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The Medical Use of Wheatgrass: Review of the Gap Between Basic and Clinical Applications

Gil Bar-Sela[1,4] , Miri Cohen[2], Eran Ben-Arye[3,4] and Ron Epelbaum[1,4]

[1]*Division of Oncology, Rambam Health Care Campus, Haifa, Israel;* [2]*School of Social Work, Faculty of Social Welfare and Health Sciences, University of Haifa, Haifa, Israel;* [3]*Integrative Oncology Program, Oncology Service, Lin Medical Center, Clalit Health Services, Haifa and Western Galilee District, Israel;* [4]*Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*

Abstract: A wide range of health benefits have been attributed to wheatgrass, the young grass of the common wheat plant *Triticum aestivum*. Its components include chlorophyll, flavonoids, and vitamins C and E. Forms of wheatgrass include fresh juice, frozen juice, tablets, and powders, with compositions varying according to their production processes, as well as to the growing conditions of the wheatgrass. Laboratory *in vitro* studies, mostly using the fermented wheat germ extract, have demonstrated anti-cancer potential and have identified apoptosis as a possible mechanism. In animal experiments, wheatgrass demonstrated benefits in cancer prevention and as an adjunct to cancer treatment, as well as benefits to immunological activity and oxidative stress. Clinical trials show that wheatgrass may induce synergistic benefits to chemotherapy and may attenuate chemotherapy-related side effects, as well as benefit rheumatoid arthritis, ulcerative colitis, hematological diseases, diabetes, obesity, and oxidative stress. However, all the trials were small and a number of methodological problems arose. No adverse events of wheatgrass have been reported, although some forms pose problems of tolerability. The popularity of wheatgrass continues to grow. Nevertheless, the advantages seen in the clinical trials need to be proved in larger studies before clinical recommendations for the public can be given.

Keywords: Apoptosis, Cancer, Hematological diseases, Immunology, Oxidative stress, *Triticum aestivum*, Wheatgrass.

INTRODUCTION

Wheatgrass is young grass, most often of the common wheat plant *Triticum aestivum*. In the 1930s, Dr. Charles Schnabel, who has been called the "father of wheatgrass", began documenting a wide range of its health benefits, based on his observations in animals and humans. Schnabel patented a dietary supplement derived from the dehydration of young wheatgrass shoots, which was marketed until the 1950s. In the 1970s, Ann Wigmore renewed the popularity of wheatgrass. Based on her personal health experience, Wigmore wrote books and lectured on the benefits of wheatgrass, as part of a raw/living food diet.

Only some of the many claimed health benefits of wheatgrass have been scientifically investigated. While a number of components of wheatgrass have demonstrated positive effects, the added value encompassed by the substance as an entity is not clear. We start this review with an examination of the qualities of the components of wheatgrass, followed by a discussion of the forms of wheatgrass and their characteristics. Next, we review the evidence for the health benefits of wheatgrass, from *in vitro* experiments, experiments in animals, and clinical trials. While healing qualities have also been attributed to the topical application of wheatgrass extract [1], the scope of this review is limited to oral administration. At the end, we raise issues to be considered in the clinical realm and in future research.

*Address correspondence to this author at the Integrated Oncology and Palliative Care Unit, Rambam Health Care Campus, POB 9602, Haifa 31096, Israel; Tel: 972-4-7773128; Fax: 972-4-7773168;
E-mail: g_barsela@rambam.health.gov.il

COMPONENTS OF WHEATGRASS AND THEIR HEALTH BENEFITS

Chlorophyll, which has been referred to as "living food" and as "green blood", is the primary nutrient in fresh wheatgrass. In addition, flavonoids, a large variety of vitamins including vitamins C and E, choline, minerals, enzymes, indoles, and a number of amino acids are considered to be responsible for the health benefits claimed. The alkaline pH of 7.4 is also considered an asset.

Among the qualities attributed to chlorophyll are the promotion of cancer prevention, protection against side effects of cancer treatment, and contribution to a positive hematological status. Extracts from wheat sprout roots and leaves were shown to inhibit metabolic activity of carcinogens, and chlorophyll was identified as the active substance [2]. Chorophyllin, a derivative of chlorophyll, was shown to protect mitochondria against oxidative damage [3] and to induce the activity of mammalian phase 2 proteins that protect cells against oxidants and electrophiles [4]. This

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anti-oxidant activity has implications in cancer prevention [5]. The capability of chlorophyll to protect mitochondrial membranes against gamma-radiation [6] may be beneficial during cancer treatment. The structural similarity between chlorophyll and hemoglobin, which differs by the inclusion of magnesium and iron, respectively, may explain observed hematological benefits of chlorophyll-rich substances, although a mechanism of such has not been elucidated.

Wheatgrass is also rich in flavonoids, particularly apigenin, which is known for its functions in anti-oxidation [7]; in anti-inflammation [8] by inhibiting cytokine-induced leukocyte adhesion [9]; in anti-carcinogenesis [10] by antiproliferative and proapoptotic activities [11] through regulation of signaling pathways such as PI3K/Akt/mTOR [12] and MAPK [13]; and in cardiovascular protection by inducing endothelial derived hyperpolarizing factor (EDHF)-mediated vascular dilatation [14].

Vitamin C (ascorbic acid), a component of fresh wheatgrass, has been associated with the prevention and treatment of cancer through a number of mechanisms that relate to antioxidant and pro-oxidant properties, stimulation of the immune system, altering carcinogen metabolism, enhancement of collagen synthesis, and interference with cancer cell signaling [15]. The possibility that antioxidant agents may interfere with chemotherapy necessitates the specific investigation of each agent and the timing of its administration. When received simultaneously with chemotherapy, vitamins C and E were found to restore antioxidant status that had decreased due to breast cancer and its treatment, and to reduce DNA damage [16].

Choline and indoles are other components of wheatgrass that have had anti-cancer activity attributed to them [17,18]. Phytochemical screening and gas chromatography-mass spectrometry analysis has verified, in the aqueous extract of wheatgrass: flavonoids, triterpenoids, anthraquinol, alkaloids, tannins, saponins, sterols, squalene, caryophyllene and amyrins, many of which have demonstrated anti-oxidant activity [19,20].

Oligosaccharides isolated from wheatgrass were found to stimulate the expression of inflammatory cytokines in human peripheral blood mononuclear cells [21]. The oligosaccharide, maltoheptaose, is the first immunostimulatory component of wheatgrass to be identified. Maltoheptaose was shown to activate monocytes via Toll-like receptor 2 (TLR-2) signaling.

WHEAT, WHEATGRASS, WHEATGRASS DERIVATIVES

The qualitative and quantitative composition of wheatgrass differs from that of other wheat products. For example, in the passage from grains to sprouts to wheatgrass, the quantity of flavonoids increases [22]. Table **1** shows the composition of a sample of fresh wheatgrass [23]. Nevertheless, the quantities of nutrients in wheatgrass products may vary according to growing conditions, such as soil composition, climate (amount of sunlight, temperature, presence of snow), duration of growth period, and height at harvest. Wheatgrass grown in soil with nutrients was found to have a higher level of ascorbic acid than wheatgrass grown in soil with tap water, or grown in tap water with nutrients [24]. Regardless of the growing medium, quantities of flavonoids and ascorbic acid were shown to increase over a 15-day growth period [24].

Table 1. Nutrient comparison of 1 oz (28.35 g) of wheatgrass juice [23].

Nutrient	Quantity
Protein	860 mg
Beta-carotene	120 IU
Vitamin E	880 mcg
Vitamin C	1 mg
Vitamin B_{12}	0.30 mcg
Phosphorus	21 mg
Magnesium	8 mg
Calcium	7.2 mg
Iron	0.66 mg
Potassium	42 mg

Wheatgrass can be consumed in a number of forms: fresh juice, frozen juice, tablets, and powders. Aqueous and ethanol extracts are available, following freeze drying or oven drying. The nutritional composition of the products varies according to the processes of their production, as well as the growing conditions of the wheatgrass from which they were derived. At least one commercial producer of tablets (http://www.wheatgrass.com) continues to grow wheatgrass under the same conditions implemented by Charles Schnabel during the 1930s: slow growth during the winter and harvest in the spring. The claim is that outdoor cultivation increases the chlorophyll content, while faster growth, on trays, as done by Ann Wigmore, results in more sugars and less fiber. On the other hand, the cultivation of wheatgrass without geographical or seasonal restrictions enables the widespread and continuous availability of fresh juice.

Frozen wheatgrass juice (WGJ) is more convenient than fresh juice, yet was found to contain a 20% lower level of amino acids [25]. Ethanol extracts of wheatgrass were found to have higher phenolic and flavonoid content than aqueous extracts [24]. In a study that compared quantities of nutrients in 7-day old fresh, freeze dried and oven dried wheatgrass [26], fresh wheatgrass samples had the highest amount of ascorbic acid and chlorophyll, but the lowest amount of total flavonoids and phenolics. Ethanolic extract from freeze-dried wheatgrass had the highest level of ferric-reducing antioxidant power assay and the lowest α-tocopherol value. Freeze-dried wheatgrass samples exhibited the highest activity of 2,2-diphenyl-1-picrylhydrazyl scavenging ability.

Commercial wheatgrass products standardize at least some of the components. Fermented wheat germ extract (FWGE) is a concentrated extract derived from the endosperm of the wheat plant, and is sold as a dietary supplement under the trade name Avemar. Fermentation with baker's yeast during preparation concentrates biologically-active benzoquinones. Inspiration for the development of Avemar came from the work of Albert SzentGyorgyi, the Nobel laureate who discovered vitamin C, and who proposed that the combination of vitamin C with methoxy-substituted benzoquinones could help treat cancer. Máté Hidvégi, the Hungarian chemist who invented Avemar in the early 1990s, aimed to develop wheat germ extracts with high and standardized levels of benzoquinone. The health benefits attributed to Avemar are primarily in the realm of cancer, as adjunct care against the disease and in the reduction of the side effects of its treatment. Animal experiments and clinical trials do not report any adverse events of Avemar, nor do toxicity studies in which dosages by body weight of 25-fold the recommendation for oral use were administered to rats [27].

HEALTH BENEFITS OF WHEATGRASS

Early evidence of the health benefits of wheatgrass comes from anecdotal reports. Numerous in vitro studies have been conducted in recent years, particularly on the standardized FWGE, Avemar. Several animal studies have been performed, as well as a few clinical trials. Here we present central findings from studies published in the English language. We note that several studies that were presented at conferences were not published as articles, raising the possibility of publication bias.

LABORATORY (IN VITRO) EXPERIMENTS

Laboratory experiments have been conducted on the involvement of wheatgrass and its derivatives, particularly FWGE, in immunological, anti-oxidative, and anti-cancer activities. Both immunological and anti-oxidative activities have been investigated in the search for mechanisms of anti-cancer activity. Both the cancer disease and its treatment are known to impair the immunological and oxidative status of patients.

Anti-Cancer Effect Through Immunological Activity

We present two examples by which immunological effects of FWGE may impede the activity of tumor cells. In the first example, FWGE was shown to downregulate cell surface MHC class-I proteins of malignant T- and B-cells, yet not affect healthy peripheral blood mononuclear cells [28]. Overexpression of MHC-1 protects tumor cells from attack by natural killer (NK) cells. Thus, the selective downregulation by FWGE of MHC-1 proteins may reduce metastatic activity by decreasing the defense of tumor cells against natural killer (NK) cells. In the second example, FWGE was shown to induce the synthesis of ICAM-1 [29]. The decreased expression of ICAM-1 protein on the endothelial cells of solid tumor vessels inhibits the passage of leukocytes through the vessel membrane. Thus, FWGE may increase the exposure of tumor cells to leukocyte infiltration. In the same study, FWGE was shown to stimulate the immune system, by means of exerting a synergistic effect on proinflammatory cytokine production, including tumor necrosis factor-alpha, a key anticancer cytokine. High concentrations of FWGE inhibited the proliferation and survival of myeloid, though not lymphoid cells.

Anti-Cancer Effect Through Apoptosis

Several experiments have identified induction of apoptosis as a mechanism of the anti-cancer activity of wheatgrass. Wheatgrass extract was found to induce apoptosis of MCF-7 breast cancer cells [30,31], human acute promyelocytic leukemia cells [32], and HeLa cervical cancer cells [31]. Apoptosis was also determined to be a mechanism by which FWGE inhibits the growth of gastric carcinoma cells [33], human lymphoma cells [34], human colon carcinoma cells [35], human ovarian cancer cells [36], and a broad spectrum of 32 human cancer cell lines, of which the highest activity was found in neuroblastoma cell lines [37]. Induction of apoptosis by FWGE was shown to be selective, occurring in leukemic human cells, but not in healthy, peripheral blood mononuclear cells [38].

One mechanism of FWGE-induced apoptosis appears to involve the Poly (Adenosine diphosphate ribose) Polymerase (PARP) enzyme. PARP promotes DNA repair in cancer cells; thus, its cleavage results in apoptosis of these cells. FWGE was shown to induce apoptosis of leukemia and hepatocellular carcinoma cells by activating cleavage of the PARP enzyme [38,39]. FWGE stimulates the PARP pathway by regulating metabolic enzymes in the glycolysis and pentose cycles of cancer cells [38]. The application of FWGE to pancreatic adenocarcinoma cells decreased glucose uptake and increased glucose oxidation and ribose recycling in the pentose cycle, in a dose-dependent manner [40]. Increased pentose cycle activity leads to an increase in superoxide dismutase scavenger activity, as well as increased lipid synthesis from glucose, which promotes cell differentiation and protects against oxidative stress. Wheatgrass has demonstrated effectiveness as a radical scavenger also in antioxidant assays [20].

FWGE was shown to enhance the effect of tamoxifen on MCF-7 breast cancer cells *in vitro*, by enhancing apoptotic activity [4]. Tamoxifen combined with FWGE significantly increased apoptosis, while tamoxifen alone showed no effect. The estrogen-receptor activity of MCF-7 cells increased with FWGE, decreased with tamoxifen, and decreased further with tamoxifen and FWGE combined [41], reflecting the synergistic effect between FWGE and tamoxifen.

Other Possible Mechanisms of an Anti-Cancer Effect

Other mechanisms that have been proposed for the anti-cancer potential of FWGE include decreasing cell motility [38] and inhibition of cyclooxygenase (COX) -2 [35], which is overexpressed in 80-85% of adenocarcinomas [42]. Gene expression data identified 2,142 genes in ovarian cancer cell lines, representing 27 biological pathways that were significantly associated with FWGE sensitivity [36].

Wheatgrass Derivatives Combined with Cancer Drugs

In vitro experiments of the combined treatment of FWGE with chemotherapy drugs show that the effects depend on the type of cancer, the specific cancer drug, and the timing of application. An aqueous wheatgrass extract was found to enhance the effect of cisplatin on MCF-7 breast and HeLa cervical cancer cells [31]. FWGE enhanced the cytotoxicity of cisplatin in the hepatocellular carcinomas: HepJ5, HepG2, and Hep3B; and the cytotoxicity of 5-Fu in HepJ5 cells only [39]. However, FWGE antagonized the cytotoxic activity of docetaxel in estrogen responsive MCF-7 cells, yet demonstrated an additive and marginally synergistic effect in triple negative HCC-38 cells and SKBR-3 Her2/neu overexpressing cells [43].

When combined with cancer treatments *in vitro*, the schedule of application of FWGE is critical to its efficacy. The application of FWGE to colon cancer cell lines, simultaneously with 5-fluorouracil (5-FU) or oxaliplatin or irinotecan, resulted in a synergistic drug interaction, particularly with 5-FU [37]. However, the effect tended toward antagonism when FWGE was applied before 5-FU. A proposed mechanism is that FWGE, when applied alone, may impair DNA synthesis by inhibiting ribonucleotide reductase, which catalyzes the reduction of ribonucleotides to deoxyribonucleotides, the building blocks of DNA synthesis. FWGE inhibition of ribonucleotide reductase was demonstrated in HT29 and HL-60 cells [28,34,35]. The resultant decrease in DNA synthesis may impair the activity of 5-FU [37].

A recently published study showed alcohol and aqueous extracts of wheatgrass to be less damaging than 5-FU to normal kidney epithelial cells, thereby confirming the safety of these extracts in anti-cancer uses [44].

ANIMAL EXPERIMENTS

Most documented animal experiments were performed on rats. Contrasting with the laboratory experiments, a great variety of health benefits, in addition to anti-cancer potential, have been investigated in animal experiments.

Anti-Cancer Potential

FWGE demonstrated benefit in both cancer prevention and as an adjunct to cancer treatment. The number of colon tumors that developed in rats injected with the carcinogen azoxymethane was greatly decreased following the administration of FWGE [45]. In mice that received wheatgrass leaf extract, the latency period of DMBA and croton oil-induced skin carcinogenesis was greater, the level of reduced lipid peroxidation was lower, and the levels of reduced glutathione, superoxide dismutase, and catalase were higher than in a control group [46], indicating positive effects of wheatgrass extract on chemoprevention and on oxidative stress.

FWGE was shown to enhance endocrine treatment of mouse estrogen receptor mammary carcinoma and human xenograft mammary carcinoma transplanted into mice [47]. As monotherapy, FWGE was more effective than three endocrine treatments: tamoxifen, exemestane, and anastrozole [47]. The application of FWGE simultaneously with 5-FU, doxorubicin or navelbine did not increase toxicity or decrease the antiproliferative activity of these cytostatic drugs in mice [48].

In immunosuppressed mice, the simultaneous treatment of FWGE and vitamin C, although not vitamin C alone, inhibited metastasis formation in a variety of tumors [49]. However, when FWGE was administered one hour prior to vitamin C, the metastatic inhibiting effect was decreased. For some tumors, smaller dosages of FWGE resulted in a greater metastatic effect [49].

Wheat sprout extract that did not contain chlorophyll was found to strongly inhibit mutagenicity of benzo[a]pyrene, a carcinogen in cigarette smoke. This indicates that compounds other than chlorophyll are responsible for the mutagenic activity of wheatgrass [50].

Immunological Activity

FWGE administered to mice significantly increased the degree of blastic transformation of peripheral blood T lymphocytes that were caused by concanavalin A [51]. Further, FWGE was found to restore the immune response, almost to normal, in mice immunocompromised by thymectomy [51]. However, the active component of FWGE, 2,6-dimethoxy-*p*-benzoquinone, did not demonstrate this restorative effect.

FWGE monotherapy was shown to be as effective as dexamethasone and indomethacin in inhibiting the development of the secondary, immune-mediated response in adjuvant arthritis in Wistar rats. FWGE inhibited COX-1 and -2, while indomethacin enhanced COX-2 gene expressions [52].

Oral administration of FWGE to mice with induced systemic lupus erythematosus (SLE) resulted in decreased autoantibody production, inhibition of the Th2 response, and less protein in the urine than in SLE mice that did not receive FWGE [53].

Effect on Bone Disease

An aqueous extract of wheatgrass was administered for 30 days, together with the bisphosphonate, risedronate, to rats with glucocorticoid-induced osteoporosis. Serum levels of bone mineral content markers were increased and serum and urinary levels of bone resorption markers decreased [54].

Effect on Oxidative Stress

In rabbits that received wheatgrass together with a high-fat diet, malondialdehyde, a marker of oxidative stress, was decreased, and glutathione and vitamin C increased, compared to rabbits that received the high-fat diet without wheatgrass supplementation. Furthermore, rabbits who received wheatgrass had lower levels of total cholesterol and higher levels of HDL cholesterol [55].

In rats that received the organophosphate insecticide, chloropyrifos (CPF), supplementation of FWGE normalized parameters of oxidative stress: lipid peroxidation level, superoxide dismutase, catalase and glutathione-s-transferase;

as well as parameters of blood, liver and kidney function, including red and white blood cell counts, erythrocyte indices; and levels of hemoglobin, hematocrit, liver enzymes, total protein, albumin, globulin, serum creatinine, and urea [56].

The administration of wheatgrass to male albino Wistar rats restored to normal levels of antioxidants: superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione, vitamin E, and vitamin C, which had decreased following alcohol and polyunsaturated fatty acid-induced oxidative stress [57]. In rats considered to be hepatotoxic, following a diet containing alcohol and heated polyunsaturated fatty acids, the administration of wheatgrass restored to normal fatty acid composition, total phospholipid levels, and phospholipase A and C [58].

Effect on Metabolic and Cardiovascular Diseases

In the deoxycorticosterone acetate (DOCA)-salt-induced rat model of chronic hypertension, treatment with FWGE improved cardiac function; decreased macrophage infiltration, which resulted in decreased collagen deposition in the ventricular myocardium; reversed increased stiffness of the left ventricle in diseased hearts, and attenuated increased plasma malondialdehyde concentrations [59]. In rats fed a high-fat/high-carbohydrate diet, FWGE reversed glucose intolerance, normalized systolic blood pressure and decreased visceral fat deposition [59].

Fresh WGJ reduced levels of total cholesterol, triglycerides, low density lipoprotein-cholesterol, and very low density lipoprotein-cholesterol in hypercholesterolemic rats. The effect was in a dose-dependent manner, and similar to that of atorvastatin [20]. Consumption of the WGJ increased fecal cholesterol excretion.

Administration of an ethanol extract of wheatgrass to streptozotocin-induced diabetic rats resulted in improved metabolic and lipid profiles, as demonstrated by an increased level of liver glycogen high density lipoprotein, and decreased levels of fasting blood glucose, glycosylated hemoglobin (HbA1c), serum triglycerides and low density lipoprotein [60]. Enhanced anti-oxidant potential was demonstrated by decreased levels of lipid peroxides, superoxide dismutase and glutathione peroxidase, and increased levels of vitamin E and catalase.

Treatment with wheatgrass restored levels of plasma glucose and insulin, serum levels of glucose oxidative enzymes, and liver glycogen levels, in male Wistar rats with diabetes [61]. A methanolic extract of 9-day old wheatgrass leaves restored markers of liver damage in carbon tetrachloride-treated rats specifically: liver transaminases and total and direct bilirubin content [62].

CLINICAL TRIALS

Wheatgrass in its many forms has become a common nutritional supplement. While the findings of only a few clinical trials have been published, the range of health benefits examined is wide. We present the findings of clinical studies published in the English language.

Effect on Cancer

Wheatgrass and its derivatives have been investigated for possible synergistic benefits to chemotherapy in humans and for the attenuation of chemotherapy-related side effects. The first clinical trial of FWGE published in the English language appeared several years after the release of this product. Six-month supplementation of FWGE to anticancer treatments resulted in lower rates of recurrence of colorectal cancer, of new metastases, and of deaths; and longer progression-free and overall survival [63]. The cohort, recruited from three oncosurgical centers, was large compared to later trials of FWGE: 66 received FWGE and 104 comprised the control. Allocation to study group was by patients' choice, which poses a selection bias.

In a randomized, open-label, phase II clinical trial of 52 patients, progression-free and overall survival were greater at the 7-year follow-up among stage III melanoma patients who had received FWGE in conjunction with dacarbazine (DTIC)-based adjuvant chemotherapy than among patients who did not receive FWGE [64]. These results are questionable, since DTIC has been found to be ineffective as adjuvant treatment for stage III melanoma; and too few patients were included in the study to achieve statistical power regarding any outcome for cancer adjuvant treatments.

The effect of WGJ on myelotoxicity induced by chemotherapy was assessed among chemotherapy-naïve breast cancer patients [25]. Thirty patients received frozen WGJ daily during the first three cycles of chemotherapy, in addition to standard supportive therapy. Thirty patients, matched by pairs according to age, disease stage, and blood count, received only standard supportive therapy together with the chemotherapy. The effect of WGJ was most profound with regard to hematological toxicity (17% versus 37%, $p=0.04$). The most important effect observed was a reduction in neutropenic fever events and in neutropenic infections. No difference in response to chemotherapy was observed between those who received WGJ and those who did not, regarding the eight patients from each group for whom such an assessment was relevant. Thus, although the sample size was small and the trial open-label, the investigators concluded that WGJ taken during FAC (5-fluorouracil, doxorubicin, and cyclophospamide) chemotherapy may reduce myelotoxicity and the need for chemotherapy dose reductions or the use of granulocyte colony-stimulating factors. Problems arose due to tolerability of the WGJ, with 22 (73%) reporting difficulties in swallowing the WGJ, and six (20%) stopped consumption of the WGJ due to nausea.

Another small open-label, matched-pair pilot clinical trial was conducted in pediatric cancer patients with chemotherapy-induced febrile neutropenia [65]. Among 11 patients who received FWGE in addition to standard anticancer treatment, febrile neutropenic episodes were fewer ($p<0.05$) and mean white blood cell and lymphocyte counts higher than among 11 patients matched by pairs, according to diagnosis, stage of disease, age, and sex.

In an open-label trial of 60 patients affected by head and neck tumors, levels of oxidative stress, as assessed by

concentrations of hydroperoxides, were decreased among those who received FWGE for two months, compared to those who received conventional oncological therapy only [66]. Quality of life, as assessed by Spitzer's index, was also higher among those who received FWGE.

Effect on Rheumatoid Arthritis

An open-label single arm study was conducted to investigate a potential benefit for FWGE on rheumatoid arthritis (RA), following the observation of improvements in RA among cancer patients taking FWGE [67]. Following one year intake of FWGE as supplementation to standard therapy, improvements in symptoms of RA were observed in 15 women who had failed at least two disease-modifying antirheumatic drugs. Dosages of steroids were reduced by some of the patients. No adverse effects were observed. The authors suggested that, since insufficient apoptosis of macrophages and fibroblasts contributes to the increased numbers of chronic inflammatory cells in the joints of patients with RA [68], the mechanisms of apoptosis at the basis of the anti-cancer effect of FWGE may contribute to the improvement of RA.

Effect on Ulcerative Colitis

Among 10 patients with ulcerative colitis who received fresh WGJ during the course of one month, greater improvements in overall symptoms and in the severity of rectal bleeding were observed compared to patients who received a placebo, which was similar in appearance but not in taste or smell [69]. Allocation of the two groups was random. The trial was inspired by reports from patients who attributed improvements in symptoms of ulcerative colitis to their intake of WGJ. The authors suggested that the anti-inflammatory and anti-oxidant activity of wheatgrass may explain their findings.

Effect on Hematological Diseases

A number of clinical trials have shown WGJ to have positive effects on red blood cell disorders. The mechanism may be by reducing the oxidative stress that is associated with such disorders [70,71]. Three before and after studies assessed the effect of wheatgrass on beta thalassemia in pediatric patients; two showed positive effects and one showed no effect. In the first study, for 8 of 16 (50%) patients with transfusion-dependent beta thalassemia major, blood transfusion requirement decreased by more than 25% during a one-year period of daily consumption of fresh WGJ produced from homegrown plants [72]. The index date for analysis of the effects of WGJ was at least six months after the start of WGJ consumption, since no response to WGJ therapy was observed during the first few months of its intake. It has been suggested that the existence of this neutral period may indicate that natural antioxidants contained in WGJ are better able to prevent cellular injury than to repair red blood cell enzymes and membranes that have already been damaged [73]. Inspiration for this study came from the positive reports about the effects of WGJ consumption from patients with b-thalassemia. The study has received criticism [74] for the inclusion of only 16 patients in the final analysis, of the 38 who enrolled in the study. Reasons for dropout could be intolerability of the WGJ or lack of response.

The second study demonstrated similar findings. The mean level of hemoglobin and the time between transfusions increased, and the mean quantity of blood transfused decreased for a one-year interval during which wheatgrass tablets were consumed compared to the year prior to their consumption [75]. Of the 40 patients enrolled in the study, seven (17.5%) died during the study period, and five were noncompliant.

In the third study, the intake of WGJ tablets over a one-year period did not affect transfusion requirements in 53 patients [74]. Contrasting with the study by Marwaha et al [72], but the same as the study by Singh et al [75], wheatgrass was consumed in the form of tablets and parameters were assessed after one year of intake, without a neutral period.

Fresh WGJ demonstrated the capability to chelate, or bind iron, in a study of 20 patients with excessive levels of iron due to recurrent blood transfusions as treatment for myelodysplastic syndrome [76]. The effect was similar to that achieved by iron chelator drugs.

Effect on Diabetes and Obesity

Mean levels of fasting glucose, post-prandial glucose, and HbA1c were found to decrease substantially in people with diabetes who ingested WGJ over six months, contrasting with the lack of change in a control group [77]. In a cohort of individuals considered obese (mean BMI 25 for males and 27 for females, in an Indian population), mean levels of triglycerides and LDL decreased, and HDL increased, among those assigned to ingest WGJ over a six-month period. No such changes were observed in a control group [77].

Effect on Oxidative Stress

In a randomized controlled study of 30 healthy 18-21 year olds, supplementation with wheatgrass powder for 30 days resulted in improvement in markers of oxidative status, including decreased blood malondialdehyde and increased vitamin C [78]. Such changes were greater than among those who received Spirulina, another functional food considered to be an anti-oxidant, and among those who received a placebo.

CLINICAL ISSUES

Anecdotal experience, rather than preclinical studies, has inspired most of the clinical trials on wheatgrass that have been conducted. Likewise, anecdotal experience, rather than research findings, may still provide the main incentive for its personal use. Currently available evidence confirms the safety of wheatgrass and its products. *In vitro* and animal experiments show additive and synergistic effects when wheatgrass supplements are added to standard medical therapy. However, wheatgrass has also been shown to antagonize certain chemotherapy drugs, if taken before rather than simultaneously with these drugs.

Many factors may affect selection of the form of wheatgrass, including availability, tolerability of swallowing the product, the expectation that a fresh product will be more beneficial, and motivation to be involved in growing wheatgrass and making juice. Future research may add another factor for consideration, by determining the forms of wheatgrass that may benefit specific conditions. For example, fermented wheat germ, with its high level of benzoquinones, may be more effective in treating cancer, while fresh WGJ, with its high level of ascorbic acid and chlorophyll, may be more effective in treating hematological and inflammatory conditions. Differences between the forms of wheatgrass may also be relevant to their effects on chemotherapy, both synergistically and possibly adversely. For some forms and conditions, the benefit may start only a period of time after daily intake. On the other hand, particularly in human use, the effect of wheatgrass as a whole may be greater than the sum of its parts, and more important than the form or timing of its intake.

RESEARCH ISSUES

Considering the extent of evidence that has emerged from *in vitro* experiments on the anti-cancer potential of wheatgrass, animal experiments and clinical trials lag behind. Since commercial wheatgrass products are classified as nutritional supplements and not as drugs, clinical trials are not required for their marketing. In fact, all published clinical trials on commercial products were conducted after their release to the market. All published trials were small and a number of methodological problems arose. Only one trial was identified that showed negative results. More high quality studies are needed to precisely examine, in animals and humans, the benefits of wheatgrass that have been demonstrated *in vitro*. Conversely, scientifically sound experiments are needed to explore *in vitro* the range of health benefits attributed to wheatgrass. Such examination may distinguish the effects of different forms of wheatgrass, as well as its components.

The investigation of wheatgrass poses particular methodological challenges. For example, the strong taste, smell, and texture of WGJ make it difficult to conduct a placebo-controlled study, and may also cause high dropout due to intolerability of swallowing it. Interviewing study participants as to the group they believed they were allocated to, study or control, as done by Ben Arye et al [69], can help to elucidate a placebo effect. Accessing information about tolerability can distinguish between those who drop out for this reason rather than other reasons, such as poor response. While the composition of commercial wheatgrass products is standardized and more tolerable, their effects may differ from those of WGJ.

As the popularity of wheatgrass continues to grow and the health benefits attributed it continue to increase, robust scientific investigation is important to verify or refute these claims. Future studies should improve study design, considering the methodological challenges posed and the scientific evidence that has accumulated. Controlled studies should also compare the immune-modulation related to wheatgrass consumption to other interventional approaches, such as behavioral or cognitive. Along this line, a controlled open-label trial is currently underway to compare parameters of immunity and quality of life among colorectal cancer patients on adjuvant chemotherapy, randomized to receive biofeedback training or wheatgrass juice (NCT01991080).

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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Declared none.

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