Trusted, and Proven Immunomodulator™

BioBran®

Rice Bran Arabinoxylan Compound
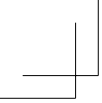
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1. INTRODUCTION

BioBran is a trusted and proven immunomodulator made from a rice bran extract enzymatically modified with an enzyme complex from shiitake mushroom.

Daiwa Pharmaceutical Co., Ltd. developed this dietary supplement in the 1990's. Since then, numerous clinical papers published in leading medical journals worldwide demonstrated and proved the immunomodulatory function of this dietary supplement. With this scientific background and established efficacy, the product has served many people worldwide, most of whom have had issues with their immune system.

It is now distributed to more than 50 countries worldwide, mostly through health practitioners. Daiwa never stops their research on BioBran and new findings are coming up all the time.

2. PRODUCTION PROCESS

A water soluble dietary fiber, hemicellulose B, is extracted from rice bran. A carbohydrase enzyme complex is obtained from shiitake mushroom mycelia culture. Through a patented and innovative production process the Hemicellulose B from rice bran is reacted with the enzyme complex and is changed into BioBran.

3. ACTIVE INGREDIENT

The component of BioBran involved in immunomodulatory activity was analyzed using macrophage stimulation as the indicator. The chemical structure of the active ingredient was also analyzed. The result shows that the main components are arabinose, galactose, and glucose. Although it is difficult to estimate the structure of the polysaccharide from these methylated monosaccharides with various linkages, the approximate structure could be predicted based on the structures of many polysaccharides that compose the plant cell wall. The results of methylation analysis suggested the following polysaccharide structures (1) arabinogalactan with a main chain of 1,4-β-galactan and side chains of arabinose, (2) arabinoxylan with a main chain of 1,4-β-xylan and side chains of arabinose, (3) arabinan with the main chain of 1,5-α-arabinan and side chains of arabinose, and (4) β-1,3:1,4-glucan. Further analysis is needed to determine whether these polysaccharides are mixed or present as parts of one molecule. However, the immunomodulatory ingredient of BioBran is estimated to be a heteropolysaccharide of complex structure.

4. TOXICOLOGY AND SAFETY STUDIES

4.1: Mutagenicity Test (Ames Test)

BioBran, up to a concentration of 10,000 μg/plate, tested negative in a bacterial reverse mutation test, both with and without metabolic stimulation.

Conducted by Consumer Product Testing Company, NJ, USA

4.2: Single Dose Test in Rats

Male and female Wistar rats were given a single oral dose of BioBran at 5, 10, 18, 27, or 36 g/kg. BioBran was regarded as non-toxic according to the reference: $LD_{50} > 36$ g/kg.

Conducted by AMA Laboratories, Inc. NY, USA

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4.3: Repeated Dose Test in Dogs

In a safety study of BioBran in dogs, male and female beagle dogs were treated with 0, 20, or 200 mg/kg of BioBran, admixed to their feed once daily for a period of 4 weeks (28 doses). Even after the administration of BioBran for 4 weeks at a dose of 20 mg/kg/day or 200 mg/kg/day, no abnormal findings were observed in general conditions, body weight, food intake, hematology, or blood biochemistry. In this study, the No Observed Adverse Effect Level (NOAEL) of the product was estimated to be 200 mg/kg/day or more.

Conducted by Kyodoken Institute. Kyoto, Japan

4.4: Human Studies

Subjects participated in a two-group, randomized intervention, where one group (n=10) consumed 1 gram/day and the other (n=10) consumed 3 gram/day. Safety and tolerability of BioBran were assessed with total bilirubin, total protein, creatinine, and liver function tests. All subjects tolerated the product without any adverse reactions.

Ali KH, Melillo AB, Leonard SM, Asthana D, Woolger JM, Wolfson AH, McDaniel HR, Lewis JE. "An Open-label, Randomized Clinical Trial to Assess the Immunomodulatory Activity of a Novel Oligosaccharide Compound in Healthy Adults", Functional Foods in Health and Disease. 2(7):265-279., 2012.

5. MECHANISMS OF ACTION

With respect to pharmacokinetics, Endo et al. demonstrated the presence of substances in serum that react to the BioBran antibody in an oral administration study of BioBran in mice, suggesting that after oral administration, constituents of BioBran enter the body. Furthermore, in a 13-day oral dose study in rats conducted by the authors, BioBran, compared with the control, significantly increased the number of Peyer's patches. This suggests that the polysaccharides in the cell wall of grains exhibit a stimulating effect on the gut immune system. Results of the studies presented above indicate that some components of BioBran are absorbed by the digestive tract and show *in vivo* activity, and others indirectly act on the gut immune system. These components, as a whole, are considered to cause the immunomodulatory effect.

6. DOSAGE AND ADMINISTRATION

The recommended dose of BioBran is 1 to 3 gram per day, taken orally.

The recommended dose was determined by the following clinical study.

6.1: Enhancement of Human Natural Killer Cell Activity by Modified Arabinoxylan from Rice Bran (BioBran)

BioBran was examined for its augmentory effect on human natural killer (NK) cell activity. Twenty-four individuals were divided into three groups of eight individuals, and given BioBran orally at three different concentrations: 15, 30 and 45 mg/kg/day for 2 months. Peripheral blood lymphocyte-NK cell activity was measured at 1 week, 1 month and 2 months posttreatment and results were compared with baseline NK activity.

BioBran at a dose of 15 mg/kg/day showed no changes at 1 week as compared with base line

values, however, a twofold increase in NK cytotoxicity was detected after 1 month of treatment. Increasing the dose to 30 mg/kg/day resulted in a significant enhancement of NK activity (310% over baseline) that was detected as early as 1 week. The activity of NK cells continued to increase with continuation of treatment. The peak response was observed at the end of the treatment period (2 months) where NK activity increased fivefold. Increasing the dose to 45 mg/kg/day demonstrated a similar increasing trend in NK activity but the values were higher in magnitude than those for 30 mg/kg/day. Discontinuation of treatment resulted in a decline of NK activity and at 1 month, NK activity returned to base line.
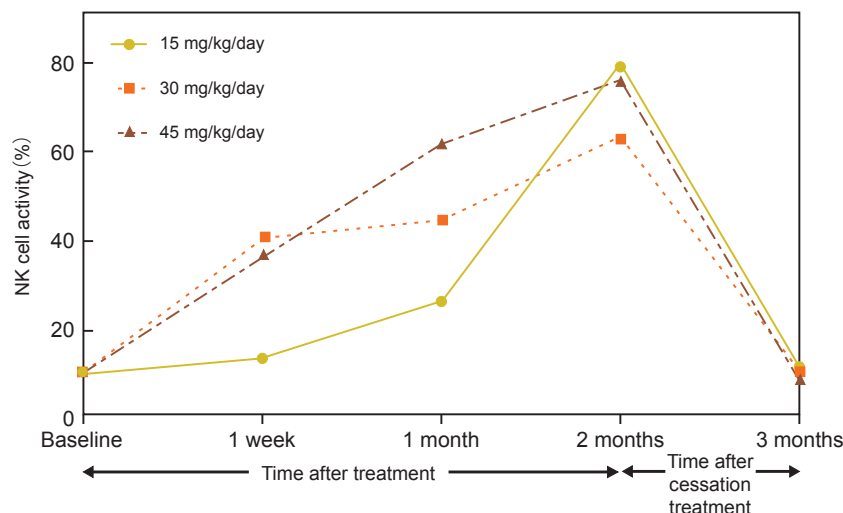

Fig. 1 Dose range and time course of NK cell activation by intake of BioBran

Ghoneum M. "Enhancement of Human Natural Killer Cell Activity by Modified Arabinoxylan from Rice Bran (MGN-3)", International Journal of Immunotherapy. XIV(2):89-99.,1998.

7. *IN VITRO* STUDIES

7.1: Production of Tumor Necrosis Factor-α and Interferon-γ from Human Peripheral Blood Lymphocytes by BioBran, a Modified Arabinoxylan from Rice Bran, and Its Synergy with Interleukin-2 *in Vitro*

This study examined the mechanism by which BioBran elevated natural killer (NK) cytotoxic activity. It was done by testing the action of BioBran on the levels of both tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) secretions and BioBran function on the expression of key cell surface receptors.

7.1.1: Increase in *In Vitro* NK Cell Cytotoxicity by BioBran and IL-2

Peripheral blood lymphocytes (PBLs) from healthy individuals were treated with BioBran in the presence and absence of interleukin (IL) -2 and were examined for NK activity. Figure 2A shows that the addition of BioBran at a concentration of 0.5 mg/ml to PBLs significantly increased the cytotoxic function of NK cells ($p<0.001$). Furthermore, treatment of PBLs with the combination of IL-2 and BioBran resulted in a higher augmentation of NK cell cytotoxicity as compared to treatment with either agent alone. Addition of BioBran to highly purified NK cells

increased NK cell cytotoxic function (see Fig. 2B). Data also show that the level of NK activation is maximized after treatment with IL-2 and that NK activity did not increase further after the combined treatment of BioBran and IL-2, as compared to use of either agent alone.
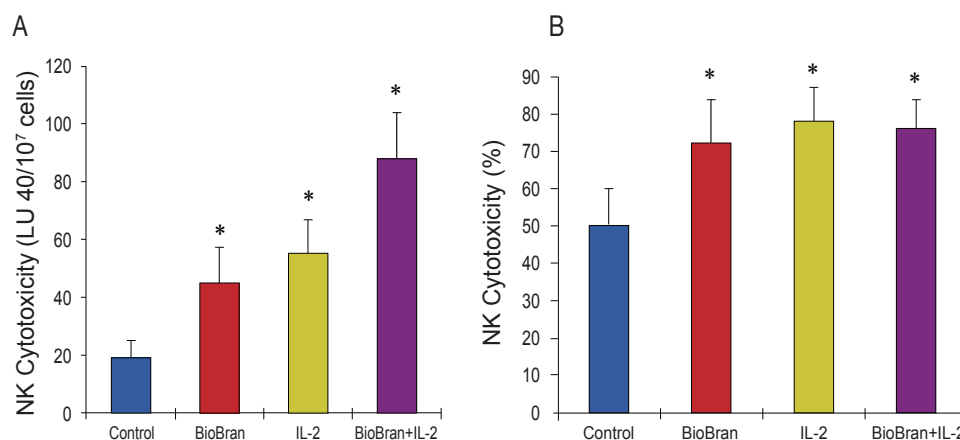

Fig. 2 Increase in *in vitro* NK cell cytotoxicity by BioBran and IL-2

(A) Increased cytotoxicity mediated by PBLs after treatment with BioBran *in vitro* in the presence or absence of IL-2. PBLs from five donors were incubated with BioBran (0.5 mg/ml) and interleukin-2 (IL-2; 500 U/ml) for 16 hours, after which they were examined for NK cell activity by 51Cr-release assay. Activity expressed as lytic units at 40%. *p<0.001
(B) Action of BioBran on activity of purified NK cells. Purified NK cells were incubated with BioBran (0.5 mg/ml) in the presence or absence of IL-2 (500 U/ml) overnight. Activity examined at effector: target (E:T) ratio of 2:1 M±standard deviation of five individuals. *p<0.01.

7.1.2: Increase in TNF-α Secretion, Titration Experiments

PBLs were incubated with BioBran for 16 hours, and supernatants were recovered and subjected to a specific and sensitive enzyme-linked immunosorbent assay (ELISA). First, titration experiments were carried out to examine the effect of a wide range of BioBran concentrations (1-1,000 μg/ml) on TNF-α production. Figure 3 demonstrates that BioBran is a potent TNF inducer. The effect was dose-dependent. The level of TNF-α did not change at 1 to 10 μg/ml but increased at a concentration of 0.1 mg/ml and maximized at concentration of 1 mg/ml. IL-2 alone had no effect on TNF-α production; however, a synergistic effect of BioBran and IL-2 was observed.

These results show that treating highly purified NK cells with BioBran also resulted in increased levels of TNF-α and IFN-γ secretion in conjunction with augmentation of NK cell cytotoxic function.
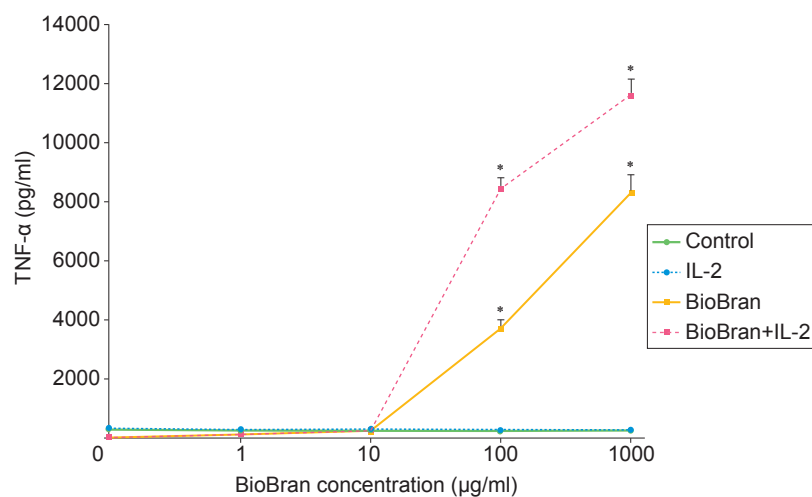
Fig. 3 Increase of TNF-α production from PBLs cultured with BioBran and/or IL-2

PBLs were incubated with a wide range of BioBran concentrations (1-1,000 µg/ml) in the presence or absence of IL-2 (500 U/ml) for 16 hours. Supernatant was harvested and subjected to ELISA. *p<0.001. PBLs: Peripheral blood lymphocytes.

Ghoneum M, Jewett A. "Production of Tumor Necrosis Factor-α and Interferon-γ from Human Peripheral Blood Lymphocytes by a Modified Arabinoxylan from Rice Bran (BioBran/MGN-3), and Its Synergy with Interleukin-2 *In Vitro*", Cancer Detection and Prevention. 24(4):314-24., 2000.

7.2: Augmentation of Macrophage Phagocytosis by Modified Arabinoxylan Rice Bran (BioBran)

The effect of BioBran on macrophage function was examined in this study.

7.2.1: Effect of BioBran on Stimulation of Phagocytosis

Human macrophage cell line (U937) cells and murine peritoueal macrophages (P-Mφ) were treated with BioBran (100 and 500 µg/ml) for two days. Cells were then cultured with yeast and the percentage of phagocytosis was examined at 2 hrs.

7.2.2: Percent of Phagocytosis

Treatment of both cell types with BioBran resulted in an increase in the percent of phagocytosis. Treatment of U937 cells with BioBran (100 µg/ml) resulted in a 200% increase in the percent of phagocytosis, and 120% as the concentration of BioBran reached 500 µg/ml, as compared with control cells. With respect to P-Mφ, BioBran in a dose dependent manner elicited a notable response. At 100 µg/ml concentration, the percent of phagocytosis climbed to 150%, as compared to the control cells (Fig. 4).

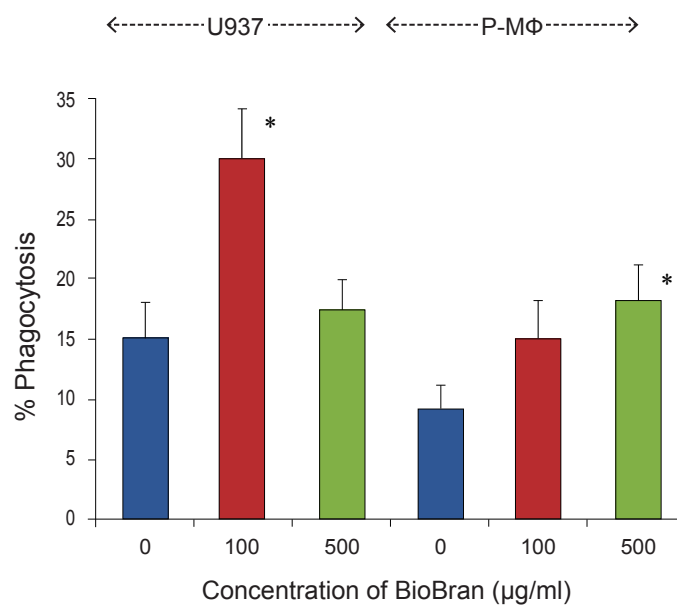This study demonstrates that BioBran is a potent inducer of phagocytic function by macrophage.

Partly modified from original papers.

Fig. 4 Effect of BioBran on percent of phagocytosis of yeast by two macrophage models

Human U937 and murine P-Mφ were treated with BioBran for 48 hr, and then incubated with yeast for 2 hr in a ratio of 1:10. Data represent the mean SD of 3 different experiments, *p<0.05; as compared to control untreated cells.

Ghoneum M, Matsuura M. "Augmentation of Macrophage Phagocytosis by Modified Arabinoxylan Rice Bran (MGN-3/BioBran)", International Journal of Immunopathology and Pharmacology. Sep-Dec;17(3):283-92., 2004.

7.3: BioBran-augmented Maturation of Human Monocyte-derived Dendritic Cells

BioBran was tested for its possible effects on *in vitro* maturation of human dendritic cells (DC).

Monocytes isolated from peripheral blood of healthy subjects were cultured for 6 days in AIM-V complete medium supplemented with granulocyte macrophage colony-stimulating factor (GM-CSF) (1,000 IU/ml) and interleukin (IL)-4 (1,000 IU/ml) to generate immature dendritic cells (iDC). On day 7 the medium was exchanged and cells were cultured in complete AIM-V medium containing GM-CSF (1,000 U/mL) and IL-4 (1,000 U/mL) in the presence or absence of maturation stimuli for further 2 days. Cytokine maturation mix 1 (CMM1; tumor necrosis factor-α (10 ng/ml), IL-1β (10 ng/ml) and IL-6 (10 ng/ml)), and cytokine maturation mix 2 (CMM2; Lipopolysaccharide (250 ng/ml) and Interferon-γ (1,000 IU/ml)) with or without addition of BioBran (10, 100, 400 and 1,000 μg/ml) were used as stimuli for maturation of DC into matDC1 and matDC2, respectively.

BioBran downregulated the expression of monocyte marker cluster of differentiation (CD) 14 (Fig. 5) on the surface of iDC, but extensively increased the expression of CD83 (Fig. 5) a marker of mature DC, both in a dose dependent manner.

These results demonstrate that BioBran is a potent enhancer of DC maturation.
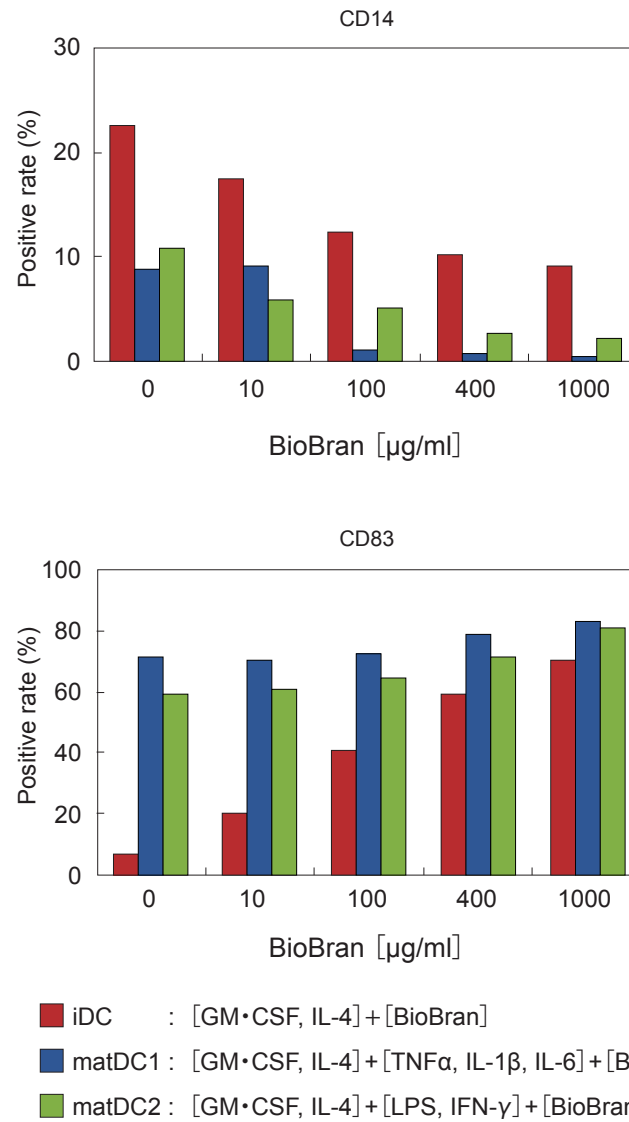
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Fig. 5 Maturation of human dendritic cells by BioBran in *in vitro*

The effect of BioBran on the expression of differentiation/maturation antigens on DC subsets. The surface expression of CD14 and DC maturation marker CD83 was examined by flow cytometry on HLA-DR positively gated iDC, matDC1 and matDC2. The percentage of cell positivity is shown and is representative of three independent experiments.

Cholujova D, Jakubikova J, Sedlak J. "BioBran-augmented Maturation of Human Monocyte-derived Dendritic Cells.", Neoplasma. 56(2):89-95., 2009.

7.4: BioBran Enhances Generation of Cytotoxic CD8+ T Cells via Upregulation of DEC-205 Expression on Dendritic Cells

This study explored the ability of BioBran-activated Dendritic Cells (DC) to prime cluster of differentiation (CD) 8+ T cells and examined the mechanisms underlying its effects.

7.4.1: DEC-205 Expression

DCs were either treated with BioBran (20 and 40 µg/ml) or lipopolysaccharide (LPS) (100 ng/ml) or left untreated. The level of DC activation was examined under all three conditions. The data depicted in Fig. 6 displays the DEC-205, a member of the macrophage mannose receptor family of C-type lectin endocytic receptors, expression. Flow cytometry analysis revealed increased expression of DEC-205 in BioBran-activated DCs in a dose-dependent manner.
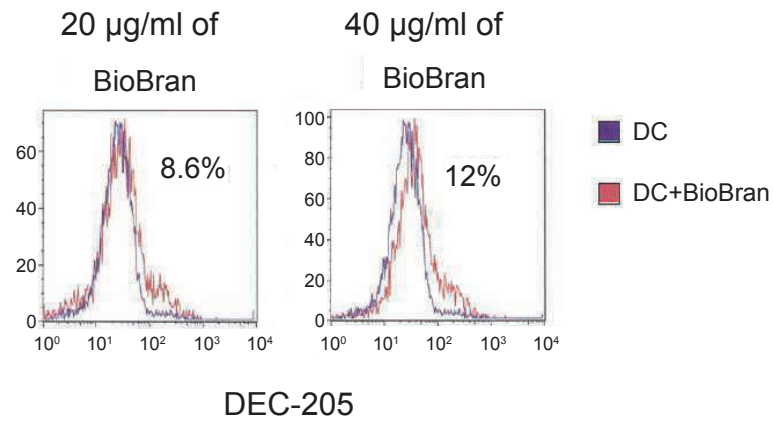

Fig. 6 Increased expression of DEC-205 in BioBran-activated DCs

BioBran activates DCs to induce DEC-205. Histogram depicts the percent positive DEC-205 expression in dose-dependent manner of BioBran (20 and 40 µg/ml).

7.4.2: BioBran Induces Granzyme-expressing Cytotoxic CD8+ T cells

DCs also induce the generation of cytotoxic CD8+ T cell responses. To explore this, we determined the effect of BioBran-stimulated DCs on CD8+ T cells. DCs cultured for 24 hrs with BioBran (at concentration of 20 mg/ml) were washed and cultured with purified CD8+ T cells. Seven days later the cells were collected and stained for intracellular granzyme B. DCs stimulated with BioBran induced significantly higher levels of granzyme B-positive CD8+ T cells ($p<0.05$) as compared to unstimulated DC-CD8+ T cells (Fig. 7). These data suggest that stimulation of DCs through BioBran is highly effective in priming cytotoxic T-cell responses.
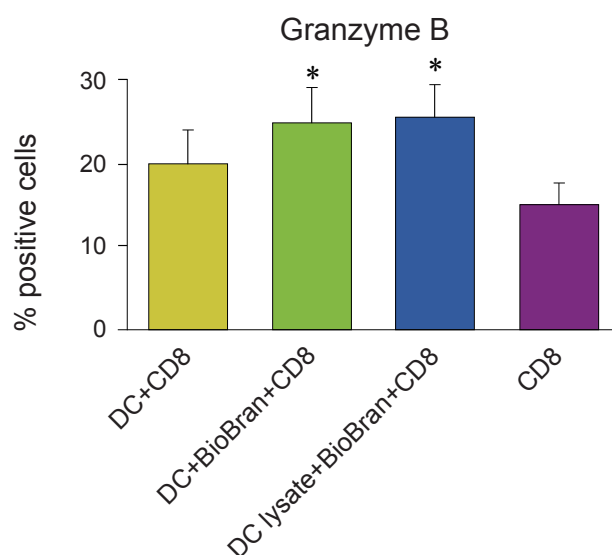
Fig. 7 Effect of BioBran in inducing granzyme expressing cytotoxic CD8+ T cells

BioBran (at concentration of 20 μg/ml) induces granzyme expressing cytotoxic CD8+ T cells. DCs activated with BioBran and tumor cell lysate and BioBran co-cultured with CD8+ T cells for 7 days.

7.4.3: BioBran-stimulated DCs Prime CD8+ T cells with Higher Cytolytic Activity

Granzyme-expressing CD8+ T cells are able to kill tumor cells. PC3 (prostate cancer cell line) cells were used as tumor target cells. PC3-specific CD8+ T cells were generated by pulsing peripheral blood mononuclear cells (PBMCs) with tumor lysate of PC3 for 10 days. To determine the killing or lysis, purified CD8+ T cells (effectors) were co-cultured with CFSE-labeled PC3 cells as targets. Target: effector (T:E) ratios were 1:25 and 1:50. Four hours later 7-AAD was added to the cells to stain dead cells. Controls included CFSE-stained PC3 cells without effectors and 7AAD-and CFSE-stained PC3 cells. Analysis was performed by gating on the target cells and measuring the 7AAD-negative vs 7AAD-positive cells. Cells positive for both 7-AAD and CFSE were considered lysed.

Figure 8 shows results (average with +/- S.E) from 5 experiments. Unstimulated DCs primed CD8+ T cells as well as CD8+ T cells alone show -24% and -18% lysis, while BioBran stimulated DCs with -40% lysis and tumor lysate pulsed BioBran stimulated DCs with -44% lysis ($p<0.05$ as compared with unstimulated DC-CD8+ T cells). These data clearly suggest that stimulation of DCs through BioBran induces highly cytolytic CD8+ T cells. Tumor lysate-pulsed BioBran-activated DCs stimulated CD8+ cell killing of tumor cells at an even higher rate.

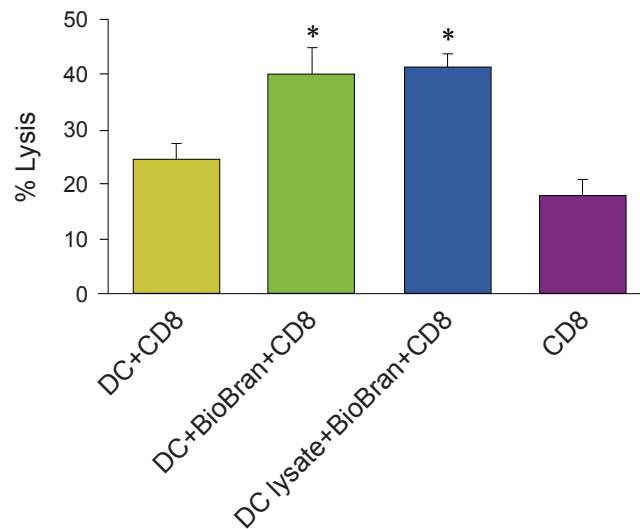The results indicate that treatment with BioBran caused DCs to prime higher granzyme B-expressing CD8+ T cells.

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Fig. 8 Effect of BioBran in priming cytolic CD8+ T cells

BioBran stimulation primes higher cytolic CD8+ T cells. DCs activated with BioBran (at concentration of 20 µg/ml) and tumor cell lysate plus BioBran co-cultured with CD8+ T cells for 7 days. Purified CD8+ T cells (effectors) were co-cultured with CFSE labeled PC3 cells (target) at target: effector ratio of 1:50. Cells positive for both 7-AAD and CFSE were considered lysed. Flow cytometry studies show the percent specific lysis of PC3. Data from one representative experiment is shown in (A) and ±S.E. of 5 experiments in (B). *(p<0.05) as compared with unstimulated DC-CD8+ T cells.

Ghoneum M, Agrawal S. "MGN-3/BioBran Enhances Generation of Cytotoxic CD8+ T Cells via Upregulation of DEC-205 Expression on Dendritic Cells", International Journal of Immunopathology and Pharmacology. Oct-Dec; 27 (4): 523-30., 2014.

7.5: Anti-HIV Activity *in Vitro* of BioBran, an Activated Arabinoxylan from Rice Bran

BioBran was tested for anti-HIV activity *in vitro*. Peripheral blood mononuclear cells (PBMCs) from 3 healthy individuals were incubated (37°C) with phytohemagglutinin (PHA) (5 µg/ml) for 3 days and then washed before incubation (37°C, 1hr) with HIV-1 SF strain (HIV-1 p24 of 3,000 pg/10^6 cells). PBMCs were then washed 3 times with phosphate buffered saline (PBS) to remove unbound virus. Infected cells were incubated (37°C, 7 days) either with or without BioBran at various concentrations (0-100 µg/ml), in a prepared medium. Half of the medium was changed twice per week with corresponding BioBran concentrations.

7.5.1: Production of HIV-1 p24 Antigen

BioBran inhibited HIV-1 replication in PBMCs in a dose dependent manner. As shown in Table 1, BioBran caused inhibition in HIV p24 antigen production in all subjects; however, there was a clear differential response among different individuals towards BioBran inhibitory effect by BioBran. The effect of BioBran at low concentration (12.5 µg/ml) on subject I was minimal (5.5%) while the same dose caused 34% antigen production in subject II. Similarly, at high concentrations (100 µg/ml) of BioBran, the percentage of p24 antigen inhibition varied greatly among the three subjects (59% - 90%). Data in Fig. 9 summarizes the mean and SD of the results depicted in Table 1. At concentrations of 25, 50, and 100 µg/ml, BioBran demonstrated 18.3, 42.8, 59 and 75% inhibition in the production of HIV-1 p24 antigen, respectively.

Table 1 Dose dependent inhibition of HIV-1 replication by BioBran

BioBran dosage (µg/ml)	HIV-1 p24, pg/ml		
	Subject I	Subject II	Subject III
0	565 (100%)	720 (100%)	433 (100%)
12.5	534 (94.5%)	480 (66.7%)	364 (84.1%)
25	325 (57.5%)	333 (46.3%)	294 (67.9%)
50	263 (46.5%)	139 (19.3%)	248 (57.3%)
100	132 (23.4%)	77 (10.7%)	178 (41.1%)

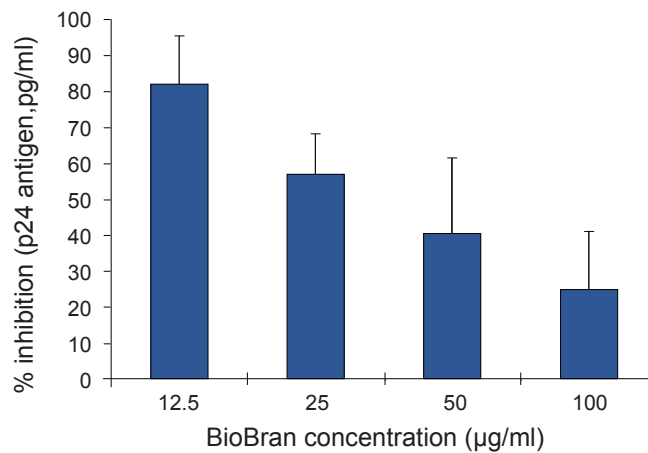Note. Data from three different subjects examined at 7 days.

Fig. 9 Effect of BioBran on production of HIV-1 p24 antigen

Data represent mean ± S.D. of three different individuals from Table 1

Ghoneum M. "Anti-HIV Activity *in Vitro* of MGN-3, an Activated Arabinoxylane from Rice Bran", Biochemical and Biophysical Research Communications. Feb 4;243(1):25-9., 1998.

7.6: Modified Arabinoxylan Rice Bran (BioBran) Sensitizes Human T Cell Leukemia Cells to Death Receptor (CD95)-induced Apoptosis

This study investigated the effect of BioBran on death receptor-induced apoptosis in the human leukemic HUT78 cell line. Expression of cluster of differentiation (CD) 95 and Bcl-2 were measured by flow cytometry.

7.6.1: BioBran Sensitizes HUT78 Cells to Death Receptor-mediated Apoptosis

Leukemia cell line (HUT78) was pre-treated with BioBran and then incubated with agonistic anti-CD95 antibody. Twenty-four hours post-incubation, apoptosis was determined by the PI technique, using a FACScan flow cytometer. Specific apoptosis was calculated as the percentage of experimental apoptosis-percentage of spontaneous apoptosis. The data in Fig. 10 shows data from three independent experiments. BioBran alone at concentrations of 100 - 1,000 µg/ml had minimal effect on apoptosis (specific apoptosis 2.5 - 4.5%). Anti-CD95 antibody induced apoptosis in 29% of HUT78 cells (specific apoptosis = 20%). However, when leukemic cells were pre-treated with BioBran, followed by the anti-CD95 antibody, a significant increase in the number of apoptotic cells (specific apoptosis 35 - 42%) ($p<0.01$) was noted.

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This represents a 200% increase as compared to anti-CD95 antibody alone. Lower concentration <100 µg/ml did not sensitize HUT78 cells to anti-CD95 antibody-induced apoptosis.
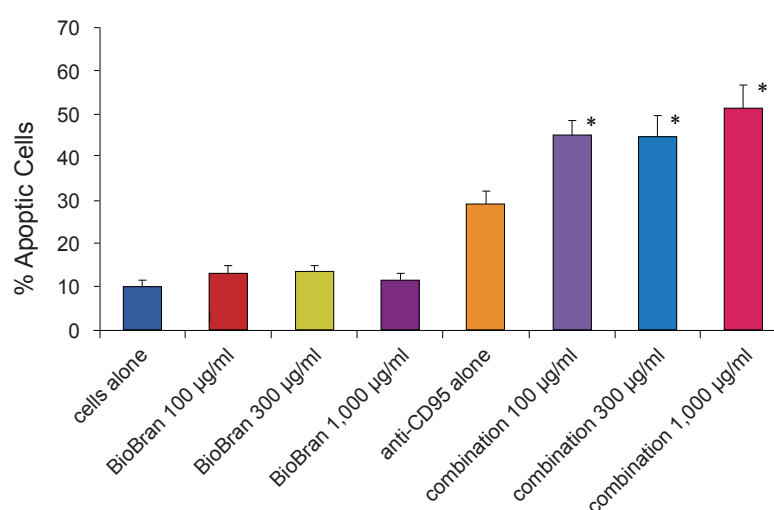

Fig. 10 Effect of BioBran on anti-CD95 antibody-induced apoptosis

Cells (HUT78) were pre-treated with BioBran for 3 hrs, and then were incubated with agonistic anti-CD95 antibody. Apoptotic cells were determined by PI technique using FACScan flow cytometer. This figure represents the mean ± SD of three experiments. Combination=BioBran at 100, 300, 1,000 µg/ml by anti-CD95 antibody. *Significant at p<0.01.

7.6.2: BioBran Downregulates Bcl-2 Expression

Bcl-2 is an anti-apoptotic molecule that is shown to protect the cells from apoptosis induced by diverse agents. To further investigate the effect of BioBran, we tested its effect on Bcl-2 expression. The data in Table 2 shows that BioBran caused a significant decrease in the level of expression of this anti-apoptotic protein.

Table 2 Effect of BioBran on the expression of Bcl-2

Cells treated with	Bcl-2 positive cells (%)	MFC
None	100	740
BioBran	100	559*

HUT78 cells (1×10^6 cells/ml) were cultured in the absence or presence of BioBran (1,000 µg/ml) for 24 hr. Expression of Bcl-2 was determined by staining the cells with anti-human Bcl-2 antibody and flow cytometry. MCF = mean fluorescence channel number. *Significantly different from control untreated cells D=0.30.

These results suggest that BioBran increases the susceptibility of cancer cells to undergo apoptosis mediated by death ligands, which may be relevant for anti-cancer activities.

Ghoneum M, Gollapudi S. "Modified Arabinoxylan Rice Bran (MGN-3/BioBran) Sensitizes Human T Cell Leukemia Cells to Death Receptor (CD95)-induced Apoptosis", Cancer Letters. Nov 10; 201(1):41-9., 2003.

7.7: BioBran, Modified Arabinoxylan from Rice Bran, Sensitizes Human Breast Cancer Cells to Chemotherapeutic Agent, Daunorubicin

This study evaluated the chemo-sensitizing activity of BioBran against human breast cancer

cells (BCCs) *in vitro*.

BCCs (MCF-7) were cultured with different concentrations of daunorubicin (DNR) (from 1×10^{-9} to 1×10^{-6} M) in the presence or absence of selected concentrations of BioBran (100 - 1,000 μg/ml) for 3 days. Cancer cell survival and the IC_{50} values were determined.

DNR inhibited the survival of MCF-7 cells in a dose-dependent manner. The IC_{50} of DNR was 1 μM. However, when MCF-7 cells were co-cultured with BioBran and DNR, the IC_{50} of DNR against MCF-7 cells was significantly reduced (IC_{50} 0.2 μm). Data in Fig. 11 shows that BioBran at concentrations of 100, 500 and 1,000 μg/ml decreased the DNR IC_{50} of MCF-7 cells by 3-, 5- and 5.5-fold, respectively, as compared with DNR alone.

These data may suggest that BioBran in conjunction with chemotherapy may be useful for the treatment of breast cancer.
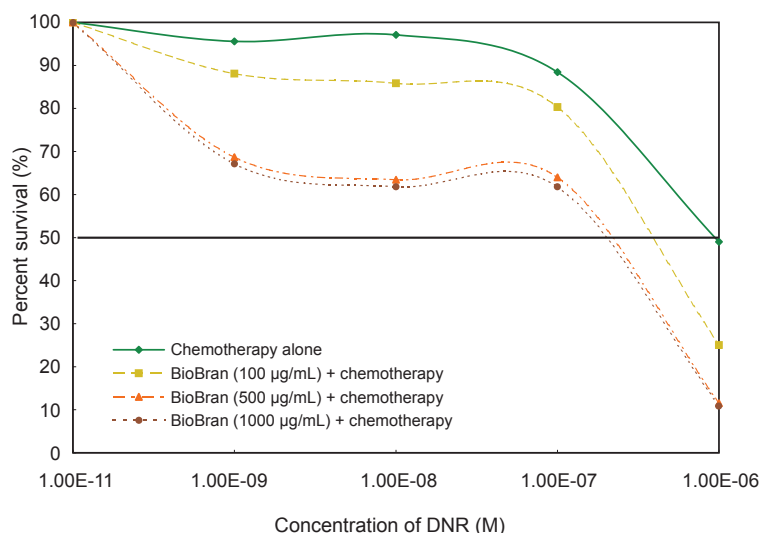

Fig. 11 Effect of BioBran on daunorubicin cytotoxicity in human breast cancer cells

MCF-7 cells (1×10^4 well^{-1}) were seeded in 96-well plates with DNR (1×10^{-9} to 1×10^{-6} M) in the presence or absence of various concentrations of BioBran (100-1000 mg/ml) for 3 days. Cell survival was determined using an MTT assay. MCF-7 cells: the data represents the mean ±S.D. of six experiments, each experiment in triplicate.

Gollapudi S, Ghoneum M. "MGN-3/BioBran, Modified Arabinoxylan from Rice Bran, Sensitizes Human Breast Cancer Cells to Chemotherapeutic Agent, Daunorubicin", Cancer Detection and Prevention. 32 (1):1-6., 2008.

7.8: Arabinoxylan Rice Bran (BioBran) Enhances Natural Killer Cell-mediated Cytotoxicity against Neuroblastoma *in Vitro* and *in Vivo*

This study evaluated the effect of BioBran on natural killer (NK) cell activation, expansion and cytotoxicity against neuroblastoma cells. NK cells were enriched with magnetic beads and stimulated with BioBran. NK cell activation was evaluated via analysis of their phenotype, and their expansion capability was tracked. The *in vitro* cytotoxic ability of the activated NK cell was

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tested against K562, Jurkat, A673, NB1691, A-204, RD and RH-30 cell lines.

The addition of BioBran-stimulated NK cells resulted in an increase in cluster of differentiation (CD) 69 and CD25 expression from a median of 9% - 88% and 6% - 90%, respectively. CD69 elevation on NK cells correlates with an increase in NK cell cytotoxicity. Proliferative potentials are indicated by CD25 expression elevation on NK cells.

To test the synergistic effect of interleukin (IL)-2 and BioBran, the study compared stimulation with high-dose IL-2 (1,000 IU/mL) with low dose IL-2 (40 IU/mL) and low dose IL-2+BioBran. Adding BioBran to low dose IL-2 further enhanced the stimulatory effect of 40 IU/mL IL-2 and resulted in comparable cytotoxicity to that obtained with 1,000 IU/mL IL-2 (Fig. 12). The result shows that BioBran upregulates NK cell activation markers, stimulates NK cell cytotoxic activity against neuroblastoma *in vitro* and selectively augments the expansion of NK cells. These result may be useful for future NK cell therapeutic strategies of the treatment of neuroblastoma.
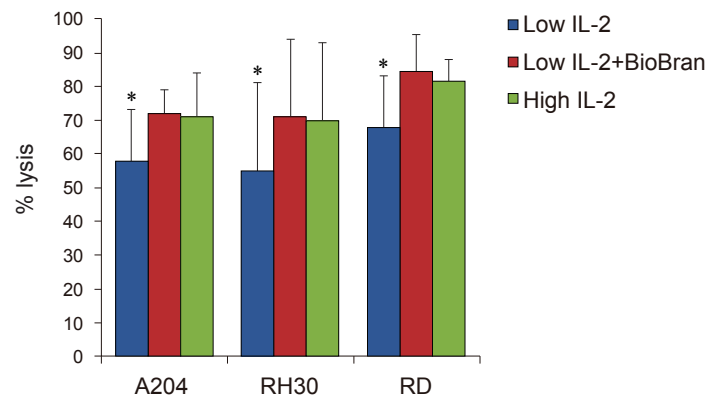

Fig. 12 IL-2- and BioBran-stimulated NK cell cytotoxic activity against A-204, RD and RH-30 (effector/ target 10:1)

Data include results from 3 healthy volunteers in 3 independent experiments. *Statistically significant

Pérez-Martínez A, Valentín J, Fernández L, Hernández-Jiménez E, López-Collazo E, Zerbes P, Schwörer E, Nuñéz F, Martín IG, Sallis H, Díaz MÁ, Handgretinger R, Pfeiffer MM. "Arabinoxylan Rice Bran (MGN-3/Biobran) Enhances Natural Killer Cell-mediated Cytotoxicity against Neuroblastoma *in Vitro* and *in Vivo*", Cytotherapy. May; 17(5):601-12., 2015.

8. ANIMAL STUDIES

8.1:Antioxidant Potential by Arabinoxylan Rice Bran (BioBran), Represents a Mechanism for Its Oncostatic Effect against Murine Solid Ehrlich Carcinoma (SEC)

This study examined the antioxidant system as another possible mechanism through which BioBran exerts its oncostatic potential.

8.1.1: The Activity of Antioxidant Scavenger Enzymes

The effect of BioBran on the activity of antioxidant scavenger enzymes in normal and Solid Ehrlich Carcinoma (SEC) tumor-bearing mice was examined. These enzyme include glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT). Data in Table 3 show that administration of BioBran solely revealed no significant change in GST, SOD and CAT activities of blood and liver when compared to the normal control group but a significant increase in liver GPx level was recorded when compared with the normal control group.

The activities of these enzymes in the blood and liver of the SEC group was significantly lower ($p<0.05$, $p<0.01$) than that of their corresponding control counterparts (untreated control group and BioBran group). Treatment with BioBran to SEC-bearing mice, elevated GPx, GST, SOD and CAT activity in blood and liver to be comparable with the normal levels; however, the effect of early treatment with BioBran at 4 days post-tumor cell inoculation was more remarkable than late treatment at 11 days as compared to the SEC control group. Early and late treatment with BioBran markedly augmented GPx, GST, SOD and CAT activity in the tumor tissue ($p<0.01$) when compared with the untreated SEC values. Such antioxidant enzyme augmentation in the tumor tissue was more pronounced with the early treatment of BioBran at day 4 post-tumor cell inoculation.

In conclusion, BioBran-induced oncostatic activity by modulating lipid peroxidation, augmenting the antioxidant defense system and protecting against oxidative stress.

Table 3 Effect of BioBran administration on antioxidant enzymes, GPx, GST, SOD and CAT activity, in blood, liver and tumor tissues of different experimental groups

Parameters / Groups	GPx Blood (U[*]/mg Hb)	GPx Liver (U[*]/mg protein)	GPx Tumor (U[*]/mg protein)	GST Plasma (U[#]/mg Hb)	GST Liver (U[#]/mg protein)	GST Tumor (U[#]/mg protein)	SOD Blood (U[@]/mg Hb)	SOD Liver (U[@]/mg proein)	SOD Tumor (U[@]/mg protein)	CAT Blood (U[†]/mg Hb)	CAT Liver (U[†]/mg protein)	CAT Tumor (U[†]/mg protein)
Vehicle Control	c 41.12±6.323	bc 6.20±0.295		4.064±0.246	c 0.58±0.028		c 5.60±0.110	c 19.38±1.675		4.34±0.085	c 2.853±0.194	
BioBran	c 40.03±2.929	aCe 8.02±0.829		C 4.43±0.402	CE 0.75±0.106		c 5.77±0.325	C 20.41±0.665		Ce 4.90±0.212	C 3.17±0.081	
% Change◇	-2.7%	29.34%		9.032%	29.34%		3.07%	5.34%		13.07%	11.18%	
SEC	abc 27.11±2.491	aBDe 4.06±0.255	De 1.33±0.076	BDe 3.31±0.144	aBD 0.40±0.013	DE 0.058±0.0031	abde 4.74±0.210	aBD 15.22±0.979	DE 24.03±1.434	BD 3.83±0.235	aBD 2.27±0.118	DE 3.54±0.212
% Change◇	-34.07%	-34.46%		-18.67%	-31.43%	59.25%	-15.46%	-21.45%		-11.62%	-20.54%	
SEC+ BioBranE	c 40.27±3.313	C 7.50±0.554	C 2.306±0.241	C 4.66±0.280	Ce 0.69±0.046	Ce 0.092±0.0021	c 5.82±0.458	C 21.45±0.424	C 32.70±0.631	Ce 4.85±0.329	C 3.18±0.173	C 4.58±0.189
% Change◇	-2.058%	20.98%	73.72%	14.72%	18.30%	59.25%	3.94%	10.67%	36.15%	11.71%	1.12%	29.55%
SEC+ BioBranL	c 37.54±2.934	bc 6.087±0.864	c 1.908±0.186	c 4.34±0.164	Bcd 0.50±0.072	Cd 0.077±0.0058	C 5.60±0.107	18.28±1.715	C 30.74±0.443	bd 4.17±0.179	2.71±0.243	C 4.39±0.192
% Change◇	-8.714%	-1.84%	43.74%	6.70%	-14.63%	33.5%	0.07%	-5.67%	27.94%	-3.78%	-5.06%	24.11%

Date are means of 6 mice/group±SEM. ◇% change of vehicle control.
a, A - significantly different from vehicle control group at $p<0.05$ and $p<0.01$, respectively.
b, B - significantly different from BioBran group at $p<0.05$ and $p<0.01$, respectively.
c, C - significantly different from SEC group at $p<0.05$ and $p<0.01$, respectively.
d, D - significantly different from SEC+BioBranE at $p<0.05$ and $p<0.01$, respectively.
e, E - significantly different from SEC+BioBranL at $p<0.05$ and $p<0.01$, respectively.
* µg of GPx consumed for oxidized 1 µmole of GSH/min.
# µmoles of CDNB-GSH conjugate formed/min.
@ Enzyme required for 50% inhibition of NBT reduction/min.
† µmoles of H_2O_2 utilized/min.
◇ µmoles of H_2O_2 utilized/min.

Noaman E, Badr El-Din NK, Bibars MA, Abou Mossallam AA, Ghoneum M. "Antioxidant Potential by Arabinoxylan Rice Bran, MGN-3/BioBran, Represents a Mechanism for Its Oncostatic Effect against Murine Solid Ehrlich Carcinoma", Cancer Letters. Sep 18;268(2):348-59., 2008.

Partly modified from original papers.

8.2: *In Vivo* Tumor Inhibitory Effects of Nutritional Rice Bran Supplement BioBran on Ehrlich Carcinoma-Bearing Mice

This study investigated the *in vivo* anti-tumor activity of BioBran.

Swiss albino mice were inoculated intramuscularly in the right thigh with Ehrlich ascites carcinoma (EAC) cells. Post-tumor cell inoculation, mice bearing a solid Ehrlich tumor received IP (intraperitoneal) injections of phosphate-buffered solution (PBS) or IP injections of BioBran at 40 mg/kg BW. Animals commenced treatment on Day 8 post-EAC cells inoculation with continued treatment 3 times a week for 3 weeks. Time interval measurements of TV (tumor volume) were conducted from Day 8 to Day 35.

Figure 13 shows that IP-administration of BioBran in tumor-bearing mice resulted in retardation of solid tumor development that became significant as early as Day 14 posttreatment. Photographs of the tumor isolated from BioBran-treated mice clearly demonstrate significant tumor regression as compared to PBS-treated mice.

The results of this study yield important information about the ability of BioBran to cause a rapid and significant tumor regression in mice.
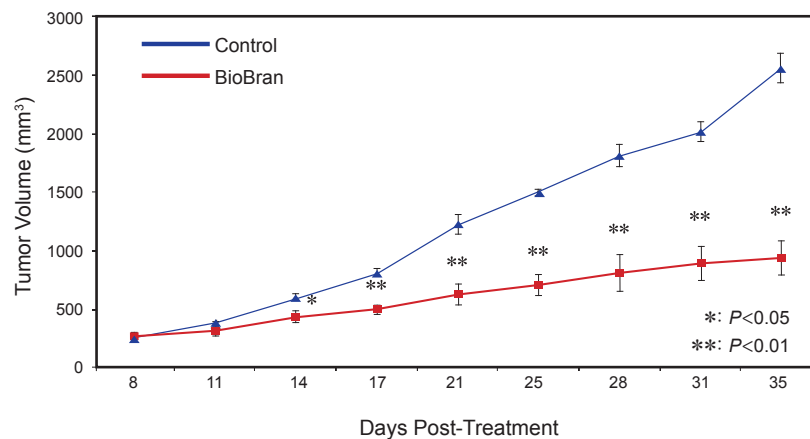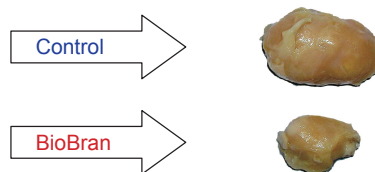

Fig. 13 *In-vivo* effect of IP injection of BioBran on tumor volume

Mice were inoculated in the right thigh with Ehrlich ascites carcinoma cells. At Day 8, after tumor cell inoculation, mice bearing solid Ehrlich carcinoma were injected with BioBran (■) and change of tumor volume (TV) mm^3 was examined at Day 8 until Day 35. The figure represents the change of TV mm^3 during the time course; results were compared with control phosphate-buffered solution (PBS)-treated mice-bearing tumor (▲). Each value represents the mean ± SE. *P<0.05, **P<0.01 as compared to PBS group at the corresponding time point.
Photograph of tumor regression. PBS-treated and BioBran-treated mice bearing tumor. Data is representative of all groups examined.

Badr El-Din NK, Noaman E, Ghoneum M. "*In Vivo* Tumor Inhibitory Effects of Nutritional Rice Bran Supplement MGN-3/BioBran on Ehrlich Carcinoma-bearing Mice", Nutrition and Cancer. 60(2):235-44., 2008.

8.3: Arabinoxylan Rice Bran (BioBran) Provides Protection against Whole-Body γ-irradiation in Mice via Restoration of Hematopoietic Tissues

This study examined the protective effect of BioBran on overall maintenance of hematopoietic tissue after γ-irradiation.

8.3.1: Body Weight Change

The 57 mice were divided into four groups (G1 - G4). Group G1 served as the untreated vehicle saline control group (receiving neither irradiation nor BioBran). Group G2 received only BioBran every other day. Group G3 received only whole body γ-irradiation at 2 weeks after the beginning of the experiment. Group G4 was pretreated with BioBran for 2 weeks then exposed to irradiation and continued to receive BioBran every other day. Animals from all groups were euthanized at 1 and 4 weeks after radiation. Parameters under investigation include fluctuations in body and organ weights, complete blood count (CBC), histopathology of multiple organs and evaluation of oxidative stress biomarkers.

The data in Table 4 show body weight changes in mice at 1 and 4 weeks post-irradiation. The groups of mice without irradiation (control and BioBran only) showed comparable weight gain from Day 0 to the end of the experiment. Irradiated mice showed early weight loss at 1 week post-irradiation (-20% of control) ($p<0.01$), which was maintained at 4 weeks post-irradiation ($p<0.05$). However, treatment with BioBran prevented the early weight loss in irradiated mice, and maintained normal body weight throughout the 4 weeks.

Table 4 Body weight change/gm in mice at 1 and 4 weeks post-irradiation

	Groups			
	Control	BioBran	Irradiation	BioBran + Irradiation
Number of mice/group	13	14	15	15
Initial body weight (no treatment)	21.01 ± 0.48	21.68 ± 0.56 (3.16%)	22.37 ± 0.53 (6.44%)	22.01 ± 0.32 (4.74%)
2 weeks post-BioBram treatment	24.40 ± 0.66	25.42 ± 0.47 (4.20%)	24.15 ± 0.44 (-1.02%)	25.12 ± 0.61 (2.96%)
1 week post-irradiation	27.02 ± 0.82	28.75 ± 0.57*, †† (6.39%)	21.61 ± 0.49** (-20.03%)	26.64 ± 0.40#, †† (-1.41%)
Number of mice/group	6	6	6	7
4 weeks post-irradiation	29.30 ± 0.79	31.45 ± 0.75* (7.32%)	27.02 ± 0.65*, ## (-7.79%)	29.14 ± 0.63†, # (-0.54%)
Number of mice/group	7	8	9	8

*Significantly different from control group at 0.05 level. **Significantly different from control group at 0.01 level. #Significantly different from BioBran group at 0.05 level. ##Significantly different from BioBran group at 0.01 level. †Significantly different from irradiation group at 0.05 level. ††Significantly different from irradiation group at 0.01 level (% change of control group). Data represent changes in body weight ± SE.

8.3.2: WBC Counts and Differential

Data shown in Fig. 14 are summaries of changes in the total white blood cell counts (WBC) and the percentages of lymphocytes, monocytes, and neutrophils in mice under different treatment

conditions. The data show that irradiation caused leukopenia in mice at 1 week (p<0.01). However, BioBran treatment prevented leukopenia of irradiated mice (p<0.05). These values were maintained but not significant at 4 weeks.

Lymphopenia is also noted in the irradiated mice at 1 week post-irradiation, and again treatment with BioBran protected its level (p<0.01). These values were also noted at 4 weeks but were not significant. Irradiation caused an early neutrophilia at 1 week (p<0.01). However, BioBran limited the marked neutrophilia caused by irradiation to half its value. At 4 weeks, these values were not significant. In addition, irradiation caused a decreased monocyte count at 1 week that was prevented by treatment with BioBran.

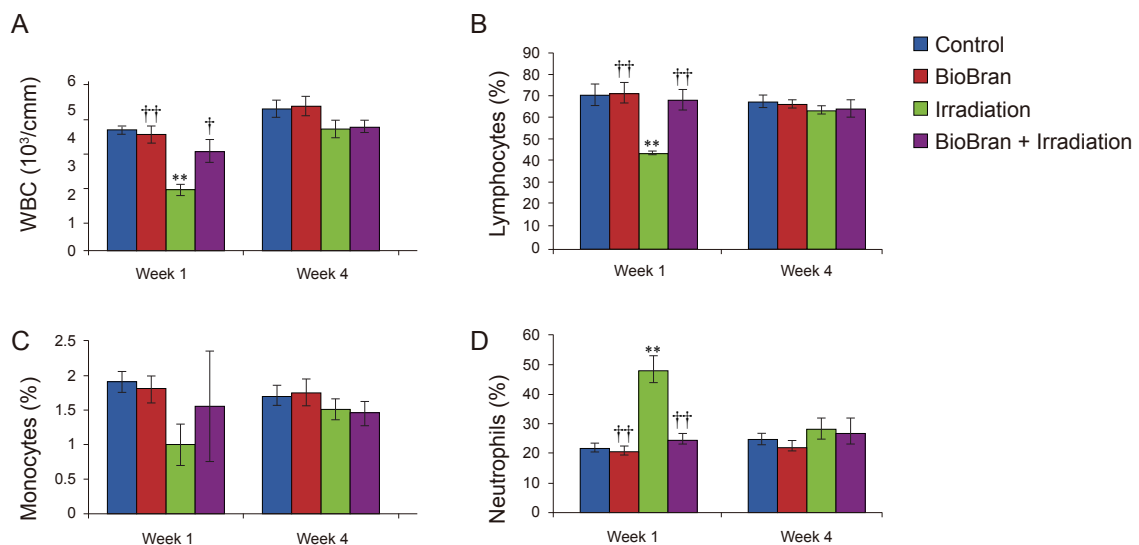The results indicated that BioBran has the ability to enhance the blood cellular radioresistance.

Fig. 14 WBC series in mice under different treatments at 1 and 4 weeks post-irradiation

A) Total WBC count. The percent B) lymphocytes, C) monocytes, and D) neutrophils were determined at 1 and 4 weeks after initial treatment. Number of mice per group is 6-9. Two asterisks indicate significant difference from the corresponding control group at the 0.01 level. One dagger indicates significant difference from the irradiation group at the 0.05 level. Two daggers indicate significant difference from the irradiation group at the 0.01 level (% difference from the control group).

Ghoneum M, Badr El-Din NK, Abdel Fattah SM, Tolentino L. "Arabinoxylan Rice Bran (MGN-3/BioBran) Provides Protection against Whole-body γ-irradiation in Mice via Restoration of Hematopoietic Tissues", Journal of Radiation Research. May; 54 (3):419-29., 2013.

9. HUMAN STUDIES

9.1: NK Immunorestoration of Cancer Patients by BioBran, A Modified Arabinoxylan Rice Bran (BioBran) (Study of 32 Patients Followed for up to 4 Years)

This study investigated the augmentory effect of BioBran on natural killer (NK) cell function and T and B cell proliferation in 32 patients. The study was carried out on 32 cancer patients. Patients had different types of malignancies: prostate, breast, multiple myeloma (MM), and leukemia. The majority of the patients first went through a debulking done using conventional therapies such as surgery, radiation, or chemotherapy.

9.1.1: NK Cell Activity

Figure 15 demonstrates the baseline values of cytotoxic responses of NK cells in 32 cancer patients. Patients demonstrated overall significant low level in NK function. Depression in NK activity was observed in patients with different types of malignancies as follows: prostate, 11.1 Lytic units (LUs); breast, 11.4 LUs; MM, 7.3 LUs; and leukemia, 4.3 LUs. Studies performed on peripheral blood lymphocytes from 12 participants one to two weeks after the primary studies revealed no statistically significant differences in NK cell activity in comparison with the initial results. Treatment with BioBran resulted in significant increase in NK activity up to tenfold. BioBran augmentory effect was detected in all types of malignancies: prostate, 41.9 LUs; breast, 33 LUs; MM, 31.9 LUs; and leukemia, 51.4 LUs. Individuals varied in response to augmentory effect of BioBran.
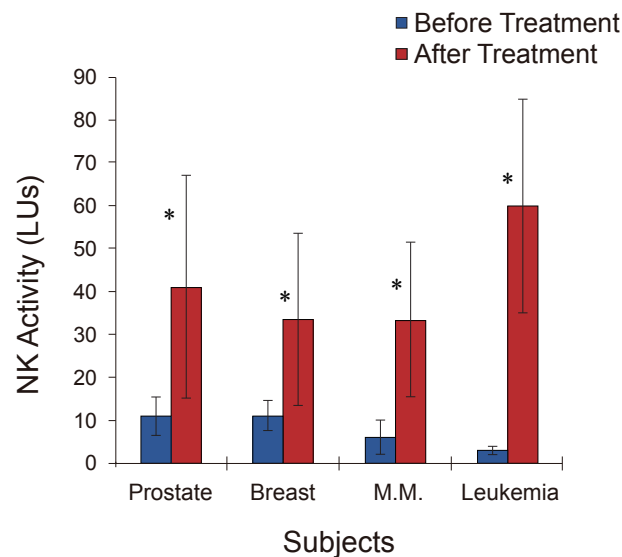

Fig. 15 Effect of BioBran on NK cell activity of 32 patients at one to two weeks after treatment

Malignancies were: prostate (10), breast (12), multiple myeloma—MM (5), and leukemia (5). LUs at 20% *p<0.001.

9.1.2: *In Vivo* T and B Lymphocyte Proliferation

Figure 16 shows that treatment with BioBran significantly increased T cell proliferation as indicated by their response to phytohemagglutinin (PHA) and concanavalin (Con) A mitogens. B cell proliferation also increased post-treatment with BioBran as indicated by their response to pokeweed mitogen (PWM), a B cell mitogen, as compared to baseline value.
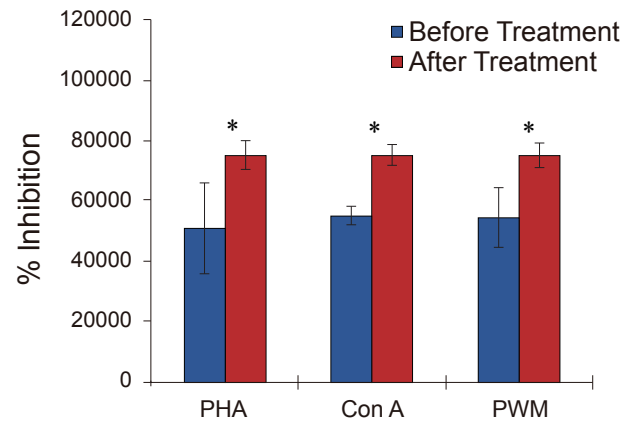

Fig. 16 *In vivo* action of BioBran on T and B cell mitogen response at one month after treatment

MNC were cultured for three days in the presence of PHA, Con A and PWM. Data represent mean (s.d. of five individuals. *p<0.001)

Ghoneum M, Brown J. "NK Immunorestoration of Cancer Patients by MGN-3, a Modified Arabinoxylan Rice Bran (Study of 32 Patients Followed for up to 4 Years)", Anti-Aging Medical Therapeutics. 3: 217-26., 1999.

9.2: The Life Prolongation and QOL Improvement Effect of Rice Bran Arabinoxylan Compound (BioBran) for Progressive Cancer

This study was designed to evaluate the effect of BioBran to improve the quality of life (QOL) of progressive and partially metastasized cancer patients.

The 205 cancer patients in late III - IV stages after surgery were treated with alternative therapies and anticancer drugs with lesser side effects. The patients were assigned to two groups; 109 patients continued the prescribed therapies (the control group), and 96 patients were administered with BioBran in addition to the prescribed therapies (the BioBran group) for 18 months. BioBran was given orally at 1 g, 3 times per day after meals. All patients were monitored for natural killer (NK) cell activity and the QOL. The QOL was evaluated by observation and enquiry during the study. Pain, malaise, and nausea were assessed by 4 grading scale and appetite by 3 to compare the scores before and after treatment.

The survival rate at 18 months of treatment was 54.2% in the BioBran group, and 33.9% in the control group, respectively. After administration of BioBran, NK activity also increased as did survival rate. The proportion of patients with unchanged or increased NK activity was higher in the BioBran group than in the control group, resulting in a 1.5 times higher survival rate in the former group (Table 5).

These findings indicate that NK activity can be used as a pathological index in progressive cancers. The QOL improvement by the administration of BioBran was also observed (Table 6).

Table 5 Relation among total survival rate, NK activity and survival rates in 2 groups

Group	BioBran group	Control group
Total survival rate	52/96 (54.2%)	19/56 (33.9%)
NK activity category		
<19.9 %	17/40 (42.5%)**	2/16 (12.5%)
20 - 40 %	18/35 (51.4%)*	7/25 (28.0%)
≥40 %	17/21 (81.0%)	10/15 (66.7%)

** : *p*<0.01   * : *p*<0.05

Table 6 QOL amelioration

QOL	Pain			Fatigue			Nausea			Appetite		
	Before	After	%	Before	After	%	Before	After	%	Before	After	%
Control group	2.9	2.5	-13.8	3.5	2.9	-17.1	2.5	2.9	+16.0	1.6	1.9	+18.8
BioBran group	2.2	1.9	-13.6	2.9	2.4	-17.2	2.3	2.0	-13.0	1.7	2.1	+23.5

(-) : indicates negative factors (pain, fatigue, and nausea) decrease
(+) : indicates improvement in appetite

Takahara K, Sano K. "The Life Prolongation and QOL Improvement Effect of Rice Bran Arabinoxylan Derivative (MGN-3, BioBran) for Progressive Cancer", Clinical Pharmacology and Therapy. 14(3):267-71., 2004.

9.3: Arabinoxylan Rice Bran (BioBran) Enhances the Effects of Interventional Therapies for the Treatment of Hepatocellular Carcinoma: A Three-year Randomized Clinical Trial

This study examined the efficacy of BioBran in conjunction with an interventional therapy (IT) for the treatment of hepatocellular carcinoma (HCC) patients.

Sixty-eight patients (54 males, 14 females) with HCC, aged 30 - 68 years, participated in the study. The patients were admitted to the 108 Military Central Hospital in Hanoi, Vietnam and were randomly divided into two groups using a computer-generated randomization list: the IT group and the IT+BioBran group. Both patients and investigators were blinded. The IT group (30 patients, age 50 ± 17 years: 24 males, 6 females) was treated with IT alone, while the IT+BioBran group (38 patients, age 49 ± 19 years: 30 males, 8 females) was treated with IT+BioBran for three years (age values represent mean ± standard deviation).

9.3.1: AFP Levels

Alpha-fetoprotein (AFP) levels in HCC patients were examined before and after treatment according to the total tumor volume, and the patients were clustered into sub-groups with tumor volume ≤200 cm^3 or >200 cm^3 in the IT group and the IT+BioBran group, and the final AFP level measurements are displayed in Table 7. Using the non-parametric Mann Whitney U-test, the IT group as a whole showed an increase in AFP levels of 7% (p = 0.2), relative to the before treatment value of AFP. Specifically, an increase in AFP levels ranging from 6 to 8% was noted in patients with a total tumor volume ≤200 cm^3 or >200 cm^3. In contrast, the IT+BioBran group showed a significant decrease of AFP levels with a change of 38% relative to values before treatment (p<0.001). In particular, a significant decrease of AFP levels ranging from 30 to 49% (p<0.05) was observed in patients with tumor volumes ≤200cm^3 and >200 cm^3 (Table 7).

Table 7 Effect of treatment on AFP levels (whole groups)

Treatment	Tumor volume (cm^3)	Patients		AFP level (ng/ml)		% Change
		Number	%	Before treatment	After treatment	
IT	≤200	17	57	292.8	316.5	+8%
	>200	13	43	431.7	456.1	+6%
	10-1320[†]	30	100	353.0	376.97	+7%
IT+BioBran	≤200	22	58	509.0	356.8	-30%
	>200	16	42	511.4	260.4	-49%
	12-1200[†]	38	100	510.0	316.2	-38%

[†]Range of tumor volumes in group. Data represent the mean AFP level. % Change = 100 × [(value after treatment) - (value before treatment)] / (value before treatment). IT: interventional therapy

9.3.2: Tumor Volume

Tumor volumes were examined in HCC patients post-treatment and the results were categorized into two sub-groups according to the whole tumor volume, ≤200 cm^3 or >200 cm^3. Surprisingly, the IT group had almost no change in tumor volume (0.2%). In contrast, the IT+BioBran group had an overall reduction of tumor volume by 36% (Table 8). Specifically, patients in the IT+BioBran group with tumor volumes ≤200 cm^3 and >200 cm^3 experienced a significant decrease in average tumor volume by 25% (p<0.05) and 40% (p<0.05), respectively. However, the patients in the corresponding IT groups showed no significant change in tumor volume. Using the Mann Whitney U-test, patients in the IT+BioBran group also showed a significant change in tumor volume compared with the control group (z = 2.5 and p = 0.01).

Table 8 Effect of treatment on tumor volume (whole groups)

Treatment	Tumor volume (cm^3)	Patients		Treatment		% Change
		Number	%	Before treatment	After treatment	
IT	≤200	17	57	92.7 ± 12.3	97.6 ± 35	+5%
	>200	13	43	460.4 ± 76.1	454.9 ± 106.7	-1%
	10-1320[†]	30	100	252.0 ± 258.7	252.5 ± 324.3	+0.2%
IT+BioBran	≤200	22	58	125.9 ± 13.6	94.1 ± 22.3	-25%
	>200	16	42	481.7 ± 56.6	288.3 ± 47.9	-40%
	12-1200[†]	38	100	275.7 ± 234.1	175.8 ± 174.9	-36%

[†]Range of tumor volumes in group. Data represent mean ± standard deviation. %Change = 100× [(value after treatment) - (value before treatment)] / (value before treatment) IT: interventional therapy

Bang MH, Van Riep T, Thinh NT, Song Le H, Dung TT, Van Truong L, Van Don L, Ky TD, Pan D, Shaheen M, Ghoneum M. "Arabinoxylan Rice Bran (MGN-3) Enhances the Effects of Interventional Therapies for the Treatment of Hepatocellular Carcinoma: A Three-year Randomized Clinical Trial", Anticancer Research. Dec;30(12):5145-51., 2010.

9.4: BioBran Arabinoxylan Rice Bran Modulates Innate Immunity in Multiple Myeloma Patients

A randomized, placebo-controlled study was performed to examine the effects of BioBran on innate immune system parameters in 48 multiple myeloma (MM) patients. This study performed immunophenotypic analysis of peripheral blood samples, determined natural killer (NK) cell activity, and assessed the cytokine profiles of plasma before and during 3 months of treatment.

The NK cell cytolytic activity against susceptible K-562 targets was analyzed in peripheral blood mononuclear cells (PBMCs) from MM patients receiving BioBran (n=32) or placebo (n=16) with a flow cytometry-based CAM assay.

This increase in NK cell activity was also confirmed by comparing the lytic units (LU); a significant increase over baseline levels (30.8 ± 7.4 LU) was observed after 1 month (47.0 ± 8.5 LU, p=0.045) and 2 months (56.6 ± 12.2 LU, p=0.029; Fig. 17) of BioBran treatment. No significant changes in NK activity were observed in the placebo group during the treatment (Fig. 17).
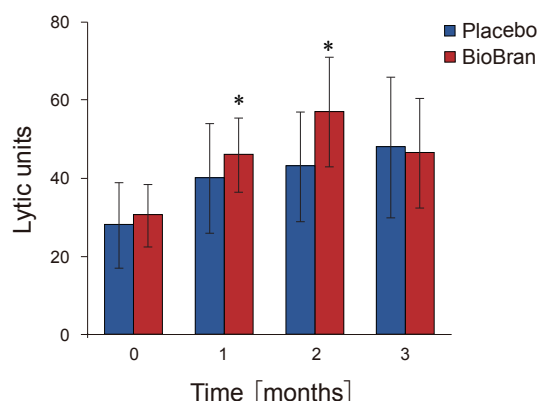
Fig. 17 Effect of BioBran treatment on NK cytolytic in MM patients

NK cell lytic activity against target K-562 cells in PBMC from MM patients receiving BioBran (n=32) and those receiving placebo (n=16) was assessed using 3-h FC-based CAM cytotoxicity assay before treatment (baseline), and after 1,2 or 3 months of treatment. Statistical significance: *p<0.05; versus baseline. b Lytic units (LU, mean ± SEM) per 10^7 of effector cells.

In this study, increased levels of several important Th1 cytokines, in particular Interleukin (IL)-1β, IL-12, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, were observed in MM patients receiving BioBran, in contrast to the placebo group. In addition, the concentration of IL-17 was significantly increased upon BioBran treatment (Fig. 18).
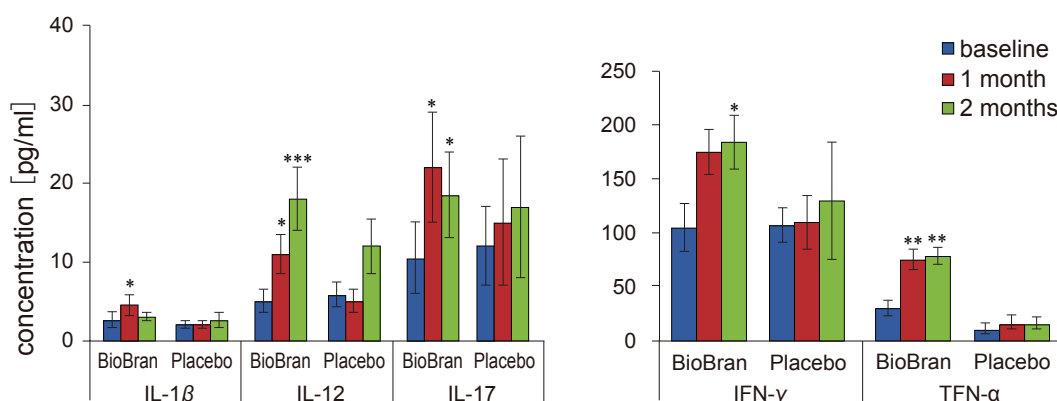

Fig. 18 Changes in Th1 and Th2 cytokine profile after BioBran treatment

Plasmatic concentration (pg/ml) of a Th1 cytokines was measured using multiplex microbead-based immunoassay in patients treated with BioBran (n=30) or placebo (n=15). Statistical significance: *p<0.05; **p<0.01, *** p<0.001.

Cholujova D, Jakubikova J, Czako B, Martisova M, Hunakova L, Duraj J, Mistrik M, Sedlak J. "MGN-3 Arabinoxylan Rice Bran Modulates Innate Immunity in Multiple Myeloma Patients" Cancer Immunol Immunother. Mar; 62(3):437-45., 2013.

9.5: Therapeutic Effects of BioBran, Modified Arabinoxylan Rice Bran, in Improving Symptoms of Diarrhea Predominant or Mixed Type Irritable Bowel Syndrome: A Pilot, Randomized Controlled Study

It was revealed that low grade mucosal inflammation and/or immune imbalance of the lower

digestive tract is one of the mechanisms involved in symptom generation in patients with irritable bowel syndrome (IBS). BioBran has been reported to have several biological actions such as anti-inflammatory and immune modulatory effects.

This study investigated the therapeutic effects of BioBran in patients with IBS. Forty patients with diarrhea predominant or mixed type IBS were randomly assigned to either a BioBran group for treatment with BioBran or placebo group. Therapeutic efficacy and IBS symptoms were assessed subjectively by the patients after 4 weeks of administration. The results show that the global assessment was effective in 63.2% of the BioBran group and in 30% of the placebo group (Table 9).

Table 9 The global assessment to treatment of either BioBran or placebo

	BioBran (n=19)	Placebo (n=20)
Markedly improved	4 (21.1%)*	2 (10.0%)
Slightly improved	8 (42.1%)*	4 (20.0%)
Unchanged	6 (31.6%)	11 (55.0%)
Not so good	1 (5.3%)	2 (10.0%)
Deteriorated		1 (5.0%)

*p=0.0465 versus placebo.

BioBran group showed a significant decrease in the score of diarrhea and constipation and in C-reactive protein (CRP) value. However, no significant changes were observed in the placebo group (Table 10).

Table 10 Changes in values of Gastrointestinal Symptom Rating Scale (1 GSRS) and State Trait Anxiety (STAI) between baseline and after 4 weeks of treatment.

		Baseline	Treatment	P
GSRS				
Diarrhea	BioBran	4.88 ± 1.98	3.51 ± 2.02	<0.001
	Placebo	4.39 ± 1.59	3.95 ± 1.40	N.S.
Constipation	BioBran	3.87 ± 1.73	3.20 ± 1.67	0.024
	Placebo	3.68 ± 1.82	3.28 ± 1.67	N.S.
Hematological examinations				
CRP (g/dl)	BioBran	0.12 ± 0.10	0.10 ± 0.13	0.042
	Placebo	0.32 ± 0.47	0.25 ± 0.36	N.S.

The results showed that the administration of BioBran improved IBS symptoms. It is likely that anti-inflammatory and/or immune modulatory effects of BioBran might be useful in IBS patients.

Kamiya T, Shikano M, Tanaka M, Ozeki K, Ebi M, Katano T, Hamano S, Nishiwaki H, Tsukamoto H, Mizoshita T, Mori Y, Kubota E, Tanida S, Kataoka H, Okuda N, Joh T. "Therapeutic Effects of BioBran, Modified Arabinoxylan Rice Bran, in Improving Symptoms of Diarrhea Predominant or Mixed Type Irritable Bowel Syndrome: A Pilot, Randomized Controlled Study", Evidence-Based Complementary Alternative Medicine. 828137., 2014.

Partly modified from original papers.

9.6: Modulation of the Anticancer Immunity by Natural Agents: Inhibition of T Regulatory Lymphocyte Generation by BioBran in Patients with Locally Limited or Metastatic Solid Tumors

The main cause responsible for the lack of an effective antitumor response in advanced cancer patients is believed to be represented by the generation of a subtype of T helper lymphocytes (CD4$^+$) with suppressive activity on anticancer immunity, the so-called T regulatory lymphocytes (T reg), which may be clinically identified as CD4$^+$CD25$^+$ cells. On this basis, this study was planned to evaluate the effect of BioBran on T reg cell count and percentage in solid tumor patients in relation to the various lymphocyte subpopulations.

The study included 24 consecutive patients, 18 of whom had a metastatic solid tumor, which did not respond to the conventional anticancer chemotherapies and for whom no other effective standard treatment was available, while the remaining 6 patients had been surgically treated for a locally limited neoplasm. Evaluable patients were 22/24, while the remaining 2 patients, both affected by untreatable disseminated liver metastases due to colorectal cancer, rapidly died for disease progression before concluding the two planned months of arabinoxylan therapy. The clinical characteristics of the evaluable patients are reported in Table 11.

Table 11 Clinical characteristics of 22 evaluable cancer patients treated by arabinoxylan

Characteristics	n
M/F	14/8
Median Age (years)	62 (24-82)
Median performance status (Karnofsky's score)	90 (70-100)
Tumor histotypes: colorectal cancer	6
lung cancer	4
prostate cancer	4
breast cancer	3
renal cell cancer	2
pancreatic cancer	2
soft tissue sarcoma	1
Disease extension: Locally limited disease	6
Metastatic disease	16
bone	2
lung	5
liver	3
lung + liver	3
brain	2
peritoneum	1

As illustrated in Fig. 19, TH and T reg mean numbers increased and decreased, respectively, after BioBran therapy, without, however statistically significant differences with respect to the values seen prior to therapy. On the contrary, a statistically significant increase in TH/T reg mean ratio was achieved after BioBran therapy ($p<0.025$). The increase in TH/T reg ratio under BioBran therapy was more pronounced in patients with an abnormally low ratio prior to therapy

with respect to that occurring in those with normal pretreatment ratio, however without statistically significant differences (2.3 +/- 0.4 vs 1.7 +/- 0.5). In more detail, before BioBran therapy, an abnormally low or TH/T reg ratio was present in 12/22 (55%) evaluable patients. BioBran treatment induced a normalization of TH/T reg ratio in 5/12 (42%) patients with an abnormally low ratio prior to therapy. The percentage of BioBran-induced TH/T reg normalization obtained in lymphocytopenic patients was not significantly different from that achieved in patients with normal pre-treatment lymphocyte count (3/7 (43%) vs 2/5 (40%)).

The results showed that BioBran may inhibit the production of T reg cells, which are responsible for cancer-related immunosuppression, with a following improvement in anticancer immunity.
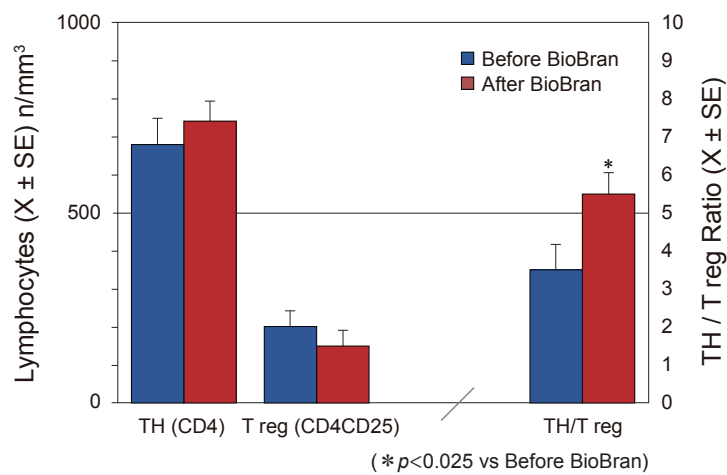
Fig. 19 Changes in the mean number of TH (CD4) and T reg and in TH (CD4)/T reg mean ratio

Lissoni P, Messina G, Brivio F, Fumagalli L, Vigore L, Rovelli F, Maruelli L, Miceli M, Marchiori P, Porro G, Held M, Fede GD, Uchiyamada T. "Modulation of the Anticancer Immunity by Natural Agents: Inhibition of T regulatory Lymphocyte Generation by Arabinoxylan in Patients with Locally Limited or Metastatic Solid Tumors", Cancer Therapy. 6: 1011-1016., 2008.

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Headquarter:

1-16-19 Sangenjaya Setagaya-ku Tokyo, 154-0024, Japan

Tel: +81-3-5430-4070, E-mail: info@daiwa-pharm.com

Tsukuba Plant:

1650-88 Okubara-cho, Ushiku-shi, Ibaraki, 300-1283, Japan

http://www.daiwa-pharm.com

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