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A Randomized Placebo-Controlled Crossover trial of Aloe Vera on Bioavailability of Vitamins C and B_{12}, Blood Glucose, and Lipid Profile in Healthy Human Subjects

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ABSTRACT. Several factors limit the absorption and bioavailability of vitamins. Vitamin C, a commonly used water-soluble supplement reduces the risk of disease. Vitamin B_{12} is necessary for the development of RBC, growth, and nervous system. Vitamin B_{12} deficiency is common among elderly. Thus, agents that improve bioavailability of vitamin C and B_{12}, especially in older individuals would be important. Aloe Vera is a botanical with immunomodulatory properties. Aloe is processed using the hand-filleted technique or whole leaf procedure. The aim of this study is to examine the effect of two different aloe vera preparations (aloe inner leaf gel, [AG] and aloe whole leaf decolorized gel, [AL]) compared to placebo on the bioavailability of vitamins, C and B_{12}, in healthy human volunteers in a randomized crossover trial. Subjects ($n = 15$) received in a random fashion either aloe whole leaf extract (AL with vitamins B_{12}, 1 mg and vitamin C 500 mg) or aloe fillet gel (AG with B_{12} 1 mg and vitamin C 500 mg) or water (with vitamin B_{12} 1 mg and vitamin C 500 mg). Blood was obtained fasting, followed by 1, 2, 4, 6, 8, and 24 hours postingestion of aloe/water. When given with vitamins C and B_{12}, AG significantly increased plasma oxygen radical absorbance capacity (ORAC) at both 4 and 24 hours and AL at 4 hours compared to baseline and placebo. AG significantly increased plasma vitamin C at 4, 6, 8, and 24 hours and AL at 4 and 6 hours compared to baseline and placebo ($p < .01$). Also, both

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aloes significantly increased serum vitamin B_{12} levels at 1 and 2 hours compared to baseline and placebo ($p < .01$). Thus, AG and AL preparations are safe, well tolerated, and enhance the bioavailability of vitamins C and B_{12} and antioxidant potential.

KEYWORDS aloe, bioavailability, gel, elderly, vitamins, whole leaf

INTRODUCTION

The number of people who take vitamin supplements is increasing due to a greater awareness of the benefit. In the United States it is currently estimated that between 51 and 61% of the population consumed vitamin supplements (Subar & Block, 1990; Hensrud, Engle, & Scheitel, 1999). The elderly population is greatly increasing in developed nations. This group is especially vulnerable to vitamin deficiency due to age-related decreases in absorption, reduced food intake, and increased drug use (Fairfield & Fletcher, 2002).

Vitamin C (ascorbic acid) is a water-soluble vitamin essential to prevent scurvy. It is a common supplement because there is epidemiological evidence that it reduces the risk of cancer, diabetes, cataracts, and Alzheimer's disease. Vitamin C has been proven to greatly increase the absorption of iron and improves poor iron status (Sandstrom, 2001). A recent report showed that Helicobacter pylori infection significantly impairs the bioavailability of vitamin C (Woodward, Tunstall-Pedoe, & McColl, 2001). Diabetics and patients with end stage renal disease are deficient in vitamin C, and hemodialysis further decreases ascorbate levels (Will & Byers, 1996; Wang, Eide, Sogn, Berg, & Sund, 1999). The recommended amount of vitamin C by the US Government has recently been increased to 75 mg per day for women and 90 mg for men. Smokers should add an additional 35 mg per day because their metabolic turnover of vitamin C is more rapid, as is their rate of oxidative stress (Food and Nutrition Board, 2000). In a comprehensive study of its pharmacokinetics in humans, it was suggested that the amount be increased to 200 mg/day that represent maximum bioavailability (Levine et al., 1996). This amount can be obtained by eating five servings of fruits and vegetables. Because the majority of Americans do not consume five servings (Stables et al., 2002), either a supplement of vitamin C or ingestion of an agent that can increase the bioavailability of vitamin C may be needed.

Vitamin B_{12} (cobalamin) is necessary for the development of RBC, growth and nervous system maintenance. Vitamin B_{12} deficiency is common especially among elderly and increases with age (Clarke et al., 2007). Vitamin B_{12} deficiency can cause serious neurological and neuropsychiatric complications and anemia. The recent Cochrane database review demonstrates the effectiveness of oral B_{12} supplementation as compared to intramuscular administration (Bial, 2006). The Institute of Medicine has recommended that people over 50 supplement their diets with fortified foods or dietary supplements in order to meet the daily requirements of vitamin B_{12}. This is because of high incidence of impaired absorption (Glade, 1999) of vitamin B_{12} in older adults. Thus, agents that improve bioavailability of B_{12}, especially in older individuals and vegetarians would be important.

Aloe vera is a botanical that is commonly used to treat skin infections/wounds and has immunomodulatory properties (Coats & Ahola, 1979; Shelton, 1991; Pugh, Ross,

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ElSohly, & Pasco, 2001). Aloe is processed using the hand-filleted technique or whole leaf procedure. Hand-filleted processing removes the inner gel while avoiding the yellow latex found next to the rind. Whole leaf extracts for consumption are prepared after sterilization and blending by grinding the entire leaf and then removing the bitter yellow component (alloin) by charcoal filtration. A single study has previously reported that aloe preparations improved the absorption of vitamin C (Vinson, Kharrat, & Andreoli, 2003). However, they failed to examine if there were any effects on B-vitamins. Thus, the aim of this study is to examine the effect of two different aloe vera preparations (aloe inner leaf gel, AG, and aloe whole leaf decolorized gel, AL) compared to placebo on the bioavailability of vitamins, C and B_{12}, in healthy human volunteers.

METHODS

Subjects

Healthy control nonsmoking subjects: age 40–70 years, and who do not currently take any vitamins/mineral supplements and who had normal lipids, glucose, renal, liver, and thyroid function ($n = 15$) were recruited after obtaining informed consent. The exclusion criteria were as follows: medical conditions, such as diabetes, cardiovascular disease, inflammatory disorders, abnormal liver/renal function, malabsorption, or steroid therapy; alcohol consumption, smoking, bleeding disorders, or pregnancy. None of the subjects were on any prescription medicines.

Study Design

This was a randomized crossover trial. Randomization was performed by the Investigational Drug Services at UC Davis using a block randomization scheme. Subjects received in a random fashion either aloe whole leaf decolorized gel (AL with vitamins B_{12}, 1 mg and vitamin C 500 mg) or aloe inner leaf gel (AG with B_{12} 1 mg and vitamin C 500 mg) or water (with vitamin B_{12} 1 mg and vitamin C 500 mg) and blood sampling was performed fasting, followed by 1, 2, 4, 6, 8, and 24 hours postingestion of aloe/water. The AL, AG, and water phases were done at least 7 days apart.

Supplements

Vitamin preparations were Good Manufacturing Practices (GMP) pharmaceutical grade in order to assure precise delivery of amount qualified and provided by Progressive Vitamins. There were two aloe preparations: AL and AG that were provided by International Aloe Science Council (IASC) in gallon containers and subjects were given 30 mL of water with vitamins or 30 mL of aloe whole leaf filtered solution or 30 mL of aloe gel fillet solution after fasting blood was withdrawn at the three visits.

The two aloe products were provided by the International Aloe Science Council (Irving, TX, USA) and investigators were blinded to randomization. These products were certified following testing for total solids, calcium and magnesium content, and L-malic acid (a marker for processing in a timely fashion without excess heat, time, and pressure). Nuclear magnetic resonance (NMR) was used to detect acetylated polysaccharides, acids, and suspected adulterants and the products were found to contain 95% aloesin.

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The inner leaf gel product is designated AG and the whole leaf decolorized gel as AL. Both were kept in a refrigerator until use.

All studies took place at the General Clinical Research Center (GCRC) at UC Davis after obtaining written informed consent from the participants. Study days were at least 7 days apart. On study days, each volunteer had a baseline blood sample taken after an overnight fast. Gel drinks or water (30 mL) were sipped slowly over 5 min. Further blood sampling was performed at 1, 2, 4, 6, 8, and 24 hour postdosing. Subjects were provided a bagel for breakfast, a light lunch that did not contain any vitamin C. At each time point, 15 mL of blood was drawn for a total of 105 mL over 24 hours. Ethylene diammine tetra acetic acid (EDTA) plasma was stored in metaphosphoric acid for estimation of vitamin C as described previously (Fuller, Grundy, Norkus, & Jialal, 1996).

Oxygen radical absorbance capacity (ORAC) was performed on samples taken at time 0, 4, and 24 hours as reported previously (O'Byrne, Devaraj, Grundy, & Jialal, 2002) to determine if aloe preparations alone enhanced ORAC values in the plasma and to determine if the vitamin ORAC values are enhanced by aloe preparations.

Samples were evaluated for blood glucose, blood lipids, plasma vitamin C, and vitamin B_{12} taken at time 0, 1, 2, 4, 6, 8, and 24 hours to determine if aloe preparations have any influence on these parameters.

Other Methods

Lipid profile and blood glucose levels were performed by routine laboratory techniques. Vitamin B_{12} assays were performed in the Clinical Laboratory at UC Davis Medical Center.

Statistical Analyses

ANOVA followed by *t* tests for parametric data and Wilcoxon signed rank for non-parametric data. Area under the curve was computed using GraphPad Prizm software.

RESULTS

The average age of the subjects in the study was 55 ± 10 years with an average body mass index (BMI) of 23 ± 4 kg/sqm. AG and AL preparations were well tolerated by subjects and none of them experienced any side effects. With regards to the lipid profile, AG and AL preparations did not show any significant differences compared to placebo [Figures 1(A) and (B)]. Also, because aloe contains several saccharides, which may affect glucose levels, we tested the effect of the aloe preparations on blood glucose levels, and they were no significant differences compared to the Placebo (Figure 2).

When given with vitamins C and B_{12}, AG significantly increased plasma ORAC at both 4 and 24 hours and AL significantly increased plasma ORAC at 4 hours compared to baseline and placebo (Figure 3). When given with vitamins C and B_{12}, AG significantly increased plasma vitamin C at 4, 6, 8, and 24 hours and AL significantly increased plasma vitamin C at 4 and 6 hours compared to baseline and placebo [Figure 4(A), Area under the curve (AUC): 1.4-fold for AL; 2.1-fold for AG; $p < .01$ compared to placebo].

Furthermore, with regards to vitamin B_{12} levels, when given with vitamins C and B_{12}, AL and AG significantly increased serum vitamin B_{12} levels at 1 and 2 hours compared

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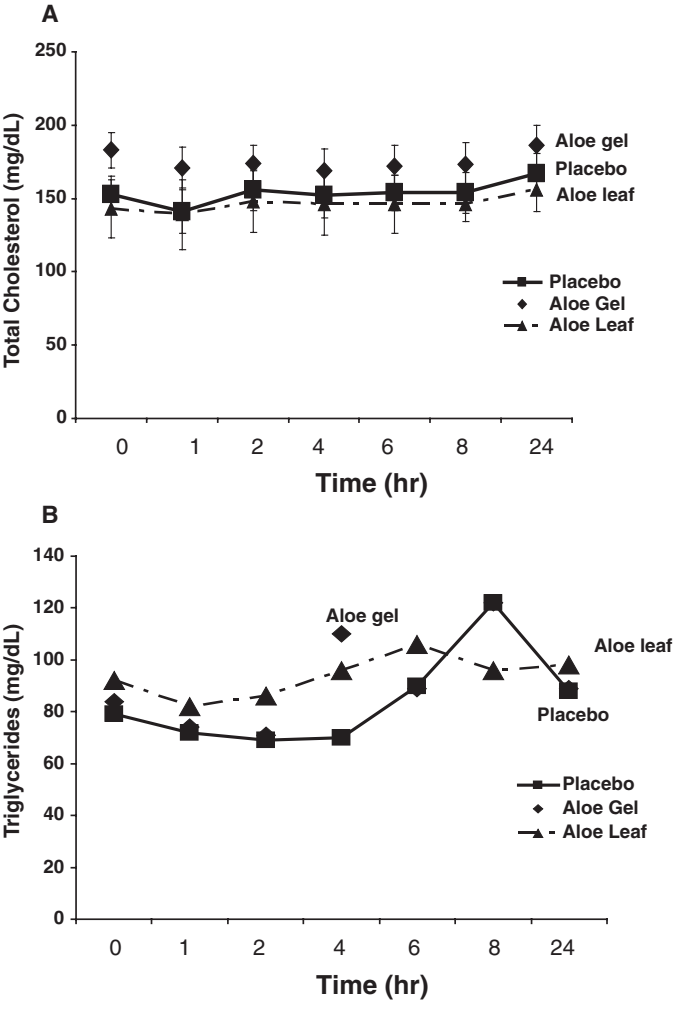
FIGURE 1. Effect of AG and AL on total cholesterol (A) and total triglyceride levels (B): subjects were given placebo with vitamins, AG with vitamins, or AL with vitamins and blood was obtained at baseline and after 1, 2, 4, 6, 8, and 24 hours of ingestion and total cholesterol/triglycerides was measured as described in methods. Data are expressed as mean ± S.D.



to baseline and placebo [Figure 4(B), AUC: 1.6-fold for AL; 1.8-fold for AG; $p < .01$ compared to placebo].

DISCUSSION

Increasing numbers of the US population consume vitamin supplements. There is also an increase in the elderly population and those that are adopting a healthy vegetarian diet, these groups are especially vulnerable to vitamin deficiencies. Aloe vera is a botanical that has been commonly used to treat skin infections/wounds and has

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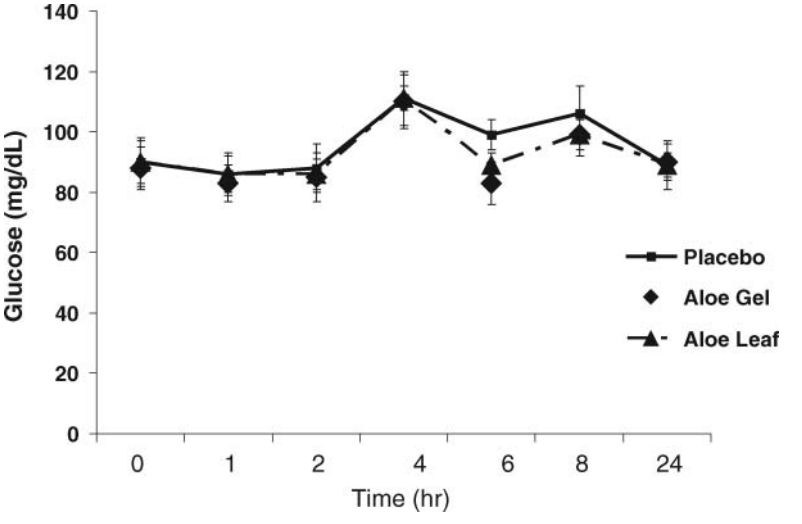
FIGURE 2. Effect of AG and AL on glucose levels: subjects were given placebo with vitamins, AG with vitamins, or AL with vitamins and blood was obtained at baseline and after 1, 2, 4, 6, 8, and 24 hours of ingestion and glucose levels were measured as described in methods. Data are expressed as mean ± S.D.



immunomodulatory properties. A single study has previously reported that aloe preparations improved the absorption of vitamin C (Vinson et al., 2003). However, they failed to examine if there were any effects on B-vitamins. In this study, we examined the acute effect of two different aloe vera preparations (AG and AL) compared to placebo on the

FIGURE 3. Effect of AG and AL on ORAC: subjects were given placebo with vitamins, AG with vitamins, or AL with vitamins and blood was obtained at baseline and after 4 and 24 hours of ingestion and ORAC was measured as described in methods. Data are expressed as mean ± S.D. $^{*}p < .001$ compared to baseline; $^{\#}p < .05$ compared to baseline.

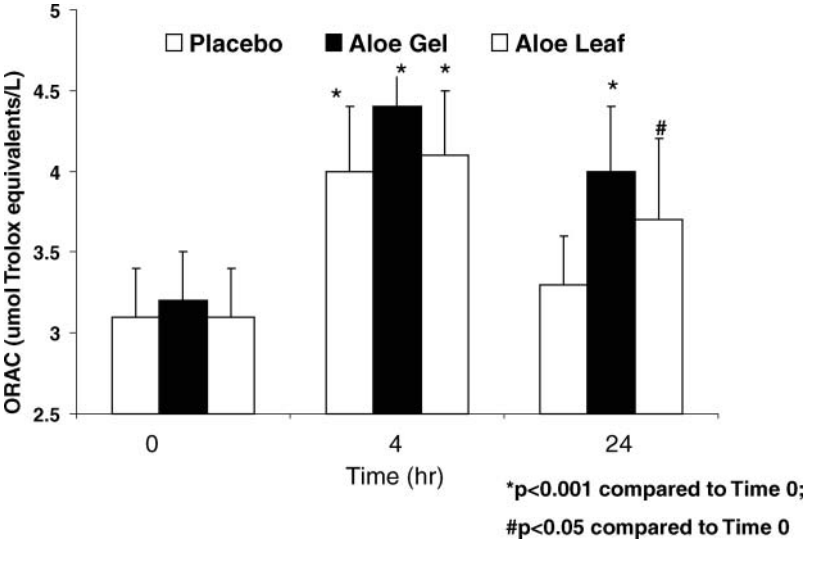


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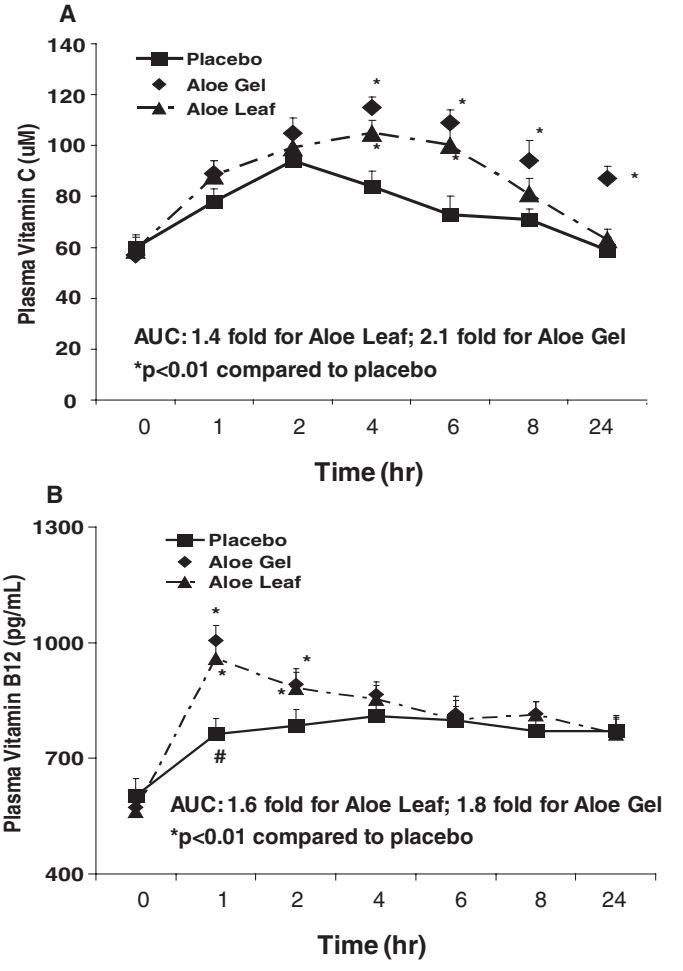
FIGURE 4. Effect of AG and AL on plasma vitamin C (A) and plasma vitamin B_{12} (B): subjects were given placebo with vitamins, AG with vitamins, or AL with vitamins and blood was obtained at baseline and after 1, 2, 4, 6, and 24 hours of ingestion and plasma vitamin C/vitamin B_{12} was measured as described in Methods. Data are expressed as mean ± S.D. Area under the curve was calculated using GraphPad Prizm software $*p < .01$ compared to placebo.



bioavailability of vitamins, C and B_{12}, in healthy human volunteers and report that while both aloe preparations improved the bioavailability of the vitamins, AG was superior to AL.

In order to assess the effect of aloe on oxidative stress and bioavailability of water-soluble antioxidant vitamins, we examined plasma ORAC. When given with vitamins C and B_{12}, AG significantly increased plasma ORAC at both 4 and 24 hours and AL significantly increased plasma ORAC at 4 hours compared to baseline and placebo. Aloes are known to contain antioxidants (Lee, Weintraub, & Yu, 2000). Previously, it has been shown that treatment of mice with low doses (30 to 60 μL/day) resulted in a reduction of lipid peroxides and an increase in thiols in vivo (Singh, Dhanalakshmi,

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& Rao, 2000). The aloe polysaccharides are also antioxidants and have been shown to reduce oxidative DNA damage in mouse liver cells (Kim, Kacew, & Lee, 1999). In this report, we show for the first time in humans, that AL and AG resulted in a significant antioxidant effect, via upregulation in plasma ORAC and it is possible that this may be directly because aloe is an antioxidant or indirectly, by improving the bioavailability of water soluble antioxidants such as vitamin C and flavonoids.

With regards to bioavailability of vitamin C, comparing the areas under the curves, AG improved the bioavailability of vitamin C two-fold when compared to control and it stayed longer in circulation (24 hours) compared to AL that was also effective in improving the bioavailability of vitamin C compared to control. Furthermore, with regards to the bioavailability of vitamin B_{12}, AG and AL significantly increased serum vitamin B_{12} levels at 1 and 2 hours compared to baseline and placebo, again, with the AG improving bioavailability of vitamin B_{12} more than AL. While the mechanisms by which it improves the bioavailability of these vitamins is not understood, previously, both the aloes have been shown to act as time-release agents for vitamin C. The factors that affect ascorbate bioavailability have recently been reviewed (Bates, 1997). The photochemical in the aloes may protect ascorbate from degradation in the intestinal tract. Furthermore, the polysaccharides in the aloe have been shown to bind to the mucosal cells in the stomach (Yagi et al., 2001) and also may bind to the ascorbic acid and slow down its absorption, thereby increasing its bioavailability. Also, binding of aloe polysaccharides to the mucosa may improve absorption of vitamin B_{12}, resulting in its increased bioavailability. Future studies will examine if aloe polysaccharides can function like intrinsic factor and thus improve vitamin B_{12} bioavailability. Lastly, due to its antioxidant effect, aloe may preserve levels of ascorbate in circulation.

Thus in conclusion, we show for the first time in humans, in an acute study, when compared to the controls, both aloes significantly improved bioavailability of vitamins C and B_{12} and AG appears to be better than the AL preparation. Furthermore, we show for the first time, a significant improvement in plasma ORAC with the aloe preparations. Future studies will examine mechanisms for these effects and also involve long-term supplementation with aloe and examine antioxidant and immunomodulatory properties. AL and AG preparations are safe, well tolerated, and enhance the bioavailability of vitamins C and B_{12} and antioxidant potential. This could have major implications with regards to supplementation of vitamins/supplements to target chronic diseases, especially in older populations.

Declaration of Interest: The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

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