

# A Novel Liquid Multi-Phytonutrient Supplement Demonstrates DNA-Protective Effects

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Published online: 3 March 2009

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**Abstract** This study explored the DNA protective (anti-mutagenic) effects of an oral, liquid, multi-phytonutrient dietary supplement containing a proprietary blend of fruits, vegetables and aloe vera concentrated components in addition to a proprietary catechin complex from green tea (VIBE Cardiac & Life, Eniva Nutraceuticals, Anoka, MN; herein described as “VIBE”). This study tested the hypothesis that VIBE would reduce DNA damage in skin cells exposed to UVR. Human epidermal cells, from the cell line A431NS, were treated with 0% (control), 0.125%, 0.5%, 1% and 2% VIBE, and then exposed to 240 J/m<sup>2</sup> UVR. The amount of DNA damage was assessed using the COMET assay. At each concentration tested, a significantly smaller amount of DNA damage was measured by the COMET assay for the VIBE treated cells compared to the control cells exposed to UVR without VIBE. The dose response curves showed a maximal response at 0.5% VIBE with a threefold reduction in COMET tail density compared to the control samples without VIBE ( $p < 0.001$ ). Additional research is warranted in human clinical trials to further

explore the results of this study which demonstrated the DNA protective and anti-mutagenic effects of VIBE for human skin cells exposed to UVR-induced DNA damage.

**Keywords** Anti-mutagenic · COMET assay · Epidermal cells · Green tea (–)-epigallocatechin-3-gallate (EGCG) · Phytonutrient supplement · UV genotoxicity

## Abbreviations

DNA deoxyribonucleic acids  
EGCG epigallocatechin-3-gallate  
LMP low melting point  
UVR ultraviolet radiation

## Introduction

Cellular stress leading to deoxyribonucleic acid (DNA) damage and genetic mutation may result in uncontrolled, pro-carcinogenic cellular proliferation. One agent known to induce carcinogenic changes in human skin cells is ultraviolet radiation (UVR). Although the exact mechanism whereby UVR exposure results in skin cancer remains unclear, UVR exposure is known to generate free radicals and DNA strand breaks which contribute to pro-carcinogenic cascades potentially leading to cancer [1–3]. The catechin, (–)-epigallocatechin-3-gallate (EGCG) has been shown to protect against UVR-induced skin and DNA damage, and may provide protection against the development of skin and other cancers [4–11]. One study demonstrated EGCG provided concentration-dependent protection against UVR-induced DNA damage in cultured human skin fibroblasts and epidermal keratinocytes. This study also demonstrated that consumption of green tea (which is a rich dietary

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source of EGCG) reduced the amount of DNA damage in blood cells of human subjects exposed to UVR [12].

This study tested the hypothesis that a liquid nutritional supplement containing essential nutrients, a proprietary blend of plant components and a proprietary green tea catechin complex including EGCG (VIBE 2.0 Cardiac & Life; Eniva Nutraceuticals, Anoka, MN) was able to reduce DNA damage in human skin cells exposed to UVR. We used the COMET assay [single-cell gel electrophoresis (SCGE)] [13] to measure the DNA protective and anti-mutagenic properties of an *in vitro* treatment with VIBE for epidermal cells exposed to UVR. The COMET assay is widely used in environmental toxicology, cancer research, and radiation biology as a sensitive and broadly accepted method for measuring DNA damage in individual cells. Briefly, cells are embedded in agarose gel on a microscope slide, lysed, electrophoresed, and stained with fluorescent DNA-binding dye. DNA from each cell migrates towards the anode during electrophoresis forming a shape of a “COMET” with a head (cell nucleus with intact DNA) and a tail (relaxed and broken DNA). The DNA percentage in the COMET tail (tail density) was the study endpoint since cells with damaged DNA have increased COMET tail density.

## Materials and Methods

**Cell Line** The human epidermoid cell line, A413NS (American Type Culture Collection, Manassas, VA) was maintained in RPMI 1640 with 10% calf serum and 2 mM glutamine.

**Test Article** The liquid multi-phytonutrient dietary supplement containing essential nutrients, a proprietary blend of fruits, vegetables and aloe vera gel concentrated components, and a proprietary green tea EGCG catechin complex (VIBE 2.0 Cardiac & Life; Eniva Nutraceuticals, Anoka, MN; Table 1), was mixed with freshly prepared RPMI 1640 growth medium (10%, *v/v*).

**UVR Treatment** A germicidal lamp with a spectrum peak at 254 nm was used as the radiation source, and a 240 J/m<sup>2</sup> energy equivalent was delivered at the surface of the cell culture medium.

**Cell Viability Assay** Viability was analyzed after test article treatment and 15 min of recovery after irradiation (Guava ViaCount Assay, Guava Technologies, Inc., Hayward, CA).

**COMET Assay** Cells were grown in 24-well plates until 60–80% confluent, then treated with 0% (positive and negative controls), 0.125%, 0.25%, 0.5%, 1.0%, and 2.0%

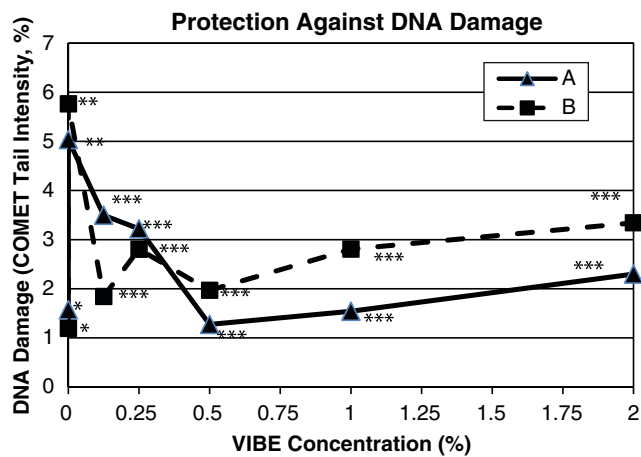
VIBE for 30 min in duplicate. The treatment medium was replaced with 100 µl of fresh medium (without VIBE), and cells were exposed to 240 J/m<sup>2</sup> UVR (“negative” controls were covered with aluminum foil to prevent exposure to UVR while “positive” controls were exposed directly to UVR). Cells were incubated 15 min to allow recovery, washed with Dulbecco’s phosphate buffered saline and trypsinized to obtain a single cell suspension. An aliquot of

**Table 1** Supplement facts for VIBE 2.0 cardiac and life

	Amount per serving <sup>a</sup>	% Daily value <sup>a</sup>
Calories	30	
Total Carbohydrate	6 g	2
Sugars	4 g	– <sup>b</sup>
Vitamin A	2,000 IU	40
Vitamin C	120 mg	200
Vitamin D	500 IU	125
Vitamin E	30 IU	100
Thiamin (Vitamin B1)	1.5 mg	100
Riboflavin (Vitamin B2)	1.7 mg	100
Niacin	18 mg	90
Vitamin B6	2 mg	100
Folic Acid	400 mcg	100
Vitamin B12	12 mcg	200
Biotin	300 mcg	100
Pantothenic Acid	10 mg	100
Calcium	100 mg	10
Phosphorus	20 mg	2
Iodine	150 mcg	100
Magnesium	155 mg	39
Zinc	5 mg	33
Selenium	25 mcg	36
Copper	0.5 mg	25
Manganese	1.8 mg	90
Chromium	120 mcg	100
Potassium	175 mg	5
Proprietary trace mineral blend	37 mg	– <sup>b</sup>
Boron, germanium, sulfur, vanadium		
AntiOX <sup>2</sup> ® proprietary blend	6,500 mg	– <sup>b</sup>
Natural extracts: green tea, cranberry, raspberry, blueberry, blackberry, strawberry, cherry, carrot, acai berry, elderberry, hibiscus (flower), lemon, lime, apple, orange, blackcurrant, oregano, chokeberry, grape, pumpkin, tomato, pomegranate, wolfberry (gojiberry), Stevia (leaf), grape seed extract, citrus bioflavonoids		
HeartPRO® proprietary blend	280 mg	– <sup>b</sup>
D-Ribose, CoQ10, L-carnitine, malic acid, isolated soy lecithin, mixed tocopherols		
CollaMAX® proprietary blend	3,500 mg	– <sup>b</sup>
Green tea leaf extract (water decaffeinated), l-lysine, L-proline, aloe vera gel (containing alanine, valine, isoleucine, glycine, leucine), glucosamine HCl (vegetable)		

<sup>a</sup> Percent daily values are based on a 2,000 calorie diet and a one fluid ounce serving

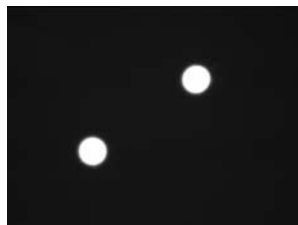
<sup>b</sup> Daily value not established



**Fig. 1** VIBE provides protection against DNA damage in human epidermal cells exposed to UVR. Two independent experiments (*A* and *B*) confirm the DNA protection provided by VIBE as measured by mean COMET tail density. The negative control (*asterisk*) was not exposed to UVR and did not contain any VIBE. The positive control (*two asterisks*) was exposed to UVR and did not contain any VIBE. All test samples with increasing concentrations of VIBE were exposed to the same amount of UVR as the positive control and showed a significantly lower amount of DNA damage compared to the positive control (*three asterisks*;  $p < 0.001$ )

10,000 to 20,000 cells were centrifuged ( $400 \times g$  for 5 min), mixed with 0.5% low melting point (LMP) agarose (in PBS at  $37^\circ\text{C}$ ), laid on an agarose-coated microscopic slide and covered with a cover glass. The slide was chilled 20 min and the cover glass was removed. Another layer of  $70\ \mu\text{L}$  LMP agarose was added above the cell-containing layer and spread thin by the addition of another cover glass and chilled. After the agarose gel hardened, the cover-glass was removed and the slide was immersed in cold lysing solution overnight. The slide was placed in a horizontal gel electrophoresis tank and electrophoresed at 25 V for 20 min. The slide was rinsed, stained with ethidium bromide ( $60\ \mu\text{L}$ ,  $20\ \mu\text{g/ml}$ ) and immediately scored using a 40-power objective with G-2A filter of a Nikon E400 fluorescence microscope equipped with a COMET III Image System (Perceptive Instruments Ltd, Haverhill, Suffolk, UK). Fifty cells from each duplicate slide were scored for tail density (percentage of DNA in the COMET tail) and this entire method was repeated for a second, independent confirmation of the experimental data.

**Fig. 2** Representative photograph of a negative control sample with no mutagen (UVR) exposure (two cells)



**Fig. 3** Representative photograph of a positive control cell showing the increased DNA density in the COMET tail after mutagen (UVR) exposure without VIBE (i.e. no DNA protective effect was evident since VIBE was not included in this sample)

**Statistics** The non-parametric Mann–Whitney test was used to compare the UVR exposed VIBE-treated cells with the UVR exposed untreated (positive) control cells.

## Results

Cell viability remained above 94.5% for all samples and cells treated with increasing concentrations of VIBE showed significantly smaller amounts of DNA damage, as measured by COMET tail density compared to the positive control cells exposed to UVR ( $p < 0.001$ , Fig. 1). Representative photographs (Figs. 2, 3 and 4) show the varying intensities of the COMET tails produced in this study. The negative control sample (Fig. 2) included cells not exposed to the UVR mutagen and showed the smallest COMET tail density compared to the positive control sample (Fig. 3—cells exposed to the UVR mutagen without any VIBE treatment) which showed the greatest COMET tail density. The test sample (Fig. 4c) included cells treated with 0.5% VIBE and showed a significantly smaller amount of COMET tail density compared to the positive control (Fig. 4 compared to Fig. 3).

## Discussion

The results of this study show that multi-phytonutrient dietary supplement, VIBE, reduced DNA damage and



**Fig. 4** Representative photograph of the anti-mutagenic effects of 0.5% VIBE in two cells showing the decreased (compared to Fig. 3) DNA density in the COMET tail after mutagen (UVR) exposure when cells were treated with VIBE (i.e. a significant DNA protective effect was evident with the VIBE treatment)

genotoxicity as measured in the COMET assay using human epidermal skin cells exposed to ultraviolet radiation. VIBE contains a broad spectrum antioxidant complex from fruits, vegetables, aloe vera gel and green tea, and the potential mechanisms behind these DNA protective properties may be related to the antioxidant activities of one or more of these antioxidant ingredients [14]. In this study, two independent COMET assays demonstrated significantly reduced amounts of DNA migrating in the COMET tails among the VIBE-treated cells compared to the amount of DNA in the COMET tails of the positive control cells exposed to UVR without VIBE (at all concentrations tested, all comparisons were  $p < 0.001$ , Fig. 1). These data suggest that VIBE promoted genetic stability and thus had antimutagenic activity in skin cells exposed to ultraviolet radiation, a common pro-carcinogenic agent with significant human exposures. These effects of VIBE on human epidermal cells warrant further investigation, especially in light of the increasing rates of skin and other cancers in the human population.

Phytonutrients have been shown to protect human skin from the cancer-causing effects of UVR, and EGCG has been shown to reduce the damaging effects of UVR when EGCG is applied directly to the skin [9, 11, 15, 16], and also when administered orally to animals [17] and humans [12]. Of interest, human population-based, case-control studies suggested regional differences in the incidence of squamous cell carcinoma of the skin that may be related to tea consumption [18, 19]. Thus, the beneficial effects of VIBE seen in this experiment will likely extend to the oral administration of VIBE (Figs. 3 and 4).

In addition, to protecting the skin against the damaging and carcinogenic effects of UVR, EGCG may decrease the risk of developing a wide variety of other cancers [4, 20] and EGCG appears to be safe and well-tolerated [21]. As suggested by studies investigating the antimutagenic and anti-neoplastic properties of orally ingested tea catechins and fruit and vegetable components, VIBE may also enhance cellular defense mechanisms against other mutagenic free-radical-generating substances [22–26].

Fruit and vegetable consumption has been associated with a decreased incidence of cancer and cardiovascular disease, and bioactive compounds have been extracted from fruits and vegetables; however, a beneficial relationship may exist between the recognized RDA (recommended daily allotment) nutrients and the additional phytonutrients that are found in the whole food matrix which may be lost during the extraction process. Recent evidence supports this idea that combinations of phytochemicals may be far more effective than isolated compounds for cancer protection [23]. The synergy between the various cofactors in the whole fruits and vegetables may be far more effective than the isolated bioactive nutrients that have been studied to

date. This cofactor synergy may explain why VIBE, which contains the natural extracts from numerous fruits and vegetables, is so effective at protecting DNA against UVR induced damage in this COMET assay. Due to the significant role free radicals play in the cellular mechanism of the pro-carcinogenic processes and other degenerative conditions, additional research is recommended.

In conclusion, the liquid multi-phytonutrient dietary supplement, VIBE, protected cultured human epidermal cells against UVR-induced DNA damage. Future studies should evaluate the beneficial potential of this product following long-term oral administration.

**Acknowledgements** The study was designed, conducted and the data were collected, analyzed and interpreted at the independent testing facility, Cancer Chemoprevention Core Laboratory, Linus Pauling Institute (Oregon State University, Corvallis, OR). Funding was provided by Eniva Nutraceuticals.

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