Safety and Efficacy of two dietary supplements, Immuno-Viva™ and Immune Lift™ used as Free Radical Scavengers in Healthy Subjects.

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Abstract

Chronic vascular disease among other things is linked to systemic stress, production of free radicals, and a reduced antioxidant defense that may ultimately lead to tissue damage and related cardiovascular complications. Administration of dietary supplements with antioxidant properties have shown to be beneficial in protecting against free radical effects.

Immuno-Viva™, a blend of cold pressed pure, black raspberry and black cumin seed oils meeting FDA DSHEA Regulations, has shown significant free radical scavenging properties in preliminary animal studies; however, supportive evidence using controlled clinical trials are not yet available. Immune Lift™, a powder product from the cold press procedure containing black raspberry and black cumin, has not been studied extensively.

The components of the two products are generally accepted as safe food ingredients or have been evaluated in animal models; however, no safety data on the final product formulations are available.

The primary aim of this study is to examine product safety in respect to maintained normal liver and kidney functions in healthy participants. Levels of ALT, AST and creatinine were measured. Secondary aims included evaluating possible effects of these oils on free radical levels immune response mechanisms. Total blood counts and lymphocyte fractions as well as malondialdehyde in urine were measured.

The study involved 30 participants consuming 3 teaspoons (15 g) of Immuno-Viva™ daily for one month, and an additional 2 tablets of Immune Lift™ for one additional month. Measurements and blood sampling was conducted on days 0, 30 and 60. The design is pre and post oil effects.

The hypothesis is that Immuno-Viva™ and Immune Lift™ will have no adverse effects on normal liver and kidney functions and support the immune response mechanisms to control free radical effects.

Table of Contents

List of Tables	(v)
Background and Significance	(1-3)
Population and Sample Selection	(3)
Study Overview	(4)
Protocol	(5-6)
Measurement Grid	(7)
Analytical Methods	(7)
Statistics	(8)
Results and Discussion	(8-10)
Investigator Biographies	(11)
Bibliography	(12)

List of Tables

Table 1 Proportions of (Normal Values/Total Sample) (vi)

Table 2 Characteristics, safety, CRP, and lipid profile data (vii)

Table 3 Hematology, immune-chemistry and urine data (viii)

Table 1. Proportions of (Normal values / Total Sample)

	Baseline	Day 30	Day 60
ALT	28/29	28/29	26/27
AST	28/29	28/29	26/27
Creatinine	29/29	29/29	27/27
Bilirubin	28/29	29/29	26/27
Eosinophils	27/29	27/29	26/27

Table 2. The Entire Cohort

Characteristics, safety, CRP, and lipid profile data on days 0, 30 and 60.

	Group means			Group differences			
	D0 N=29	D30 N=29	D60 N=27	D30-D0	D60-D30	D60-D0	F-value P-value
BMI (kg/m^2)	28.3 (4.7)	28.2 (4.6)	28.2 (4.7)	-0.1	0	-0.1	0.49 .616
BPsystolic (mmHg)	110.1 (13.3)	106.9 (12.8)	108.8 (15.0)	-3.2	1.9	-1.3	1.71 0.192
BP diastolic (mmHg)	70.6 (10.9)	67.0 (9.4)	69.3 (8.9)	-3.6	2.3	-1.3	4.03 0.024
Bilirubin (mg/dl)	0.9 (.23)	0.8 (.24)	0.9 (.22)	-0.1	0.1	0	0.79 0.459
ALT (IU/l)	23.8 (20.8)	23.1 (20.1)	25.9 (34.2)	-0.7	2.8	2.1	0.74 0.484
AST (IU/l)	25.3 (15.6)	24.3 (12.7)	25.6 (19.6)	-1	1.3	0.3	0.56 0.573
Creatinine (mg/dl)	0.9 (.14)	1.0 (.17)	0.9 (.15)	0.1	-0.1	0	4.26 0.019
Triglyceride (mg/dl)	92.4 (46.1)	88.9 (39.0)	98.6 (49.3)	-3.5	9.7	6.2	1.75 0.186
Cholesterol (mg/dl)	187.0 (34.5)	182.2 (31.1)	183.1 (27.8)	-4.8	0.9	-3.9	1.11 0.337
HDL (mg/dl)	55.0 (20.8)	53.7 (20.1)	54.6 (20.5)	-1.3	0.9	-0.4	0.75 0.479
LDL (mg/dl)	113.6 (28.7)	111.1 (28.1)	108.6 (28.4)	-2.5	-2.5	-5	1.63 0.206
VLDL (mg/dl)	18.5 (9.3)	17.7 (7.7)	19.8 (9.9)	-0.8	2.1	0.3	1.99 0.147
CRP	2.5 (2.7)	2.9 (2.7)	2.5 (3.3)	0.4	-0.4	0	1.07 0.350

Legend:
D0 : Baseline values (means with (standard deviations)) prior to the intervention.
D30: Values obtained after 1 month of Immuno Viva intervention.
D60: Values obtained after 1 month of Immuno Viva + Immune Lift intervention.

Table 3. Entire Cohort

Hematology, immuno chemistry and urine data on days 0, 30 and 60.

	Group means			Group differences			
	D0 N=29	D30 N=29	D60 N=27	D30-D0	D60-D30	D60-D0	F-value P-value
WBC (k/cmm)	5.9 (1.6)	5.9 (1.3)	5.6 (1.4)	0	-0.3	-0.3	1.23 0.302
Neutrophil (%)	60.6 (7.2)	60.4 (7.7)	58.4 (7.3)	-0.2	-2	-2.2	2.58 0.085
Lymphocyte (%)	27.5 (7.1)	27.8 (7.6)	29.4 (7.0)	0.3	1.6	1.9	2.37 0.103
Monocyte (%)	8.5 (3.0)	8.6 (2.2)	8.5 (2.2)	0.1	-0.1	0	0.15 0.860
Eosinophil (%)	2.7 (1.4)	2.6 (1.4)	3.2 (1.8)	-.01	0.6	0.5	5.35 0.008
Basophil (%)	0.6 (.32)	0.6 (.31)	0.50 (.36)	0	-0.1	-0.1	1.80 0.176
CD3	73.5 (5.7)	74.0 (5.5)	73.6 (5.4)	0.5	-0.4	0.1	0.74 0.482
CD4	50.7 (6.9)	51.2 (7.2)	50.5 (7.7)	0.5	-0.7	-0.2	0.76 0.471
CD8	21.1 (6.1)	21.8 (5.9)	21.6 (5.9)	0.7	-0.2	0.5	1.11 0.336
CD19	14.7 (4.1)	14.8 (3.9)	15.0 (3.9)	0.1	0.2	0.3	0.35 0.708
NK	10.6 (5.2)	10.0 (4.8)	10.6 (4.3)	-0.6	0.6	0	0.59 0.559
CD4:CD8	2.6 (1.0)	2.6 (1.1)	2.6 (1.1)	0	0	0	0.42 0.656
Malonyldiald.	2.6 (1.5)	2.9 (1.3)	3.0 (1.3)	0.3	-0.1	0.4	0.64 0.531
CD3absol	241.2 (144)	251.8 (159)	253.7 (139)	10.6	1.9	12.5	0.64 0.529

Legend:
D0 : Baseline values (means with (standard deviations)) prior to the intervention.
D30: Values obtained after 1 month of Immuno Viva intervention.

D60: Values obtained after 1 month of Immuno Viva + Immune Lift intervention.

WBC: White Blood Cell Count

NK: Natural Killer Cell

Background and Significance

Public health in the United States is characterized by highly prevalent chronic disease conditions. More than 90 million Americans live with chronic illnesses which result in 70% of the deaths in the United States each year. The medical care costs of people with chronic diseases account for more than 75% of the nation's $1.4 trillion medical care costs. [1] Many of these conditions are linked to systemic stress and production of free radicals, reduced antioxidant defense, and attenuated nitric oxide synthase (NOS) activity. [2]

Free radicals (oxidation) are highly charged molecules with open negative charges which are involved with cell and tissue damaging biochemical reactions. [2] Of particular interest are lipid peroxidation reactions resulting in an increase in LDL cholesterol as well as a reduction of the compliance of the arterial walls by increased cross-linking between cells. [3]

Previous work by Niki et al has shown that various antioxidants with different functions inhibit lipid peroxidation and the deleterious effects caused by the lipid peroxidation products. [3] Work performed by Hillyer et al demonstrated in laboratory procedures that black raspberry seed oil has strong free radical quenching activity. [4]

The biological roles of lipid peroxidation products have recently received a great deal of attention, but its clinical significance must be demonstrated in future studies. [3] Animal studies on antioxidants have demonstrated that treatment initiated to reduce oxidative stress prevents the age-associated development of high blood pressure in genetically hypertensive rats. [5]

Clinical trials with humans focusing on antioxidants and cardiovascular disease have been unsuccessful due to the dose-dependent effects of antioxidants, for example studies on vitamin E.[6] Weinberg's review of clinical trials found correlation between dosage of antioxidant consumption and the effect of reduction in cholesterol for hyperlipidemic mice and rabbits. [6]

The antioxidant supplements, Immuno-Viva™ and Immune Lift™, investigated in this study are a pure blend of black raspberry and black cumin seed oils using a patented Nature FRESH-Cold Press™ processing technology. Both products are manufactured by Botanic Oil Innovations, Inc., Spooner, Wisconsin, and the Immuno-Viva™ product has been commercially available for two years. The patented process is carried out in an oxygen deprived nitrogen/carbon dioxide atmosphere which optimizes nutrient yield. The process does not involve solvents, bleaching agents or high temperatures. [7, 8]

Further preliminary pilot tests have evaluated a possible synergistic effect of Immuno-Viva™ oil and an attenuated Salmonella Typhimurium that synthesizes interleukin 2 (IL-2). It was shown that the IL-2 reduced hepatic metastases by 58% in mice, and an additional 30% reduction in metastases was found including the oil. [9]

Given the lack of safety data in healthy individuals, the present uncontrolled study was conducted to evaluate the safety of the final product formulations of Immuno-Viva™ and Immune Lift™ in respect to maintained normal liver and kidney functions. The study also served to provide preliminary data on free radical levels and immune response reactions elicited by the free radicals.

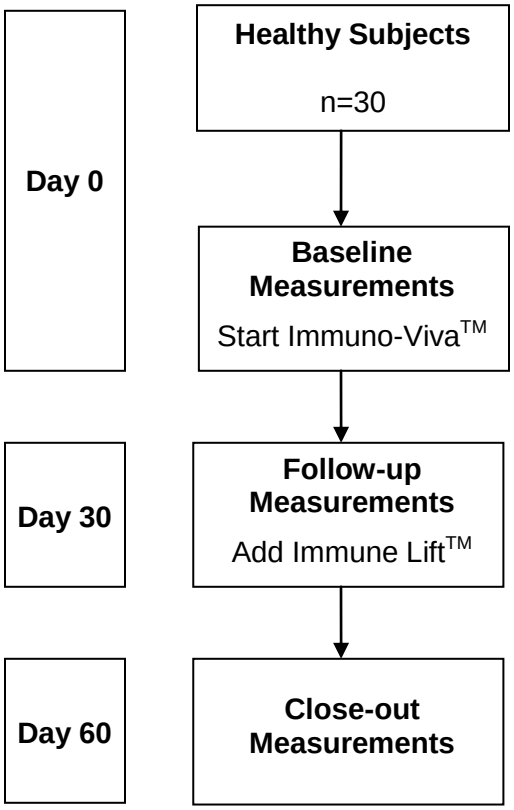
Population and sample selection.

The Minneapolis-St Paul area population was over 2.8 million in the 2000 census. The gender distribution is 51% female and 49% male, and ethnicity distributions were roughly: White 85%, African American 6%, American Indian 0.7%, Asian 4.5%, Hispanic 4%, Pacific Islander 0.05%, Other 4%.

Primary population sources were employees and former study participants at the Berman Center for Outcomes and Clinical Research. Additional sampling was sought among relatives and friends of the primary enrollments.

Study Overview

Thirty potential participants were screened. Thirty participants were eligible and initiated the study. One participant never started the procedure and returned the study oil unused. Two participants discontinued the study after one month for medical or personal reasons. Thus, twenty nine participants completed month one of the study and twenty seven participants completed the entire study.



Protocol

Day 0 the prospective participant visited the Berman Center for Outcomes and Clinical Research after an overnight fast of at least 10 hours and not more than 12 hours.

The investigator contacted the participant by telephone to verify adherence to fasting prior to arriving at the clinic.

Baseline measurements and procedures

Informed Consent and HIPAA forms, approved by the Hennepin County Medical Center and University of Minnesota Institutional Review Boards, were reviewed and signed and medical history and current medication log was obtained. The following measurements were performed:

- Body weight using a calibrated lever scale
- Body height using a statiometer
- Blood pressures and pulse rate using a digital blood pressure monitor (HEM-907, Omron, Vernon Hills, IL) (Three consecutive measurements after a five minutes rest in the sitting position).

A blood sample was taken and analyzed later for alanine transaminase (ALT), aspartate aminotransferase (AST), creatinine, Complete Blood Count (CBC), bilirubin, C-reactive protein, lipids, and lymphocytes using analytical methods described below, at the Hennepin Faculty Associates (HFA) Laboratory and the

University of Minnesota. A urine sample was obtained and analyzed for malondialdehyde (MDA) at the Berman Center. The participant received the Immuno-Viva™ product and instructions.

Days 1-29 Participants were instructed to consume 1.5 teaspoons (7.5 g) of oil twice a day in combination with 8 ounces (2.5 dl) of water. No other dietary or life style changes were prescribed.

Day 30 Participants were seen at the Berman Center after overnight fasting and it was important that no oil had been consumed 10-12 hours prior to arrival at the clinic. The procedure from day 0 was repeated. At this visit the participant was also given the Immune Lift™ product and instructions.

Days 30-59 Participants continued the daily routine, consuming 1.5 tsp of Immuno-Viva™ twice a day in combination with water. However, two tablets of Immune Lift™ were added in the morning only. No other dietary or life style changes were recommended.

Day 60 Participants attended a clinic visit at the Berman Center after fasting overnight and no oil or tablets had been consumed 10-12 hours prior to arrival at the clinic. The procedures from day 0 (baseline) were repeated. The measures done at each visit are detailed in the grid below.

Measurement Grid

	Day 0	Day 30	Day 60
	Baseline Visit	Follow-up Visit	Close-out Visit
Body Height	X		
Body Weight	x	x	x
Blood Pressure	x	x	x
Blood Sample	X	X	X
CBC w/differential	x	x	x
Lymphocyte	x	x	x
ALT	x	x	x
AST	x	x	x
Creatinine	x	x	x
Total Bilirubin	x	x	x
Cardiac C-reactive protein	x	x	x
Lipid Profile	x	x	x
Urine Sample	X	X	X
Malondialdehyde (urine)	x	x	x

Analytical Methods

CBC w/differential	Flow Cytometry
Lymphocyte	Multi-color Immunofluorescence and Flow Cytometry
ALT (SGPT)	Rate reflectance spectrophotometry
AST (SGOT)	Rate reflectance spectrophotometry
Creatinine	Rate reflectance spectrophotometry
Malondialdehyde	Colorimetric Assay (Visual reading)
Bilirubin	Spectrophotometry
Cardiac C-reactive Protein	Enhanced Turbometric Immuno Assay
Lipid Profile	Colorimetric rate reflectance spectrophotometry

Statistics

The endpoint data obtained on days 0, 30, 60 was examined for normal distribution in order to determine the appropriate statistic to apply. The data was interpreted using ANOVA statistics for continuous data, and significant differences were sought at the alpha=0.05 level with a power of 80%.
The detectable differences of the variables were calculated according to the formula:

$$D^2 = (z_{a/2} + z_b)^2 * s^2 * (1/n_1 + 1/n_2) * DEFF$$

Where D is the detectable difference, $z_{a/2}$ and z_b are the critical points on the Gaussian distributions for the type 1 and 2 error rates, s is the estimated standard deviation of the variable, n_1 and n_2 are the sample sizes at days 30 and 60, and DEFF is the variance inflation factor for the particular variable.

Results and Discussion

Our primary outcome was to look at the safety of Immuno-Viva™ and Immune Lift™. Table 1 shows the proportions of individuals who were in the normal ranges for the safety outcomes. The individual who had out of normal range values for ALT and AST at baseline was the same individual who had out of normal range values at day 30 and day 60. Creatinine was found to be statistically significantly higher than baseline values at day 30 and then

normalized to baseline values by day 60. Table 1 shows none of the participants went over the upper boundary of normal for creatinine. Therefore, we conclude that Immuno-Viva™ and/or Immune Lift™ are safe dietary supplements for healthy individuals.

Our secondary outcomes were collected to look at trends for future research studies. Repeated measure ANOVA was performed on each variable for the entire cohort Tables 2 and 3 summarize the results obtained from these analyses.

Looking at the entire cohort, we found a statistically significant difference in one of our primary outcomes and two of our secondary outcomes. As was mentioned above, creatinine as a continuous mean level increased significantly over the course of the study but categorically all individual values were within normal safe ranges. Eosinophils increased significantly for the whole population over the course of the study but the proportion of those in normal range remained constant from 27/29 at baseline to 27/29 at day 30 to 26/27 at day 60 (Table 1). Therefore, we can conclude that Immuno-Viva™ and/or Immune Lift™ are safe to take in the amounts given.

Table 2 shows that diastolic blood pressure dropped significantly from baseline to day 60. This is intriguing to the investigators as a potential future research

question looking at hypertensive individuals and endothelial dysfunction associated with free oxygen radicals by affecting nitric oxide levels.

This research project determined it is safe to use Immuno-Viva™ and Immune Lift™ as dietary supplements in healthy individuals and suggests directions in the area of hypertension and cholesterol reduction for future research with these products.

Biographical Paragraph

Dr. Grimm is the Medical Director for the Berman Center for Outcomes and Clinical Research and a faculty member at the University of Minnesota. He can be reached at grimm001@umn.edu.

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