 × initial concentration | Decolorized whole leaf extract; aloin A and B at ~1 ppm, material sterilized by filtration | Sehgal et al. (2013b) | + |
| Chromosomal aberrations, Chinese hamster lung cells | negative | negative | 10 mg/plate | Qmatrix® inner leaf fillet; aloins < 10 ppm | Williams et al. (2010) | + |
| Mouse lymphoma L5178Y/TK$^{+/-}$cells | positive | negative | 6 mg/mL | Decolorized whole leaf extract; aloin A at 63 ppm | Guo et al. (2014) | + |
| Male ICR mice, micronucleus formation in bone marrow cells | negative | | 5,000 mg/kg bw, po | Qmatrix® inner leaf fillet; aloins < 10 ppm | Williams et al. (2010) | - |

LED, lowest effective dose; HID, highest ineffective dose; NR, not reported; po, per oral.
Study quality ratings: ++, definitely low risk of bias; +, probably low risk of bias; - or NR, probably high risk of bias; --, definitely high risk of bias. Adapted from OHAT Risk Tool, 2015.

## MATERIAL & METHODS

### Test article

- Purified *Aloe vera* Juice Concentrate (Whole Leaf ), Lot# 715HB10YK03, was a light tan color powder, provided by Herbalife Nutrition as the test article (TA). Chemical characterization is present in **Table 2**.
- The TA was prepared in deionized water to achieve final dosing concentrations of 50, 100 and 200 mg/mL.

**Table 2. Chemical Composition of Purified *Aloe vera* Whole Leaf Juice Concentrate**

| Test | Specification Limit | Result |
|---|---|---|
| Appearance | Light tan free flowing powder | Light tan free flowing powder |
| Moisture content (w/w%) | ≤ 10% | 4% |
| pH | 4.0 – 5.0 | 4.59 |
| Ash (w/w%) | ≤ 45% | 32% |
| Total aloin A and B[a] | ≤ 1.5 ppm | 0.3 ppm |
| Aloe-emodin[a] | report | Not detected (LOD = 0.01ppm, LOQ = 0.02 ppm) |
| Aloe polysaccharides (w/w%) | report | 6% |
| Cadmium | ≤ 0.5 ppm | < 0.1 ppm |
| Arsenic | ≤ 3.0 ppm | 1.7 ppm |
| Lead | ≤ 1.25 ppm | 0.01 ppm |
| Mercury | ≤ 1.0 ppm | < 0.5 ppm |

### Study design (see Figure 1)

- Comet assay was performed by Integrated Laboratory Systems following the protocol in accordance with OECD 489 and applicable GLP regulations. Animal husbandry procedures were in compliance with USDA (2017) Animal Welfare Act Regulations, and animals were handled and treated according to the NRC Guide (2011) for the Care and Use of Laboratory Animals.
- Male Fischer 344/N Hsd rats were selected because this model was used in a 2-year carcinogenicity study of a non-decolorized *Aloe vera* whole leaf extract in which clear evidence of carcinogenicity was observed. Male rats appeared to be more sensitive than females (Boudreau et al., 2013).
- The colon and kidney were selected as target organs for comet analysis based upon:
  - The colon as the target site of the carcinogenic effect of aloins and non-decolorized *Aloe vera* whole leaf extract (Boudreau et al., 2013 & 2017);
  - The kidney as a target organ in a comet assay following orally administered aloe-emodin in mice (Nesslany et al., 2009).
- Systemic exposure to the TA or aloins was not measured in that the carcinogenic effect of ingested aloe HADs was only detected at the site of direct contact in the rat model, the colon, and ingested aloins are known not to be bioavailable (Boudreau et al., 2013; IARC, 2017).
- The assay acceptance and evaluation criteria were applied in adherence to OECD Guideline 489.

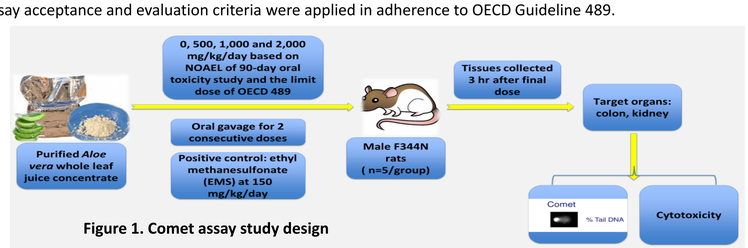


**Figure 1. Comet assay study design**

### Tissue collection and slide preparation

- The colon epithelial layer and kidney tissue were harvested from individual animals in the mincing solution, and frozen in liquid nitrogen until analysis. A portion of the cell suspension of each thawed tissue sample was diluted with 0.5% NuSieve GTG low melting point agarose, dissolved in phosphate buffer at 37 ± 2°C, and layered onto CometSlides™ slides.
- Slides were immersed in chilled lysing solution overnight under a light proof condition, then rinsed in neutralization solution. Slides were placed in the electrophoresis unit with cold electrophoresis solution at ≤ 10 °C for 20 min to allow the DNA to unwind, then subject to electrophoresis.
- After electrophoresis, slides were neutralized, dehydrated by immersion in absolute ethanol and allowed to air dry. Air-dried slides were then stained with SYBR™ Gold for the image analysis.

### Comet analysis

- The % of migrated DNA in the comet tail of 150 cells was measured per tissue sample using Comet IV Image Analysis Software (Version 4.3.2). "Hedgehogs" were tabulated but not scored. Slides were coded and scored without knowledge of their identity.

## RESULTS

- All animals survived to the scheduled termination without signs of moribundity. There were no treatment-related abnormal observations in animals administered the TA or EMS.
- No statistically significant changes in initial and final body weight or body weight gain were observed in the TA treated groups, while EMS induced a statistically lower mean body weight gain.
- EMS resulted in a statistically significant increase in the % tail DNA in both tissues analyzed ($p < 0.05$).
- Oral dosing of the TA up to 2,000 mg/kg/day did not induce statistically significant induction of DNA damage in the colon or kidney compared to the concurrent vehicle control ($p > 0.05$). A statistically significant decrease in DNA damage was observed in the kidney of the animals administered 1,000 mg/kg/day relative to the vehicle control ($p = 0.01$) but no dose-response was observed. See **Figure 2**.

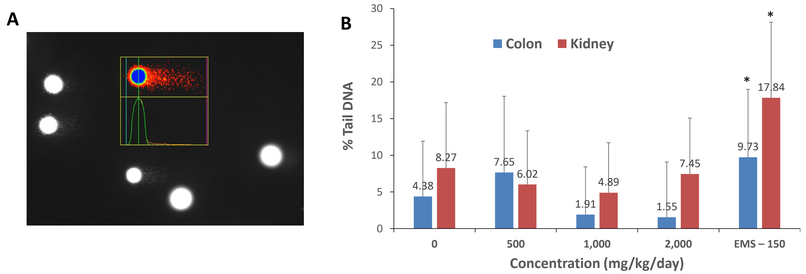


**Figure 2. Comet assay results. A. Example of automated scoring of comet cells. B. Mean percent tail DNA (mean ± SD) in male F344N rats after oral exposure to purified aloe whole leaf juice concentrate. * P < 0.05 compared to the concurrent vehicle control.**

## CONCLUSIONS

- Purified *Aloe Vera* (Whole Leaf) Juice Concentrate containing residual aloins (0.3 ppm) as impurities did not induce DNA damage in the colon or kidney tissues of male F344N rats when orally administered at doses up to 2,000 mg/kg/day.

## DISCLOSURE

This research was funded by Herbalife Nutrition which sells ingestible non-laxative products containing purified *Aloe vera* juice ingredients.

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