

Putative Prophylaxes of Aloe Vera Juice with L-arginine to Chronic Fatigue Syndrome

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INTRODUCTION

Chronic fatigue syndrome (CFS) remains an elusive health problem, despite of increasing medical and therapeutic studies. Because of no simple test for CFS and diagnosis on clinical evaluation, no single cause for CFS is known and it is generally accepted that CFS develops through the exposure of many convergent factors. According to the American Center for Disease Control (CDC), CFS is defined by "the presence of unexplained persistent fatigue that is not relieved by rest and that results in a substantial reduction in occupational, social and personal activities". The CDC states that four of the following symptoms at least must have been present for a minimum of six consecutive months with a history of previous welling to allow diagnosis of CFS. Short-term memory loss; Difficulties concentrating; Sore throat; Tender neck or armpit lymph nodes; Muscle pain or weakness; Headache; Lost or depressed vision; Visual intolerance to light; Unusual irritability; Un-refreshing sleep; Post-extortional malaise lasting more than 24 hours. Many factors are recognized to contribute to CFS^[1] and many patients and doctors report an improvement in symptoms with dietary supplements. Doctors currently recommend a combination of malic acid, magnesium and calcium for their CFS patients. Since proper magnesium metabolism is dependent on calcium, a magnesium supplement should be balanced with calcium supplement. Malic acid is an intergral component of Krebs cycle that transforms fats and glucose into energy. The combination creates a partnership important to muscle health and is potentially helpful in reducing the muscle pain associated with a deficiency of ATP-intensive nutritions.

It is clear that the immune system is affected in CFS, although a paradox exists. In many suffers there are symptoms of immune

ABSTRACT

L-Arginine is one of the most metabolically versatile amino acids. Several in vitro/in vivo experiments have indicated that exogenous L-arginine intake has multiple beneficial pharmacological and pharmaco-kinetic effects. Such effects include reduction in the risk of vascular and heart diseases and chronic fatigue syndrome. The effects of a dietary supplementation of aloe vera juice with L-arginine to chronic fatigue syndrome were demonstrated and a questionnaire was given to adult subjects. The questionnaire included 10 points regarding their mental, circulatory and muscle functions. Table 1 and 2 summarize the most noteworthy information on supplementation of aloe vera juice with L-arginine. The demonstrated benefits of aloe vera juice with L-arginine show promises to chronic fatigue syndrome in adult subjects.

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Key Words: Aloe Vera Juice; L-arginine Supplement; Bioavailability; Immune Adjuvant and Natural Killer Cells Activity; Nitro Oxide Production; Questionnaire

suppression such as a functional deficiency in natural killer cells responsible for protection against viruses. Stress and environment are also being researched, because of their impact on the immune system. Avoidance of environmental irritants and sensitizing foods that causes an individual allergic reaction are known to relieve symptoms of CFS. The recovery of adrenal function and improvement of CFS symptoms through a connection between Hypothalamic-Adrenal-Pituitary Axis (HPA) activity and CFS symptoms is the most important factor. However, recent prospective studies of high-risk cohorts suggest that there are no HPA axis changing present during the early stages of the genesis of fatigue illness. The investigation presented the case that there is no specific change to the HPA axis in CFS and that the observed changes are of multifactorial aetiology, with some factors occurring as a consequence of the illness. The HPA axis might play a role in exacerbating or perpetuating symptoms late on in the course of the illness^[2].

On the standpoint of putative prophylaxes of aloe vera juice with L-arginine to CFS, clinical discussion on HPA axis and CFS was deleted in the present review. Up-to-dated report of CFS and HPA was summarized in CFS and Stress: A new frontier for treatment by Kresser K^[3].

The efficacies of aloe vera as a super-nutrient to immune imbalances, oxidative stress, and CFS are presented as following: Aloe vera on bioavailability of vitamin C, E, and B12: Effect of aloe vera gel to immune adjuvant and NK cells activity: Effect of aloe vera gel and L-arginine on nitro oxide production: Effect of aloe vera juice to chronic fatigue syndrome. In addition, as one of the nutritional supplements, the ingestion of aloe vera gel juice alone or the juice with L-arginine was carried out by 507 and 2166 volunteers, respectively for three months and the results in a survey of a large number of people by questionnaire were summarized in Table 1 and 2.

ALOE VERA ON BIOAVAILABILITY OF VITAMIN C, E, AND B₁₂

The plasma bioavailability of vitamins C and E were determined in normal fasting subjects, with eight subjects for vitamin C and ten subjects for vitamin E. In a random crossover design, the subjects consumed either 500mg of ascorbic acid or 420mg of vitamin E acetate alone (control), or combined with 2 oz of two different Aloe preparations (a whole leaf extract or an inner fillet gel). Blood was collected periodically up to 24 h after consumption. Plasma was analyzed for ascorbate and tocopherol by HPLC with UV detection. There was no significant difference in the areas under the plasma ascorbate-time curves among the groups sincerely due to large differences within the groups. The Aloes slowed down the absorption of both vitamins with maximum concentrations 2-4 h later than the control. There was no difference between the two types of Aloe. The results indicate that the Aloe improve the absorption of both vitamins C and E. Vinson JA., and his colleagues demonstrated that Aloe is the only known supplement to increase the absorption of both of these vitamins and should be considered as a complement to them^[4].

The effect of two different aloe vera preparations (aloe inner leaf gel[AG] and aloe whole leaf decolorized gel[AL] was compared to placebo on the bioavailability of vitamins C and B₁₂ in healthy human volunteers in a randomized crossover trial by Yun JM. and his colleagues^[5]. Subjects ($n = 15$) received in a random fashion either aloe whole leaf extract(AL with vitamins B₁₂, 1 mg and vitamin C 500 mg) or aloe fillet gel (AG with B₁₂ 1mg and vitamin C 500 mg or water (with vitamin B₁₂ 1mg and vitamin C 500 mg). Blood was obtained fasting, followed by 1, 2, 4, 6, 8, and 24 hr post-ingestion of aloe/wa-

Table 1 Response of Aloe vera juice to chronic fatigue subjects.

Aloe vera juice	Positive efficacy ratio in single respondents	
Fatigue and languid syndrome	$n = 119$	23.5%
Constipation	$n = 108$	21.3%
Skin irritation	$n = 88$	17.4%
A stiff shoulder and muscle pains	$n = 77$	15.2%
A poor blood circulation	$n = 70$	13.8%
Headache	$n = 48$	9.4%

Questionnaire assessment of volunteer 507 subjects of male: 66, female: 423, unknown: 18; age-rangeing from 20th to 70th years old; recovery ratio:13.8% in single respondent from the total 3681, was carried out during September 1 to November 27, 2014. One hundred ml to 500 ml of International Aloe Science Council (IASC)-certificated aloe vera juice (Forever Living Product, Japan) was orally ingested once a day for three months. Questionnaire evaluation was based on the following scheme:1. No change, 2. Slightly better, 3. Very much better, 4. Slightly worse, 5. Very much worse. In order to fairly evaluate the juice, only responses to question No.3 were adopted as positive in each health condition. There was no adverse effect through three months trial period.

Table 2 Response of Aloe vera juice with L-Arginine ingestion to chronic fatigue subjects.

Aloe vera juice with L-Arginine	Positive efficacy ratio in plural respondents	
Fatigue and languid syndrome	$n = 943$	43.5%
Constipation	$n = 490$	22.6%
Skin irritation	$n = 430$	19.8%
A stiff shoulder and muscle pains	$n = 432$	19.9%
A poor blood circulation	$n = 362$	16.7%
Headache	$n = 287$	13.3%

Questionnaire assessment of volunteer 2166 subjects of male:154, female:1974, unknown, 38; age-range from 20th to 70th years old; recovery ratio: 58.8%, in plural respondent in the total 3681, was carried out during September 1 to November 27, 2014. Two hundred ml of IASC-certificated aloe vera juice with 5g of L-arginine powder (Forever Living Product, Japan) was orally ingested once a day for three months. Questionnaire evaluation was based on the following scheme: 1.No change, 2. Slightly better, 3. Very much better, 4.Slightly worse, 5.Very much worse. In order to fairly evaluate the juice, only responses to question No.3 were adopted as positive in each health condition. There was no adverse effect through three months trial period.

ter. When given with vitamins C and B₁₂, AG significantly increased plasma oxygen radical absorbance capacity (ORAC) at both 4 and 24 hr and AL at 4 hr compared to baseline and placebo. AG significantly increased plasma vitamin C at 4, 6, 8, and 24 hr and AL at 4 and 6 hr compared to baseline and placebo ($p < 0.01$). Also both aloes significantly increased serum vitamin B₁₂ levels at 1, and 2 hr compared to baseline and placebo ($p < 0.01$). Thus, AG and AL preparations are safe, well tolerated, and enhance the bioavailability of vitamins C and B₁₂ and antioxidant potential.

Aloe vera and vitamin supplements may play an immunoregulatory role by stimulating the functionality of the natural killer cells (NKC). Toliopoulos I., and his colleagues evaluated the *in vitro* and *in vivo* effects of vitamin C (ranging from 3×10^{-3} M to 3×10^{-9} M) and aloe vera juice (50 μ l), and examined the modulation of the NKC's functionality, taken from blood samples collected from 12 healthy volunteers. The *in vivo* experiments were performed on 15 healthy volunteers, who took supplements of a combination of 1g/day of vitamin C and 50 ml of aloe vera juice for 45 consecutive days. The *in vitro* results showed that both substances increased NKC's cytotoxicity against K562 cancer cell line. Furthermore, in the *in vivo* experiment the cytotoxicity of the NKC's was significantly increased compared to the pre-supplementation values ($p < 0.05$) under all three conditions tested. These results indicate that vitamin C and aloe vera juice can modulate NKC's cytotoxicity and has the potential to enhance the immune system^[6].

The effects of vitamin C and B₁₂ on the regulation of human nasal ciliary beat frequency (CBF) by Jiao J., and his colleagues. At

the concentrations of 0.01%, vitamin C induced an initial increase, followed by a gradual decrease of CBF to the baseline level, while 1.00% vitamin C induced a reversible decrease of CBF. Vitamin B₁₂, at the concentrations of 0.01% and 0.10%, did not influence CBF during the 20-min observation period, while a 1.00% vitamin B₁₂ treatment caused a time-dependent but reversible decrease of CBF. In conclusion, treatment with vitamin C or B₁₂ caused a concentration-dependent but reversible decrease of CBF in cultured human nasal epithelial cells. Therefore, it is necessary to choose a concentration that is safe, effective, and non-ciliotoxic when applying these drugs topically in the nasal cavity^[7].

The polymeric films containing Aloe vera and vitamin E was evaluated to treat wound caused by burns. Around 30% of vitamin E acetate was released from the polymeric films within 12h. The in vivo experiments with tape stripping indicated an effective accumulation in the stratum corneum when compared to a commercial cream containing the same quantity of vitamin E acetate. Vitamin E was found in higher quantities in the deep layers of the stratum corneum when the film formulation was applied. The results obtained by Pereira GG., and his colleagues show that the bioadhesive films containing vitamin E acetate and Aloe vera could be an innovative therapeutic system for the treatment of burns^[8].

Ascorbic acid is taken as a model drug for its high solubility. Different concentrations such as 30, 40, and 50% of matrix tablets of aloe vera powder are made by wet granulation technique using starch paste as a binder. The formulated granules were further subjected to quality control test such as Angle of response, Bulk density, Carr's index and Haser ratio. These matrix tablets are then subjected to in vitro drug release USP dissolution apparatus. The amount of ascorbic acid released from the matrix is estimated by using UV spectrometer and this results is compared with marketed ascorbic acid tablets. Anurupa C., and his colleagues demonstrated that formulation containing 40% matrix was found to be good as compared to other formulations and shows better controlled release of drug^[9].

As biological active constituents isolated from leaf and root of Aloe arborescens var. natalensis, aloenin and magnesium lactate, exhibited an inhibitory action on the gastric juice secretion by Hirata T. and Suga T^[10]. A highly potent cardiotoxic substance, calcium isocitrate, was isolated from Aloe saponaria, using solvent partition, nonionic porous resin, and gel permeation chromatographies. Cardiac stimulant activity of synthesized stereo-isomers of calcium isocitrate was demonstrated in isolated guinea pig atria by Yagi A., and his colleagues^[11].

Dykman KD., and his colleagues reported the effect of nutritional supplements on the symptoms of fibromyalgia (FM) and chronic fatigue syndrome (CFS). Fifty subjects with a physician diagnosis of FM and/or CFS were interviewed using a structured interview form. Subjects had, on their own, consumed nutritional supplements including freeze-dried aloe vera gel extract; a combination of freeze-dried aloe vera gel extract and additional plant-derived saccharides; freeze-dried fruits and vegetables in combination with the saccharides; and a formulation of dioscorea complex containing the saccharides and a vitamin/mineral complex. With medical treatments, approximately 25 percent of FM patients improve, but the beneficial effects of medical treatment rarely persist more than a few months. All subjects in this study had received some form of medical treatment prior to taking the nutritional supplements, but none with enduring success. Nutritional supplements resulted in a remarkable reduction in initial symptom severity, with continued improvement in the period between initial assessment and the follow-up. Further research is needed to verify these results, specially crossover designs in well-defined popu-

lations^[12].

Magnesium and oxidative status were investigated by Cernak I., and his colleagues. In young volunteers exposed to chronic stress or sub-chronic stress consisting of everyday mortal danger in military actions lasting more than 3 months. Significant decreases in plasma ionized Mg²⁺, total Mg²⁺ and ionized Ca²⁺ concentrations were found in both groups. Similarly, both study groups exhibited oxidative stress as assessed by increased plasma superoxide anions and malondialdehyde and modified antioxidant defense. There were no significant differences between the two stress groups. A negative correlation between magnesium balance and oxidative stress was observed suggesting that the same etiological factor (chronic stress) initiate decreases in both free and total magnesium concentrations and simultaneously increase oxidative stress intensity. These findings support the need for magnesium supplementation with antioxidant vitamins for people living in conditions of chronic stress^[13].

Intravenous micronutrient therapy (IVMT), and specifically the Myer's Cocktail, (composed of Mg²⁺, Ca²⁺, vitamin C, B group (B_{5,6} and B₁₂), is a popular approach for treating fibromyalgia syndrome (FMS) among complementary and alternative medicine practitioners, but its efficacy is uncertain. The trial by Ali A., and his colleagues, assessed the feasibility, safety, and provided insights into the efficacy of this therapy. The subjects were 34 adults with American college of rheumatology-defined FMS. Clinically significant improvements were noted (of a magnitude similar to other effective interventions). However, in part because of the high placebo response and the small sample size, no statistically significant differences were seen between groups, in any outcome measure, at 8 and 16 weeks. Statistically significant within-group differences were seen in both the intervention and placebo groups, demonstrating a treatment effect for both IVMT and placebo. At 8 weeks, the IVMT group experienced significantly improved tender points, pain, depression, and quality of life directly following treatment all, $p \leq 0.02$, while the placebo group experienced significantly improved tender points only $p \leq 0.05$. The treatment effects of IVMT persisted at 4 weeks post-intervention for tender points, pain, and quality of life, while placebo effects persisted only for tender points. A single minor adverse event was noted in one subject in the intervention group. In conclusion, this first controlled pilot study established the safety and feasibility of treating FMS with IVMT. Most subjects experienced relief as compared to baseline, but no statistically significant differences were seen between IVMT and placebo. The efficacy of IVMT for fibromyalgia, relative to placebo, is as yet uncertain^[14].

Dysfunction of natural killer activity in a family with chronic fatigue syndrome (CFS) was investigated by Levine PH., and his colleagues. A family was identified with 5 of 6 siblings and 3 other immediate family members who had developed chronic fatigue syndrome (CFS) as adults. All 8 met criteria for the CFS case definition as recommended by the Centers for Disease Control and Prevention. Sixty-eight blood samples were obtained over a period of 2 years from 20 family members (8 affected, 12 unaffected) and 8 normal controls. All blood samples were tested for NK activity in 4-h 51 Cr-release assays and for the number of circulating CD3-CD56(+) and CD3-CD16(+) by flow cytometry. NK activity of the affected immediate family members (cases, $n=8$) was significantly lower ($p=0.006$, two-sided) than that of the concurrently tested normal controls. The results for unaffected family members were intermediate between these two groups, and the pair-wise comparison of unaffected family members to either cases or controls showed no statistically significant difference ($p = 0.29$, two sided). No differences were seen between the groups in the absolute number of CD3-CD56(+) or CD3-CD16(+)

lymphocytes in the peripheral blood. Familial CFS was associated with persistently low NK activity, which was documented in 6/8 cases and in 4/12 unaffected family members. In the family with 5 of 6 siblings who had documented CFS, 2 of their offspring had pediatric malignancies. Low NK activity in this family may be a result of a genetically determined immunologic abnormality predisposing to CFS and cancer^[15].

EFFECT OF ALOE VERA GEL TO IMMUNE ADJUVANT AND NATURAL KILLER (NK) CELLS ACTIVITY

Djeraba A. and Quere P. demonstrated a quick and lasting *in vivo* priming effect of acemannan (ACM), a β -(1,4)-acetylated mannan isolated from Aloe vera, on macrophage response after intramuscular inoculation in chickens (500 μ g/2-month-old bird). In response to IFN- γ *in vitro*, monocytes from ACM treated chickens exhibited a strong enhancement of NO production for splenocytes only day 3 *p.i.* By that time, splenocytes exhibited a strong higher capacity to proliferate in response to the T cell-mitogen PHA. At the same time, the *in vivo* capacity to produce NO, measured by the (NO₂+NO₃) serum level after intravenous LPS injection, increased greatly from 3 to 9 days *p.i.* In conclusion, ACM was able to efficiently and durably to increase the activation capacity of macrophages from the systemic immune compartment (in particular from the blood and spleen after an intramuscular injection) in chickens, especially for NO production. These findings provide a better understanding of the adjuvant activity of ACM for viral and tumoral diseases^[16].

The acute anti-inflammatory activity of Aloe vera leafy exudate (AVL) was evaluated using carrageenan and dextran as phlogistic agents while its chronic anti-inflammatory effect was investigated in a complete Freund's adjuvant-induced model of arthritis. The degree of inflammation in all models was measured plethysmographically. The effect of AVL on nitric oxide production in mouse peritoneal macrophages was measured by the Griess reagent. AVL (25 mg/kg) significantly reduced carrageenan and dextran-induced pedal edema in rats by 61.9% and 61.7%, respectively. In the Freund's adjuvant-induced model of chronic inflammation, AVL showed chronic anti-inflammatory activity but failed to decrease the arthritic index indicating the absence of anti-arthritic activity. AVL (10 μ g/ml) caused a decrease in NO production in macrophages without causing toxicity. In conclusion, AVL possesses acute and chronic anti-inflammatory activity, which is partly mediated by reduced production of NO, which in turn prevents the release of inflammatory mediators^[17].

Chronic Fatigue Syndrome (CFS)/Myalgic Encephalomyelitis (ME) is an etiologically unexplained disorder characterised by irregularities in various aspects of the immunological functions. Brenu EW., and his colleagues investigated natural killer (NK) cell cytotoxic activity, NK cell subsets (CD56brightCD16- and CD56dimCD16+) and cytokines, over the course of a 12 month period in patients with CFS/ME. NK cytotoxic activity was significantly decreased in the CFS/ME patients at T1, T2 and T3 compared to the non-fatigued group. Additionally, in comparison to the non-fatigued controls, the CFS/ME group had significantly lower numbers of CD56brightCD16-NK cells at both T1 and T2. Interestingly, following mitogenic stimulation, cytokine secretion revealed significant increase in IL-10, IFN- γ and TNF- α at T1 in the CFS/ME group. A significant decrease was observed at T2 in the CFS/ME group for IL-10 and IL-17A while at T3, IL-2 was increased in the CFS/ME group in comparison to the non-fatigued controls. Overall cytotoxic activity was significantly

decreased at T3 compared to T1 and T2. CD56brightCD16NK cells were much lower at T2 compared to T1 and T3. IL-10 and IL-17A secretion was elevated at T2 in comparison to T1 and T3. In conclusion, these results confirm decreases in immune function in CFS/ME patients suggesting an increased susceptibility to viral and other infections. Furthermore, NK cytotoxic activity may be a suitable biomarker for diagnosis CFS/ME as it was consistently decreased during the course of the 12 months study^[18].

Campbell KS. and Hasegawa J., reviewed recent discoveries, including a better comprehension of the "education" of NK cells to achieve functional competence during their maturation and the discovery of "memory" responses by NK cells, suggesting that they might also contribute to adaptive immunity. The improved understanding of NK cell biology has forged greater awareness that these cells play integral early roles in immune responses. In addition, several promising clinical therapies have been used to exploit NK cell functions in treating patients with cancer. As a molecular understanding improves, these and future immuno-therapies should be continue to provide promising strategies to exploit the unique functions of NK cells to treat cancer, infections, and other pathologic conditions^[19].

Petrovsky N., and Cooper PD., reported that carbohydrate structures play critical roles in immune system function and have the virtue of strong safety and tolerability record. A number of carbohydrate compounds from plant (such as acemannan and β -glucan), bacteria, and yeast have emerged as promising vaccine adjuvant candidates^[20].

EFFECT OF ALOE VERA GEL AND L-ARGININE ON NITRO OXIDE PRODUCTION

Natural killer (NK) cell function was evaluated by Ogawa M., and his colleagues in 20 subjects with chronic fatigue syndrome (CFS) and compared with that in 21 healthy individuals. In healthy control subjects, NK activity was significantly increased after treatment with L-Arginine (Arg) an NK function enhancer, for 24 h, whereas the same treatment failed to enhance NK activity in the CFS patients. Ogawa's group focused on Arg metabolism, which involves nitric oxide (NO) production through NO synthase (NOS). The expression of inducible NO synthase (iNOS) transcripts in peripheral blood mononuclear cells was not significantly different between healthy control subjects and CFS patients. The Arg-mediated NK cell activation was abolished by addition of NG-monomethyl-L-arginine, an inhibitor for iNOS. Furthermore, incubation with S-nitroso-N-acetyl-penicilline, an NO donor, stimulated NK activity in healthy control subjects but not in CFS patients. In conclusion, these results demonstrate that the Arg-induced activation of NK activity is mediated by NO and that a possible dysfunction exists in the NO-mediated NK cell activation in CFS patients^[21].

Al-Shair K., and his colleagues explored the association between systemic inflammation and symptoms of depression and fatigue in patients with mainly moderate and clinically stable chronic obstructive pulmonary disease (COPD) using a range of inflammatory biomarkers, 2 depression and 2 fatigue scales. One hundred twenty patients with moderate COPD (FEV₁% 52, men 62 %, age 66). were assessed. Depression was assessed using the BASDEC and CES-D scales. Fatigue was assessed using the Manchester COPD-fatigue scale (MCFS) and the Borg scale before and after 6MWT. Systemic TNF- α , CRP, TNF- α -R1, TNF- α -R2 and IL-6 were

measured. A multivariate linear model of all biomarkers showed that TNF- α only had a positive correlation with BASDEC depression score ($p = 0.007$). TNF- α remained positively correlated with depression ($p = 0.024$) after further adjusting for TNF- α -R1, TNF- α -R2, 6MWD, FEV1%, and pack-years. Even after adding the MCFS score, body mass and body composition to the model TNF- α was still associated with the BASDEC score ($p = 0.004$). Furthermore, patients with higher TNF- α level (> 3 pg/mL, $n = 7$) had higher mean CES-D depression score than the rest of the sample ($p = 0.03$). Borg fatigue score at baseline were weakly correlated with TNF- α and CRP, and with TNF- α only after 6MWT. Patients with higher TNF- α had more fatigue after 6MWT ($p = 0.054$). In conclusion, the study indicates a possible association between TNF- α and two frequent and major comorbidities in COPD; i.e., depression and fatigue^[22].

Karaca K., and his colleagues reported that cultures of normal chicken spleen cells and HD11 line cells produce nitric oxide (NO) in response to acemannan (ACM), a complex carbohydrate derived from Aloe vera. Neither cell type produced detectable amounts of NO in response to similar concentrations of yeast mannan, another complex carbohydrate. NO production was dose dependent and inhibitable by the NO synthase inhibitor N^G-methyl-L-arginine. In addition, the production of NO was inhibited by preincubation of ACM with concanavalin A in a dose-dependent manner. These results suggest that ACM-induced NO synthesis may be mediated through macrophage mannose receptors, and macrophage activation may be accountable for some of the immunomodulatory effects of ACM in chickens^[23].

Inducible NO synthase is generally expressed after transcription induction and is known to mediate some of cytotoxic action of activated macrophages. Ramamoorthy L., and his colleague reported that acemannan (ACM) in the presence of interferon- γ , greatly increased the synthesis of NO in RAW264.7 cells. This increase was preceded by increased expression of mRNA for the inducible form of macrophage NO synthase. Preincubation with pyrrolidine dithiocarbamate inhibited the induction, indicating the involvement of nuclear factor-kappa B. These results suggest that ACM causes the activation of macrophages by increasing the level of NO synthase at the level of transcription^[24].

Effect of aloe vera gel was studied by Kaithwas G., and his colleagues on sodium nitrite (SN) induced hypoxia on elevated plus maze with pyritinol as positive control. Aloe vera gel attenuated SN induced memory impairment ($p < 0.001$). To study the effect of aloe vera gel peripheral cholinergic system, concentration response curve of acetylcholine (ACh) was plotted in presence and absence of aloe vera gel. Dose ratio of ACh with aloe vera gel (1 and 2 mg/ml) was found to be 0.846 and 0.692, respectively. This shows muscarinic receptor sensitizing effect of aloe vera gel. Moreover, SN induced elevation of brain acetylcholinesterase (AChE) activity was significantly ($p < 0.05$) lowered by pyritinol (100 mg/mL, p.o.) and aloe vera gel (100 and 200 mg/kg, p.o.), indicating the counteracting action on the cholinergic system. Attenuation of SN induced memory impairment and cholinergic muscarinic receptors sensitization suggest the role of aloe vera gel for inflammatory memory disorders like Alzheimer's^[25].

Pall ML. reported the NO/ONOO-cycle as the central cause of heart failure (HF) in recent review. The NO/ONOO-cycle is a primarily local, biochemical vicious cycle mechanism, centered on elevated peroxynitrite and oxidative stress, but also involving 10 additional elements. All of these elements have causal roles in heart failure. None of the elements of the cycle or the mechanisms linking them are original, but they collectively produce the robust nature of

the NO/ONOO-cycle diseases. Elevated peroxynitrite/NO ratio and consequent oxidative stress are essential to both HF and the NO/ONOO-cycle^[26].

Ohtsuka Y. and Nakaya J., studied 16 elderly patients (mean \pm SD age of 79 ± 7 years; 13 women and 3 men) with cerebrovascular disease who had been living in a nursing home for 2 to 4 years. Patients were treated with oral L-arginine at a dose of 1.6 g/day for 3 months. At baseline, 3 months, and 6 months, they measured cognitive function using the revised version of Hasegawa's Dementia Scale (HDS-R), which widely used in Japan and is comparable with the Mini-Mental State Examination. A perfect score is 30 points, and scores < 20 are considered to reflect dementia. Serum lipid peroxide levels were also measured. Following 3-month treatment with L-arginine, lipid peroxide levels declined from 4.3 ± 0.3 to 4.1 ± 0.3 nmol/ml ($p < 0.001$). Three months later, the mean level had increased to 4.9 ± 1.9 nmol/mL. Cognitive function increased in all patients after L-arginine administration, from a score of 16 ± 8 to 23 ± 7 ($p < 0.0001$), and returned to 17 ± 7 by 3 months after the end of the treatment ($p < 0.0001$ versus after treatment). In general, patients showed more expressive faces and quicker responses. No side effects of L-arginine administration were observed. The authors hypothesize that increases in the concentrations of NO as a neurotransmitter, increases in brain blood flow due to increased NO levels, or reductions in oxidative stress may have resulted in the apparent benefits in cognitive function^[27].

L-Arginine is one of the most metabolically versatile amino acids. In addition to its role in the synthesis of nitric oxide, L-arginine serves as a precursor for the synthesis of polyamines, prolin, glutamate, creatinine, agmatine and urea. Several human and experimental animal studies have indicated that exogenous L-arginine intake has multiple beneficial pharmacological effects when taken in doses larger than normal dietary consumption. Such effects included reduction in the risk of vascular and heart diseases, reduction in erectile dysfunction, improvement in immune response and inhibition of gastric hyper-acidity. Gad MZ. summarized several positive studies and personal experiences of L-arginine in a questionnaire given to the subjects to be completed weekly for 4 weeks in review article, L-Arginine: anti-aging pilot study^[28].

L-Arginine supplementation leads to redirection of AMP deamination on account of increased AMP dephosphorylation and subsequent adenosine production and may increase ATP regulation via activation of AMP kinase (AMPK) pathway. The central role of AMPK in regulating cellular ATP regeneration, makes this enzyme as a central control point in energy homeostasis. Hirstina K., and his colleagues reported that the effects of L-arginine supplementation on energy expenditure were successful independently of age or previous disease, in young sport active, elderly, older population and patients with angina pectoris^[29].

Mariotti F., and his colleagues investigated the kinetics and dose-dependency of dietary arginine utilization for NO compared with urea synthesis and studied the characteristics of the arginine-NO metabolic system in healthy humans. They traced the metabolic fate and analyzed the utilization dynamics of dietary arginine after its ingestion at 2 nutritional amounts in healthy humans ($n = 9$) in a crossover design by using [¹⁵N-¹⁵N-(guanido)]-arginine, isotope ratio mass spectrometry techniques, and data analysis with a compartmental modeling approach. The conversion of oral/dietary arginine into NO is not limited by the systemic availability of arginine but by a tight metabolic compartmentation at the systemic level. They proposed an organization of NO homeostasis in healthy humans^[30].

EFFECT OF ALOE VERA JUICE TO CHRONIC FATIGUE SYNDROME

Nonspecific immunomodulators are substances that induce non-antigen-specific enhancement of the body's native or acquired defense mechanisms. Immunomodulant preparations are most often used for treatment of chronic, viral, or bacterial infection with evidence of secondary immunosuppression. The proposed mechanism of action these products is macrophage activation and subsequent release of cytokines that enhance humoral and cell-mediated immunity. In equine medicine, nonspecific immunostimulant products are used for prevention and treatment of infectious respiratory disease and treatment of sarcoid skin tumors. Rush BR. and Flaminio MJ. reported chronic fatigue syndrome in horses: A case study of Aloe vera gel (acemannan)^[31].

Bloomer RJ., and his colleagues investigated the effects of a dietary supplement (Ambrotose AO containing of an antioxidant blend and a proprietary blend of polysaccharide, such as aloe vera inner gel and etc.) and exercise-induced blood antioxidant capacity and oxidative stress in exercise-trained and untrained men and women. Ambrotose AO at a daily dosage of 4 capsules per day increases resting blood antioxidant capacity and may enhance post exercise antioxidant capacity. However, no statistically detected difference is observed in resting or exercise-induced oxidative stress biomarkers, in quality of life, or in tread mill test time to exhaustion^[32].

Probiotic lactic acid producing bacteria have been shown to prevent and alleviate gastrointestinal disturbances and to normalize the cytokine profile which might be an advantage for patients suffering from chronic fatigue syndrome (CFS). Sullivan A., and his colleagues evaluated the effect of *Lactobacillus lactis* Bb12 on fatigue and physical activity in CFS patients. Fifteen patients fulfilling the criteria set by international researchers in the field at US Centre for Disease Control and Prevention in 1994 for CFS, were included in the study. The patients had high fatigue severity scores and high disability scores. During the first two weeks baseline observations without treatment were assessed, succeeded by four weeks of intake of a probiotic product and a four-week follow-up period. The fatigue, health and physical activity was assessed by the use of the Visual Analogue Scales and the SF-12 Health Survey. Faecal samples were collected and the normal microflora was analysed. Neurocognitive functions improved during the study period while there were no significant changes in fatigue and physical activity scores. No major changes occurred in the gastrointestinal microflora. At the end of the study 6 of 15 patients reported that they had improved according to the assessment described. The findings in this study that improvement of health is possible to achieve should encourage further studies with interventions with probiotics in patients with CFS^[33].

Patients with chronic fatigue syndrome (CFS) and so-called functional somatic disorders have alterations in the intestinal microbial flora. Emerging studies have suggested that pathogenic and non-pathogenic gut bacteria might influence mood-related symptoms and even behaviour in animals and humans. In this pilot study, 39 CFS patients were randomized to receive either 24 billion colony forming units of *Lactobacillus casei* strain Shirota (LcS) or a placebo daily for two months. Patients provided stool samples and completed the Beck Depression and Beck Anxiety Inventories before and after the intervention. They found a significant rise in both *Lactobacillus* and *Bifidobacteria* in those taking the LcS, and there was also a significant decrease in anxiety symptoms among those taking the probiotic vs control ($p = 0.01$). These results lend further support to

the presence of a gut-brain interface, one that may be mediated by microbes that reside or pass through the intestinal tract^[34].

Recent technology to study the gut microbiota have defined new milestones for understanding the microbial ecology of the gastrointestinal ecosystem and assessing how the microbial world within us impacts our everyday life. A novel immune-enhancing polysaccharides and an importance of gut microbiota including gut immunity are discussed on the basis to apply Aloe vera for dietary supplement^[35].

The questionnaire included 10 points regarding their health conditions and the scoring was recorded from top six conditions in Table 1 and 2. Table 1 and 2 demonstrated the personal experiences on administration of aloe vera juice with and without L-arginine under the agreement of the principles of the Helsinki declaration. Specially, the results in Table 2 having an available recovery ratio showed high responses in plural answer. At the end of the study, none of the subjects experienced any side effects or aggravation of health problems from aloe vera juice with L-arginine administration. Table 2 presented the noteworthy informations of aloe vera juice ingestion with L-arginine.

CONCLUSION AND FUTURE PERSPECTIVES

In earlier review articles, we presented aloe vera as bio-stimulant, putative prophylaxes on age-related diseases, and immune modulator^[36,37]. Some of debilitation of body function, such as chronic fatigue syndrome is reflected from shortage of immune sensitivity by aging. In the present review, we put an emphasis on aloe vera juice ingestion with L-arginine as NO-production function, and effect of aloe vera to immune adjuvant and NK cell activity, and bio-availability of vitamins C, E, and B₁₂. NO is an important regulator of physiological processes in the central nervous system. Currently, two pharmaceutical companies: one of which is interested in marketing products of L-arginine with vitamins C and E^[38], and the second company is recruiting participants for phase IV clinical trial of oral L-arginine aspartate in the muscular fatigue of quadriceps, expressed in terms of the fatigue resistance factor^[39]. The results of questionnaire demonstrated that anti-CFS benefits of aloe juice with L-arginine may have potential as putative prophylaxes on Table 2. Aloe vera juice with L-arginine may have a positive effect on cerebral as well as system circulation.

Future investigations on exploration of chronic fatigue syndrome with aloe vera juice with L-arginine, in long-term ingestion influenced by the intestinal microbiota during debilitation by aging are extensively required to verify putative prophylaxes of aloe vera juice with L-arginine.

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CONFLICT OF INTERESTS

The authors declare that they do not have conflict of interests.

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