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Epigenetic effects of four nutraceutical products on human liver cells

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ABSTRACT

Background: There are numerous botanical extracts and fractions that have been shown to have a diverse range of bioactivities. Many nutraceutical products and food supplements contain phytochemicals - often multiple numbers of them as constituents of a single product. There is a paucity of data on the net bioactivities of these multi-component products. This paper reports the effects of four such products on the expression of six genes critical to maintaining good health.

Objectives: We investigated several products that contain a number of constituents derived from plant species as well as essential minerals on the biochemical activities of liver cells in culture. The combination of these bioactives into one product results in multiple strong biological effects. Examples of these are presented in this paper with a discussion of how the constituents have provided this effect.

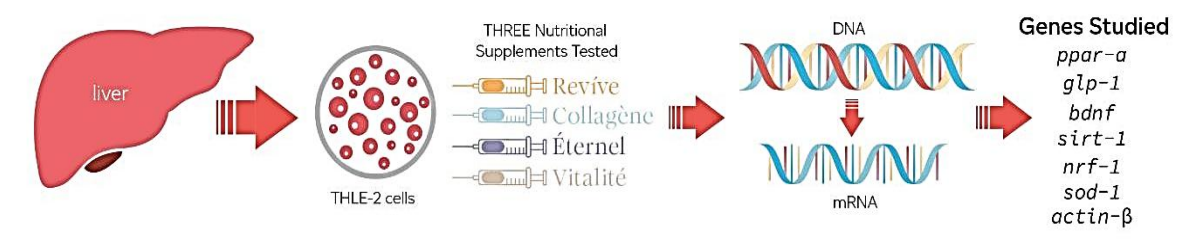
Methods: The expression of six genes (*ppar-α, glp-1, bdnf, sirt-1, nrf1, sod-1*) in a human liver cell line (THLE-2 cells) were cultured for 24 hours in the presence of Vitalité, Reviv, Revíve and Collagène (products from THREE International). The RNA was isolated from these cells and from the control unsupplemented cells. The levels of transcripts of these genes in these isolates were measured by RT-PCR. The changes in the concentrations of the mRNA from the supplemented cells were compared with those from reference cells.

Results: Of the four products, Revíve boosted the transcription of the *glp-1,* the *bdnf,* the *sirt-1,* the *nrf1,* and the *sod-1* genes. Éternel boosted the transcription of *ppar-α,* and the *sod-1* genes. Vitalité increased the transcription of the *glp-1*, the *bdnf,* the *nrf1,* and the *sod-1* genes. Collagène stimulated the expression of the *bdnf,* and the *sod-1* genes.

Nutraceutical products, either as a complement or as a substitute for pharmaceuticals, have been receiving increased attention for maintaining good health. These products are multi-component. Evidence for their clinical efficacy is frequently lacking.

Conclusion: The results of this project provide evidence that the phytochemicals in the four products investigated can provide the bio-effects that regulate the expression of key genes that control fundamental essential metabolic activities and so can justify the claims made for them.

Keywords: Epigenetics, nutritional supplements, phytonutrients, liver health



Graphical Abstract: Epigenetic effects of four nutraceutical products on human liver cells

INTRODUCTION

As well as being important for sustaining an active lifestyle, appropriate nutrition is essential for maintaining good health. For many, the food component of an individual's nutrition is sub-optimal, either because of insufficiency or deficiencies in its composition. To counter this, one remedy is to consume nutritional supplements that address the shortages.

Nutritional supplements can be derived from various sources and can be either synthetically produced or obtained from natural materials [1-3]. The supplements from natural origins can be from marine organisms, animals, plants, or microbial species. The European Food Safety Authority [2] defines these supplements as concentrated sources of nutrients or other substances with nutritional or physiological effects. They are intended to correct nutritional deficiencies, maintain an adequate intake of certain nutrients, or support physiological functions [1].

Different plants are sources of diverse supplements because of their distinctive compositions. Additionally, the methods of preparation of the extracts or fractions from a particular plant can yield products of different compositions and structures and consequently manifest different biological effects. In order to achieve biomedical effects, specific extracts from botanical species are evaluated so as to establish their efficacies, modes of action, and safety [4]. Additionally, combinations of extracts and fractions can achieve increased effectiveness as well as offer opportunities for eliciting more than one health benefit.

As an example, curcumin is a compound that is extracted from turmeric (*Curcumina longa*). Multiple bioactivities have been reported with its anti-inflammatory and pain relief effects being well-characterized [5-6]. It is also proposed to have effects on gastrointestinal tract pathologies, colorectal cancer, diabetes, depression, and viral infections [6-9]. A

hindrance to the wide acceptance of the benefits of curcumin is its poor bioavailability [10-11]. A relatively small amount of piperine from the black pepper (*Piper nigrum*) plant has been shown to improve the uptake of curcumin [10,12].

The liver is an essential organ for all mammals, which is responsible for multiple crucial functions. There are six key functions that are ascribed to it:

1. Metabolism.
2. Detoxification.
3. Filtration.
4. Digestion.
5. Protein synthesis.
6. Storage of minerals and vitamins.

The primary metabolism of the major food classes (fats, proteins, and carbohydrates) occurs in the liver [13-15]. It is also the primary organ for the activation and metabolism of drugs and xenobiotics and is responsible for the deactivation of substances and compounds that are harmful. The liver has an array of enzymes that can detoxify these unwanted compounds, which are then excreted [16,17]. It converts ammonia to urea which is removed in the urine via the kidneys. Additionally, the liver filters the blood, detoxifies any toxic and harmful substances, and secretes them into the blood or bile.

Bile is a fluid that is produced by the liver and is stored in the gall bladder. When food is consumed, bile is transported from the gall bladder to the intestines as it is essential for the breakdown and digestion of fats [18].

Proteins are essential constituents of organs and cells as well as being the enzymes that are critical for all biochemical reactions. They are also involved in physiological activities such as those conducted by blood and are engaged in transport functions [19]. So, the synthesis of proteins by the liver is necessary for the performance of these multiple actions.

A healthy liver stores, produces, and releases vitamins and minerals [20-21]. These are needed for a wide variety of molecular processes. Because of these activities, the optimal maintenance of its function is important. Consequently, the consumption of supplements, especially those comprised of a multiple number of botanical extracts, will assist in achieving this.

MATERIALS AND METHODS

Test samples: The products investigated in this project were Vitalité, Éternel, Revíve and Collagène. These were provided by THREE International (Utah, USA).

Cell Culture: THLE-2 cells were obtained from American Type Culture Collection (ATCC, Bethesda, MD) and cultured initially in culture flasks that had been pre-coated with 0.01 mg/mL fibronectin, 0.03% bovine type I collagen and 0.01% bovine serum albumin in bronchial epithelial growth medium (BEGM) (Lonza, Wellington, New Zealand) plus 10% fetal bovine serum (FBS) (Moregate, Hamilton, New Zealand) and penicillin/streptomycin (Sigma-Aldrich, St Louis, MO). They were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The cells were harvested when they reached 80% confluence.

To detach the adherent cells, the medium was removed from the culture flask, and the adherent cells were washed with phosphate-buffered saline (PBS) (Gibco, Auckland, New Zealand) to remove any traces of FBS. Then 4 mL of 0.05% trypsin/EDTA solution (Sigma-Aldrich) was added and incubated at 37°C for 5 min until all the cells detached. The trypsin was then neutralized by adding 5-6 mL of pre-warmed Eagle's Modified Essential Medium (EMEM) (ATCC) + 10% FBS and antibiotics and centrifuged at 125g (500 rpm) for 7 min at 4°C.

The supernatant was discarded, and the cell pellet was re-suspended with 10 mL of pre-warmed EMEM + 10% FBS and antibiotics. The cell number was counted and adjusted to 1 x 10^5 cells/mL for the culturing.

For the experiments, flat-bottomed 24-well plates treated for cell adhesion were used for the mRNA determinations. 96 well plates were used for the cell proliferation assays. For each test sample, triplicate cells were set up.

For the mRNA determination assays, 720 µL of the cell suspension (1 x 105 cells/ mL) were plated into wells, giving approximately 7.2 x 10^4 cells/well. 720 µL of the appropriate medium only was added to other wells. The plates were placed in the incubator for 24 hours to allow the cells to adhere to the wells.

The plates were then removed from the incubator. 80 µL of the test samples (final concentrations 50 µg/ mL or 150 µg/ mL) were added to the appropriate wells. 80 µL of 0.5% dimethyl sulfoxide (DMSO) in culture media was added to the control wells. The plates were incubated at 37°C in 95% Air/5% CO_2 for 24 hours. At the time of termination, the culture medium was aspirated from the wells.

Simultaneously a 96-well plate was set up for the cell concentration determinations. 180 µL of the cell suspension (1 x 10^5 cells/ mL) were plated into wells giving approximately 1.8 x 10^4 cells/well. 180 µL of the medium only was added to other wells. The plates were placed in the incubator for 24 hours to allow the cells to adhere to the wells. The plates were removed from the incubator, and 20 µ µL of the test samples (final concentrations 50 µg/ mL or 150 µg/ mL) were added to the appropriate wells. 20 µL of 0.5% DMSO in culture media was added to the control wells, the plates were incubated at 37°C in 95% Air/5% CO_2 for 24 hours. At the time of termination, the culture medium was aspirated from the wells, and the cells were incubated with the dimethyl thiazolyl diphenyl tetrazolium (MTT) Reagent (Sigma-Aldrich) to determine the cell concentrations.

RNA Isolation and Gene Expression: Using the cells from the 24 well plates, RNA isolations were carried out with ReliaPrep miRNA Cell & Tissue Miniprep kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol. RNA was eluted with RNase-free water (ThermoFisher, Auckland, New Zealand) and quantified using a 260 nm/280 nm ratio with a spectrophotometer.

Reverse transcription reactions were performed using 1 µg of RNA with the SuperScript VILO cDNA synthesis kit (Invitrogen, Auckland, New Zealand). The cDNA was then diluted 10-fold with water (ThermoFisher). PCR reactions involved 8 µL diluted cDNA, 1 µL gene primer (Fluorescein amidite (FAM) labelled) (see Table 1), 1 µL actin-β (hACT) primer (2'-chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC) labelled) and 10 µL TaqMan Fast Advanced Master Mix (ThermoFisher).

Table 1. Source and identification of primers

Primers	Supplier	Catalog no.
ppar-α	Applied Biosystems	Hs00231882-m1
glp-1	Applied Biosystems	Hs00913461-g1
bdnf	Applied Biosystems	Hs00380947-m1
sirt-1	Applied Biosystems	Hs01009003-m1
nrf1	Applied Biosystems	Hs01031046-m1
sod-1	Applied Biosystems	Hs00916176-m1
actin-β	Applied Biosystems	Hs99999903m1

Reactions were run on a QuantStudio 3 (ThermoFisher) with the following conditions: 50°C 2 min, 95°C 20 sec, and then 40 cycles of (95°C 3 sec, 60°C 30 sec). The threshold cycle was determined by the instrument software. The mRNA level of each gene was measured as a CT value and normalized to β-actin as an endogenous control. Values are the mean of triplicate determinations.

RESULTS AND DISCUSSION

We have investigated the effects of four nutraceutical products on six key metabolic activities and how the constituents of the products may be responsible for those effects.

The products were added to the human liver cells (THLE), and the effect on the transcription of the six genes was measured.

Ppar-α (peroxisome proliferator-activated receptor-α):

The effects of three products (Éternel, Vitalité, and Collagène) on the transcription of *ppar-α* are summarized in Figure 1 and Table 2.

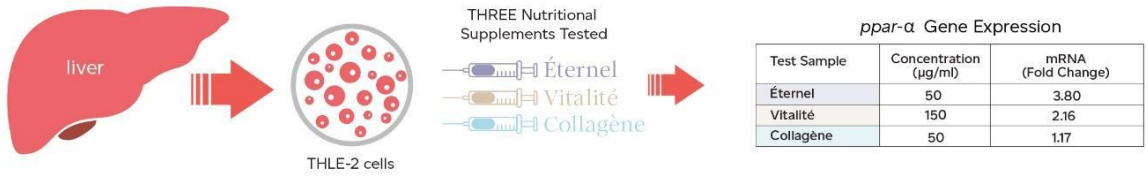


Figure 1. Effects of test samples on transcription of *ppar-α* by human liver cells (THLE)

Table 2. Effects of test samples on transcription of *ppar-α* by human liver cells (THLE)

Test Sample	Concentration (µg/mL)	mRNA (fold change)
Éternel	50	3.80
Vitalité	150	2.16
Collagène	150	1.17

This gene is an important regulator of energy homeostasis. Under conditions of low metabolic energy, it needs to be stimulated. Its elevated expression leads to increased catabolism of fatty acids [22-23].

The product Éternel contains several constituents which are known to stimulate ppar-α production. Most of the major constituents of Éternel have been reported to have a positive effect on the biosynthesis of ppar-α. Glutathione is best known as a free radical scavenger and antioxidant and so can influence the ageing process. This tripeptide has been reported as a stimulator of ppar-α [24]. Three of the fruits in Éternel also have antioxidant activities. Two of them, acai [25], and mangosteen [26] have been reported as activators of this gene. So, these and other fruits present in this product have a positive effect on this gene, which results in a stimulation of energy production.

Coenzyme Q10, which is also a component of Éternel is an essential constituent of mitochondrial energy production. Consequently, if there is a deficit in energy production, supplementation with coenzyme Q10 would be expected to boost the energy. This coenzyme can also boost energy metabolism by stimulating *ppar-α* [27].

Plant-derived lecithin, which is included in Éternel, is known to have a positive effect on *ppar-α* [28]. This nutraceutical contains several constituents that affect the expression of this key gene. It is, therefore, quite conceivable that the strong effect of this product on the biosynthesis of this regulator is either in an additive or synergistic manner.

By way of contrast, Vitalité is composed of multiple constituents including B and D vitamins, a range of 72 trace minerals, 16 amino acids, enzymes, and probiotics. There are also several berries, such as goji, pomegranate, amla, and acai, as well as wheatgrass, kale, spirulina, alfalfa, and fish oil in it. With the exception of acai [29], there is negligible evidence that any of these constituents have a significant effect on the transcription of *ppar-α* As this combination of natural products has such a profound effect on the gene, it might be assumed that the combination of these components may act synergistically to produce such an unexpected significant effect.

Two constituents of Collagène have been shown to be stimulators of producing ppar-α. For some time, ascorbic acid has been known to be a stimulator of it [30]. There are also several reports that pomegranate is an activator of the production of it [31-32].

In summary, these three nutraceutical products substantially stimulate the transcription of this important gene that is concerned with increasing energy production, especially through the oxidation of fatty acids. However, their mechanisms of action would seem to be different. Vitalité does not have any components that have been reported to activate this gene. Consequently, the fact that the product has a positive effect would seem to be due to the interaction between several of these components.

In contrast, Éternel, Vitalité, and Collagène have several constituents that are known activators of *ppar-α*. So, their effects are likely to result from having multiple stimulators in one product.

Glp-1 (glucagon-like peptide-1): This hormone peptide promotes cellular insulin production and inhibits glucagon release [33-34]. This will result in a decrease in blood sugar levels. The control of glp-1 is a current approach to the management of weight and other aspects of metabolic syndrome [35-37]. It also has neurogenic and neuroprotective effects [38]. There is a strong emphasis on using prescription pharmaceuticals such as Ozempic and Mounjaro. While effective, there are side effects such as nausea, diarrhea, and headaches that can arise from their use. Natural products and nutraceutical formulations that can modulate glp-1 expression provide an alternative to these drugs.

Two widely distributed nutraceutical products (Vitalité and Revíve) have been investigated with respect to their effects on the transcription of *glp-1* (Figure 2).

Figure 2. Effects of test samples on transcription of *glp-1* by human liver cells (THLE). The outcomes are summarized in Table 3.

Table 3. Effects of test samples on transcription of *glp-1* by human liver cells (THLE)

Test Sample	Concentration (µg/mL)	mRNA (fold change)
Vitalité	150	3.48
Revíve	50	2.93

The product Vitalité at 150 µg/mL caused a 3.48-fold increase in *glp-1* transcription in cultured liver cells. Of the vitamins in Vitalité, vitamin D has been shown to cause an increase in this peptide (39). Among the trace elements in this product, selenium is a promoter of this key hormone (40-41).

The Central South American acai berry (*Euterpo oleracea*) [42] and the Indian gooseberry (amLa) (*Phyllanthus emblica*) [43] are also known to activate *glp-1* production. So, the effect of this product on the transcription of *glp-1* is not unexpected when the bioactivities of several of its constituents are considered.

Another example of the effectiveness of a nutraceutical on *glp-1* transcription is Revíve. There was a significant increase in the transcription of this gene in liver cells. This product also contains acai, and there is a seminal report by de Bem Costa on the effect of this fruit on *glp-1* production [42]. Among the other components of this product, ginger (*Zingiber officinale*) has a positive effect on *glp-1* [44], and polyphenol resveratrol has a similar effect [45-46].

It is apparent that, although the constituents of Revíve are quite different to those of Vitalité, it has compounds and extracts that are capable of being responsible for the activating effect on *glp-1* expression. So, there is well-established evidence for the effects of these two products on *glp-1*.

Bdnf (brain-derived neurotrophic factor): Bdnf is a modulator of nerve transmission and is important for both learning and memory [47]. It is expressed by several organs, such as the central nervous system and gut [48]. Additionally, it is key to the control of glucose and energy metabolism [49]. For neurodegenerative diseases such as Parkinsons disease, Alzheimer's disease, Huntington's disease, and multiple sclerosis, as well as diabetes, maintaining adequate levels of bdnf is important in the prevention and management of them.

Three nutraceutical products currently on the market were investigated for their effects on the transcription of the *bdnf* gene in human liver cells (Figure 3).

Figure 3. Effects of test samples on transcription of *bdnf* by human liver cells (THLE). The responses are presented in Table 4.

Table 4. Effects of test samples on transcription of *bdnf* by human liver cells (THLE).

Test Sample	Concentration (µg/mL)	mRNA (fold change)
Vitalité	150	8.40
Collagène	150	5.70
Revíve	50	4.30

Pomegranate has been demonstrated to stimulate the expression of *bdnf* [50-51]. It is a constituent of both Collagène and Vitalité. The former at 150 µg/mL caused a strong increase in the transcription of *bdnf* (5.7-fold increase), and the latter at the same concentration produced a substantial increase in the gene expression (8.4 times). Some vitamins also have a positive effect on this gene. Ascorbic acid (vitamin C), a constituent of Collagène is a promoter of it [52]. Vitamin D and a multiple number of B vitamins have been shown to modulate *bdnf* expression [53-57]. These vitamins are active ingredients of the product Vitalité.

Vitalité also contains several components that have been shown to modulate positively the expression of

bdnf. These include spirulina [58-59], wheatgrass [60], and goji berries [61-62].

There are several other extracts and compounds derived from botanical sources that increase *bdnf* expression. These include curcumin [63,64], resveratrol [65-66], ginger [67-68], acai berries [69] as well as the popular shiitake mushrooms [70]. All five of these are constituents of Revíve. So, it is not surprising that this nutraceutical product at 50 µg/mL stimulated the transcription of *bdnf* by 4.3 times.

There is an extensive and diverse range of natural products that can positively affect the expression of this gene. Combining them into individual products results in either an additive or possibly a synergistic modulation of the *bdnf* gene, although the mechanisms involved may not be identical.

Sirt1 (sirtuin 1): The actual functions of sirt1 are not well characterized, but its expression is downregulated in cells that show insulin resistance [71-72]. The sirt1 protein has deacetylation activity as it removes acetyl groups from several key metabolic regulators [73] and the p53 protein [74]. The nutraceutical product Revíve was investigated for its effect on the transcription of *sirt1*. The outcome is summarized in Figure 4 and Table 5.

Figure 4. Effects of test sample on transcription of *sirt1* by human liver cells (THLE)

Table 5. Effects of test sample on transcription of *sirt1* by human liver cells (THLE)

Test Sample	Concentration (µg/mL)	mRNA (fold change)
Revíve	50	1.60

The nutraceutical Revíve at 50 µg/mL elevated the transcription of *sirt1* by 1.6 times. This product is composed of five key ingredients, three of which have been shown to increase sirt1 levels.

These include curcumin [75-76], ginger [77] and resveratrol [78,79]. So, the stimulatory effect of Revíve on the *sirt1* gene expression is supported by the established characterized effects of its major ingredients.

Nrf1 (nuclear respiratory factor 1): The protein that is the product of the *nrf1* gene is a transcription factor that is responsible for regulating several metabolic activities. It is important for the genes involved in respiration as well as DNA transcription by mitochondria [80] and cell proliferation [81]. Additionally, it has a role in the growth of neurites [82] as well as being a critical factor for many biochemical reactions. Two nutraceutical products (Revíve and Vitalité) were investigated for their effects on the transcription of *nrf1* (Figure 5).

Figure 5. Effects of test samples on transcription of *nrf1* by human liver cells (THLE). The outcomes are summarized in Table 6.

Table 6. Effects of test samples on transcription of *nrf1* by human liver cells (THLE)

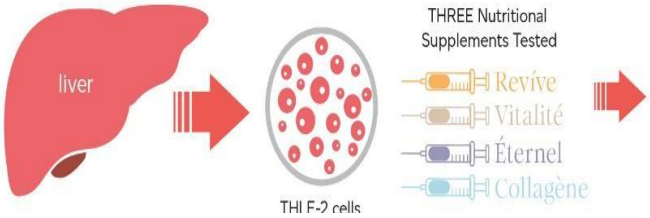
Test Sample	Concentration (µg/mL)	mRNA (fold change)
Revíve	50	2.29
Vitalité	150	2.24

At 50 µg/mL, Revíve also had a significant effect on the expression of *nrf1*. The increase was 2.29 times. Several phytochemicals that are ingredients of this product are known to affect the production of this factor. These include curcumin [83-85], ginger [86], boswellia [87], resveratrol [88-89], and grape seed [90-92]. Consequently, it is not surprising that a product that contains all of these known stimulators can activate the gene expression, as recorded here.

Vitalité at 150 µg/mL produced 2.24 times increase in the expression by the *nrf1* gene. This product has a number of constituents that have been reported to be activators of this gene. The trace elements, selenium and chromium, which are included in the product, have well-established positive effects on the *nrf1* gene [93-96]. Vitamin D, which is important to many biochemical activities and is a constituent of Vitalité, is also a stimulator of *nrf1* [97-98]. Of the botanical components of the nutraceutical product, astragalus [99], alfalfa [100], goji [101-102], and broccoli [103] have been shown to stimulate the expression of *nrf1*.

So, with this combination of known effectors of *nrf1*, it is not unexpected that Vitalité has such an effect on *nrf1* in liver cells.

Sod1 (superoxide dismutase 1): The excessive production of reactive oxygen species has multiple damaging consequences. Oxidative damage to many tissues and organs is a feature of multiple pathologies. Inhibition of this damage is beneficial and is the basis of a number of therapeutic approaches. One of the natural *in vivo* means of achieving this is the enzyme superoxide dismutase. The increase in this enzyme's action is advantageous in the control of the level of reactive oxygen, superoxide, and other detrimental oxygen radicals [104]. All four test products had positive effects on the expression of this gene (Figure 6 and Table 7).



sod 1 Gene Expression

Test Sample	Concentration (µg/ml)	mRNA (Fold Change)
Revíve	50	1.85
Vitalité	50	1.38
Éternel	50	1.23
Collagène	50	1.22

Figure 6. Effect of test sample on transcription of *sod1* by human liver cells (THLE)

Table 7. Effect of test sample on transcription of *sod1* by human liver cells (THLE)

Test Sample	Concentration (µg/mL)	mRNA (fold change)
Revíve	50	1.85
Vitalité	50	1.38
Éternel	50	1.23
Collagène	50	1.22

Acai, which is a constituent of Revíve, has been shown to stimulate *sod1* transcription [69, 105]. As well at least three other constituents have been shown to activate this gene. These include resveratrol [106], ginger [107-108] and curcumin [109-110]. So, the presence of these four natural phytochemicals is likely to be the

cause of the effects of Revíve on *sod1* gene expression.

Several of the components of Vitalité are known stimulators of *sod1*. There are publications demonstrating that both Vitamin B [111-112] and Vitamin D [113-114] activate this enzyme. As it contains the essential minerals selenium and chromium, both of which are well-known to promote the expression of *sod1* [115-116], these are likely to be significant contributors to the effect of Vitalité on this gene's transcription.

This product contains a range of botanical extracts, some of which have been reported to be activators of sod1. These include acai [69,105], kale [117], and spirulina [118-119]. So, there is strong data to provide a rationale for the promotional effects of this product on *sod1*.

Goji and acai are constituents of Éternel and are contributors to the effects of sod1 in the liver cells. Glutathione is also in Éternel, and it is known to activate sod1 [120-121].

Coenzyme Q10, which is also present in Éternel, has multiple bioactivities, including affecting the expression and activity of *sod1* [122-123]. Other constituents that have been demonstrated to have a positive effect on sod1 are mangosteen [124] and collagen [125].

There are at least six components of Éternel that are known activators of sod1, so it is to be expected that the combination of them results in an activation of this gene.

Collagène at 50 µg/mL was able to stimulate the *sod1* gene by 1.22 fold in cultured liver cells. Collagen, which is a major constituent of Collagène, is known to influence sod concentrations [126-127]. This product also contains another well-characterized structural protein, keratin. In 2021 Cheng, Qing *et al*. reported that keratin can increase the activity of sod [128]. Another important component of tissue matrices is hyaluronic acid which is a stimulator of sod1 [129-130].

Two vitamins that are part of this nutraceutical product also influence sod1. Vitamin C (ascorbic acid) has been shown to stimulate superoxide dismutase [131-134]. Vitamin H (biotin) has also been reported to modulate this dismutase [135].

Additionally, pomegranate extract, which has antioxidant properties, has been reported to activate sod1. So, its inclusion in Collagène adds to the stimulation of sod1 production. Collagène, which is composed of matrix constituents as well as two vitamins, has a positive effect on *sod1* transcription. These constituents individually all influence this transcription, so the activation produced by this product can be explained by the properties of its components.

CONCLUSION

In this investigation, the effects of four nutraceutical products on the expression of six important regulatory genes involved in key metabolic processes have been determined. The expression of these genes and their translated products are necessary for the maintenance of healthy organs and tissues. Dysfunction of these genes can lead to disturbances that translate to ill-health, some of which are chronic conditions. Modulation of the expression of these crucial genes, especially in a critical organ such as the liver, can result in the optimization of the biochemical pathways that they control.

This study has clearly shown that combining botanical extracts and phytochemicals that have established bioactivities can produce products that have potent epigenetic effects on the transcription of genes that have essential controlling effects on metabolism. As an outcome, these nutraceutical preparations can be important alternatives in the prevention and amelioration of a number of chronic health conditions.

Having determined the basis for the epigenetic effects of four nutraceutical products using liver cells in culture, it is of considerable importance to confirm these results with in vivo studies. As well, epigenetic effects of other formulations (including prototypes being developed) could be investigated using this approach so as to establish scientific evidence for their efficacies.

Future studies include the investigation of other nutraceutical formulations to regulate the epigenetic function of genes critical for human health both in vitro and in vivo.

List of Abbreviations: BGEM: Bronchial epithelial growth medium; DMSO: dimethyl sulfoxide; EMEM: Eagle's modified essential medium; FAM: Fluorescein amidite; FBS: fetal bovine serum; hACT: human actin; MTT: dimethyl thiazolyl diphenyl tetrazolium; PBS: phosphate buffered saline; VIC: 2'-chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein.

Authors Contributions: C E Davis, P F Davis, and D A Gubler designed the study and prepared the manuscript; C E Davis undertook the experimental work. C E Davis and P F Davis analyzed the data.

Conflicts of Interest: CED and PFD do not have any conflicts of interest. DAG is an employee of THREE International, which provided financial support for this work and the products that were tested.

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