

## LETTER TO THE EDITOR

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### WHEAT GRASS SUPPLEMENTATION DECREASES OXIDATIVE STRESS IN HEALTHY SUBJECTS: A COMPARATIVE STUDY WITH SPIRULINA

Dear Editor,

Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense in different human diseases. Supplementation using natural antioxidants as functional foods such as wheat grass (*Triticum aestivum*) and Spirulina (*Spirulina maxima*) holds great promise in overcoming the ill effects of oxygen toxicity.

Hanninen et al. reported that humans consuming uncooked vegan food called living food including wheat grass juice showed increased levels of carotenoids, vitamins C and E, and lowered cholesterol concentration in their sera.<sup>1</sup> Wheat grass juice appeared effective and safe as a single or adjuvant treatment of distal ulcerative colitis.<sup>2</sup> Rauma et al. found that consumption of uncooked vegan diet significantly increased the intakes of energy and many nutrients without gain in weight in Finnish rheumatoid patients.<sup>3</sup> Various scientific groups evaluated the antioxidant activity of the Spirulina and reported that it provides some antioxidant protection both *in vitro* and *in vivo*.<sup>4–6</sup> Since wheat grass and Spirulina are good sources of natural antioxidants as they contain vitamin E,  $\beta$ -carotene, and minerals, the effect of these functional foods on human health with respect to their role as antioxidants was evaluated in the present study.

We conducted a randomized, double-blind, placebo-controlled study with the volunteers and study team being blinded to the type of functional food intervention the subjects received. The study was conducted with 30 healthy volunteers ages 18–21. The subjects were consuming food from the same place. Their socioeconomic status was the same. The subjects who participated in the study were medical assistants in the Indian Navy. They were undergoing training at School of Medical Assistants, Institute of Naval Medicine, Mumbai, India. Naval recruits are selected for the medical branch in the Navy after completion of Basic/Sea training. The School of Medical Assistants at Mumbai imparts higher-rank professional and specialist training to medical assistants of the Navy to handle all types of emergencies on ship and/or on shore in war and peace. The subjects were

engaged in routine physical exercise (i.e., morning physical therapy, morning drill, parade training, classroom instructions, afternoon muster, evening games, and night study). Written consent was obtained from the volunteers after giving full details of protocol approved by the Institute's Ethic Committee.

The volunteers were divided into three groups of 10 each. Group I took placebo (calcium gluconate), Group II took wheat grass, and Group III took Spirulina. All three supplements were given as a dry powder for 30 days, at 500 mg twice daily, in capsules that were identical in appearance, before breakfast and dinner. The manufacturers of the wheat grass and Spirulina were M/s Sanat Products Ltd., Delhi, India, and M/s Nutraceuticals Bio-Tech, Mumbai, India, respectively. The physical parameters were recorded between 7:00 AM and 8:00 AM before taking food. Various biochemical tests were performed before and after 30 days of supplementation.

Blood samples were collected in heparinized tubes at 8:00 AM to 8:30 AM from an antecubital vein after 12 hours of fasting and were processed for various analytes (e.g.; reduced glutathione [GSH] and malondialdehyde [MDA]), and estimations for these were made on the same day. While for other biochemical variables, plasma and red blood cells were separated immediately by centrifugation at 1000g for 15 minutes and were frozen at  $-80^{\circ}\text{C}$  until the assay. For vitamin C, plasma samples were treated with 10% metaphosphoric acid and then they were frozen at  $-80^{\circ}\text{C}$  until the analysis was completed. MDA was estimated by using Utley et al.'s method,<sup>7</sup> and GSH was assayed using an Ellman reagent.<sup>8</sup> The total antioxidant status in plasma and erythrocytes superoxide dismutase (EC 1.15.1.1; SOD), were estimated using commercially available kits supplied by Randox Laboratories (Ardmore, UK). Erythrocytes glutathione reductase (EC 1.6.4.2; GR) was estimated using Racker et al.'s method.<sup>9</sup> Plasma vitamin C was estimated by a method used by Zannoni et al.<sup>10</sup>

Statistical analysis was carried out using a paired *t*-test within each group to make conclusions that there was a significant change from baseline. Data were reported as mean  $\pm$  standard error of the mean. A value of  $p < 0.05$  was considered statistically significant.

All three groups were homogeneous with respect to age and body-mass index (Table 1). We found that supplementation with wheat grass for 30 days (Group II) resulted in decreased blood MDA and enhanced concentrations of

TABLE 1. PHYSICAL CHARACTERISTICS OF THE VOLUNTEERS

Group	Age (years)	Weight (kg)	Height (m)	BMI (kg/m <sup>2</sup> )
Placebo	19.80 ± 0.24	57.82 ± 1.43	1.67 ± 0.019	20.66 ± 0.34
Wheat grass	19.70 ± 0.21	58.70 ± 1.68	1.70 ± 0.017	20.30 ± 0.34
Spirulina	19.85 ± 0.25	58.25 ± 1.41	1.68 ± 0.017	20.61 ± 0.45

Values are mean ± standard error of the mean; *n* = 10 in each group.  
BMI, body-mass index.

plasma total antioxidant status, vitamin C, erythrocytes activity of SOD significantly (*p* < 0.05) from baseline (Table 2).

Blood MDA is a good marker of lipid oxidation and MDA measurement may provide further indication of oxidative injury. The present study showed that Spirulina supplementation did not alter the blood concentration of MDA significantly, but a trend toward lower values was evident. However, wheat grass supplementation showed significant reductions in blood concentrations of MDA.

Plasma total antioxidant status is usually considered to provide indications of the body's global antioxidant status. In the present study, plasma total antioxidant status improved significantly after wheat grass supplementation. This may be due to the fact that wheat grass supplementation significantly increased the plasma concentration of major antioxidants, vitamin C and erythrocytes activity of the antioxidant enzyme, SOD. The contribution to the plasma total antioxidant status of small amounts of other antioxidant compounds absorbed from wheat grass may be the reason

for the increased total antioxidant status of plasma. Vitamin C, β-carotene, and α-tocopherol are well-characterized antioxidants in wheat grass.

Spirulina contains phenolic acids, tocopherols, and β-carotene, which are known to exhibit antioxidant properties. In our study, supplementation with Spirulina did not bring about any significant change in the plasma total antioxidant status, although a trend toward higher values was evident.

The study highlights the potent antioxidant properties of wheat grass in healthy subjects. Supplementation with wheat grass provided better protection against lipid peroxidation and thereby decreased oxidative stress as shown by a decreased concentration of MDA and increased concentrations of endogenous antioxidant levels such as plasma total antioxidant status and vitamin C, and improvement in activities of erythrocytes SOD. Spirulina supplementation also increased plasma total antioxidant status; however, it was statistically nonsignificant. Hence, this study showed that wheat grass is a better antioxidant as compared to Spirulina.

TABLE 2. CONCENTRATIONS OF VARIOUS OXIDATIVE AND ANTIOXIDANT BIOMARKERS IN HEALTHY HUMANS BEFORE AND AFTER 30 DAYS OF SUPPLEMENTATION WITH WHEAT GRASS AND SPIRULINA

	Placebo		Wheat grass		Spirulina	
	Initial	After 30 days	Initial	After 30 days	Initial	After 30 days
Blood malondialdehyde (μmol/L)	1.73 ± 0.05	1.90 ± 0.25	1.93 ± 0.10	1.44 ± 0.11*	1.94 ± 0.24	1.59 ± 0.19
Total antioxidant status (mmol/L)	0.76 ± 0.09	0.70 ± 0.06	0.63 ± 0.03	0.78 ± 0.06*	0.72 ± 0.09	0.82 ± 0.10
Reduced glutathione (mg/dL)	37.55 ± 0.95	34.04 ± 0.82*	39.02 ± 2.21	31.96 ± 0.89*	38.01 ± 1.53	34.27 ± 1.40*
Vitamin C (mg/dL)	1.01 ± 0.20	0.98 ± 0.12	0.81 ± 0.08	1.31 ± 0.17*	0.84 ± 0.11	0.99 ± 0.09
Glutathione reductase (μM NADPH oxidized/min/mL)	0.27 ± 0.03	0.24 ± 0.01	0.26 ± 0.02	0.28 ± 0.03	0.26 ± 0.02	0.25 ± 0.02
Superoxide dismutase (U/mL)	260.77 ± 4.50	260.58 ± 3.67	257.18 ± 3.70	267.67 ± 3.90*	253.83 ± 3.10	267.99 ± 4.99

Values are mean ± standard error of the mean; *n* = 10 in each group.

NADPH, nicotinamide adenine dinucleotide phosphate, reduced.

\**p* < 0.05 as compared to initial.

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