A Basic Study of Modified Arabinoxylan from Rice Bran (BioBran/MGN-3) on the Activation of Vital Defence

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Introduction

Modified arabinoxylan from rice bran (BioBran/MGN-3) is a plant polysaccharide processed food with a biophylactic action, which mainly contains polysaccharides such as hemicellulose, arabinoxylan and glycoprotein. BioBran/MGN-3 is known to have various biophylactic actions such as *in vitro* NK cell activity stimulation and an anti-HIV effect[1-2]. In the present study, the vital defence activating effect of BioBran/MGN-3 was studied in 3 animal experiments: Experiment 1 examined its effect on the survival in a lipopolysaccharide-induced lethal sepsis model, Experiment 2 studied the anti-stress effect on restraint stress, and Experiment 3 examined its effect on the survival of autoimmune disease-prone (NZB × NZW) F1 mice.

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Methods

1. Experimental procedures

Experiment 1

In the experiment, BALB/c mice (male, 5-7 weeks old) were used. 20 mg/kg and 200 mg/kg of BioBran/MGN-3 (provided by Daiwa Pharmaceutical Co., Ltd.) were dissolved into 0.5 ml of PBS, and via an oral zonde, administered every other day for 2 weeks, in total 7 times. Then 0.5 ml of PBS was administered orally over the same interval for the control group. 200 μg/mouse of LPS was administered intraperitoneally 12 hours after the final oral administration, and the conditions of the mice were observed over time. In another experiment, 100 μg/mouse of LPS was administered intraperitoneally in the BioBran/MGN-3 group and control group, the mice were euthanized 0, 2, 4, 8 hr after the LPS administrations, and peripheral blood was collected from the heart. Serum was separated, and IL-6 and TNF were measured. IL-6 activity was measured using the B9 cell line[3] and TNF activity was measured by bioassay in the WEHI164-13 cell line[4].

Experiment 2

BALB/c mice (male, 5 weeks old) were fed foods containing 0%, 0.25%, and 0.5% BioBran/MGN-3 and subjected to restraint stress in a metal cage for 12 hours, as previously reported[5]. The thymus and spleen were isolated before and after restraint to study the number of cells and lymphocyte subsets. The number of cells was counted using a hemocytometer and lymphocyte subsets were analyzed by flow cytometry (FACS caliber).

Experiment 3

Autoimmune disease-prone (NZB × NZW) F1 mice (female, 5 weeks old) were given the same foods as in Experiment 2 to observe body weight, proteinuria, and survival rate over time.

2. Statistical analysis

Measurements were expressed as means ± standard deviations. Survival was analyzed using the Kaplan-Meiyer method, and comparison between two groups was performed using Mantel-Cox tests. Others were analyzed by factorial ANOVA and then comparison was made between the two groups using unpaired *t* tests.

Results

1. Improvement of survival rates by BioBran/MGN-3 in a lipopolysaccharide-induced lethal sepsis model

As shown in **Fig. 1**, when 200 μg/mouse of LPS was administered, the survival rate significantly improved in groups where 20 mg/kg or 200 mg/kg of BioBran/MGN-3 was administered daily, compared with that in the control group (20 mg/kg BioBran/MGN-3 group vs. control group, $p = 0.0456$; 200 mg/kg BioBran/MGN-3 group vs. control group, $p = 0.0232$, by Mantel-Cox test). Also, when 100 μg/mouse of LPS was administered, all the mice survived in groups where 20 mg/kg or 200 mg/kg of BioBran/MGN-3 was administered daily, while 3 out of 10 mice died in the control group. Next, to establish the mechanism for improvement of survival rate by BioBran/MGN-3, blood concentrations of IL-6 and TNF were measured (**Fig. 2**). In the experimental group where BioBran/MGN-3 was administered, compared with

the control group, the circulating IL-6 level was significantly lower 2 hours after the administration of LPS (control group 702.9 ± 24.7 ng/ml, BioBran/MGN-3 group 403.1 ± 59.6* ng/ml; $p < 0.01$); however, 8 hr after the administration, it significantly increased (control group 88.5 ± 50.0 ng/ml, BioBran/MGN-3 group 441.0 ± 115.0* ng/ml; $p < 0.05$). Meanwhile, 4 hr after LPS administration, the blood TNF level significantly increased in the BioBran/MGN-3 group compared with that in the control group (control group 492 ± 187, BioBran/MGN-3 group 1816 ± 307* pg/ml; $p < 0.01$).

2. Study on anti-stress effect by BioBran/MGN-3

Subsequently, we studied the possible inhibitory effect of BioBran/MGN-3 in lymphocyte reduction in thymus and spleen caused by restraint stress, one of the non-inflammatory stresses. As shown in **Fig. 3**, in the 0.5% BioBran/MGN-3 group, the number of thymocytes decreased significantly with BioBran/MGN-3 intake alone. However, in either group, the number of cells in thymus and spleen significantly decreased by loading the restraint stress. Meanwhile, for the lymphocyte subset, although the characteristic fluctuation of subset accompanied by stress loading was observed[6], there was no difference between the BioBran/MGN-3 group and control group.

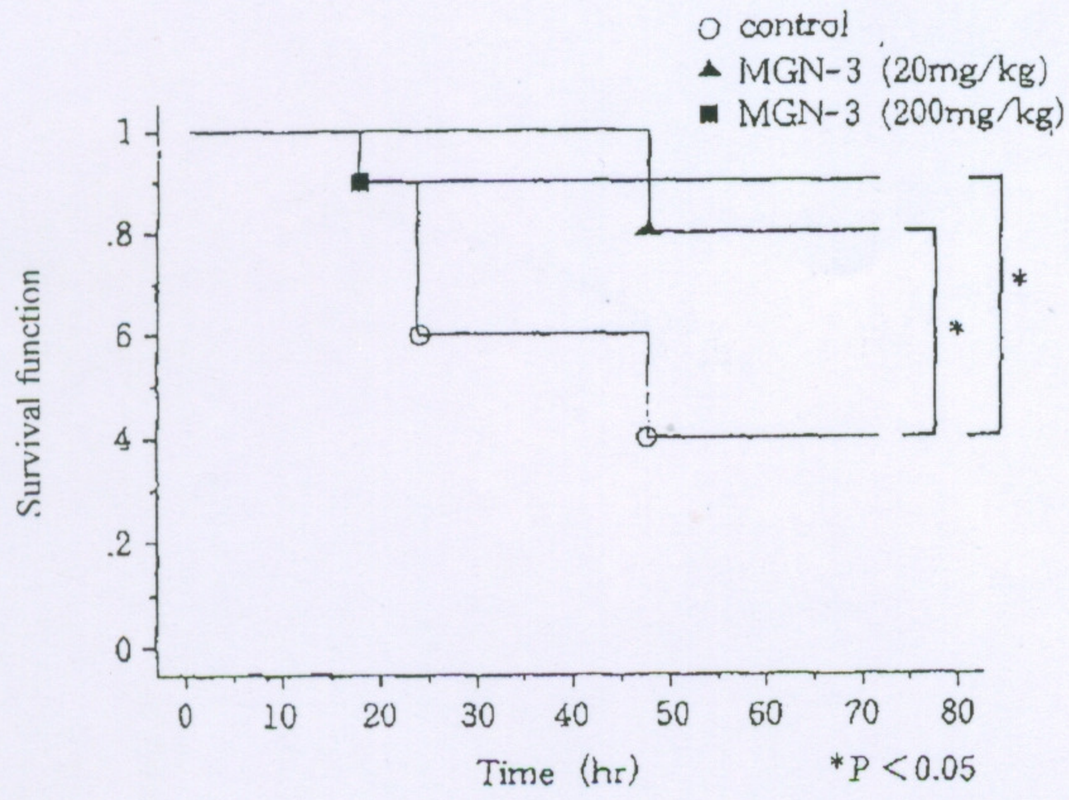

Fig. 1 Improvement of survival rates by MGN-3

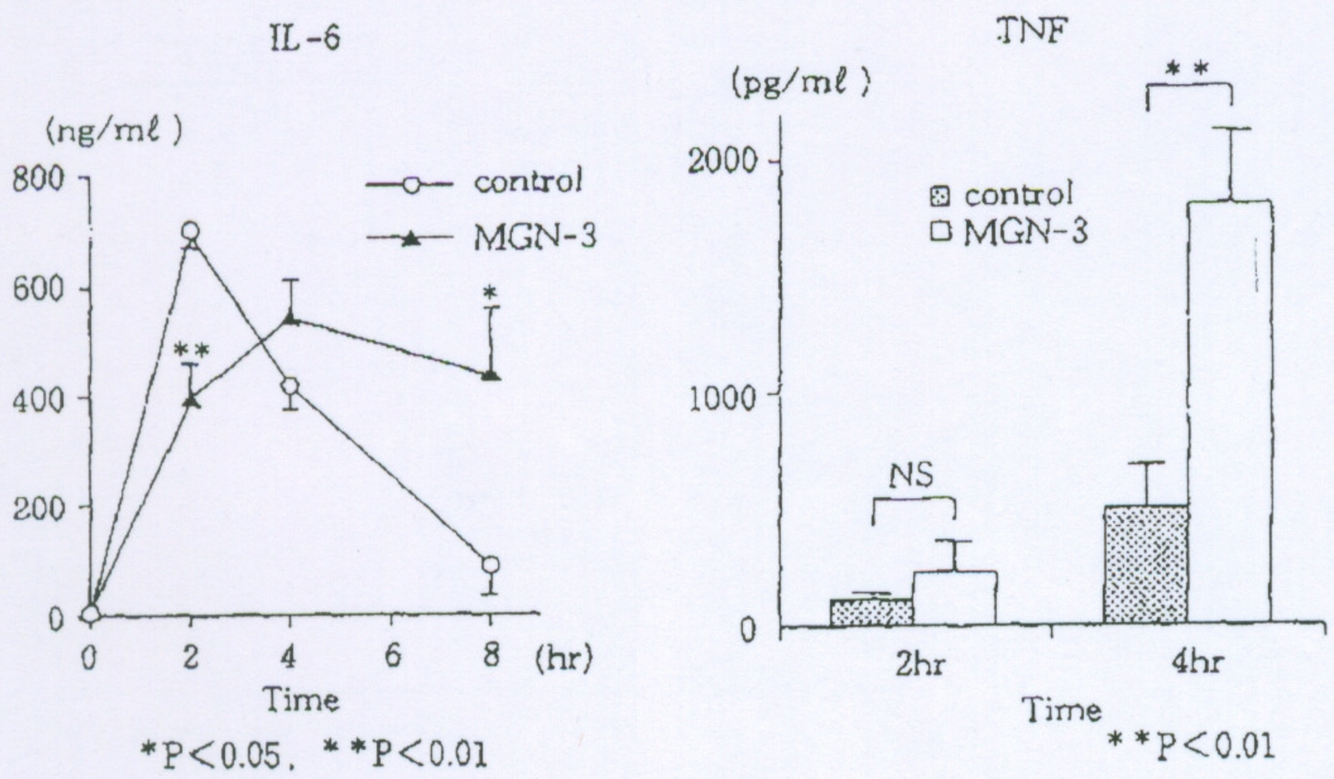

Fig. 2 Changes in blood levels of IL-6 and TNF

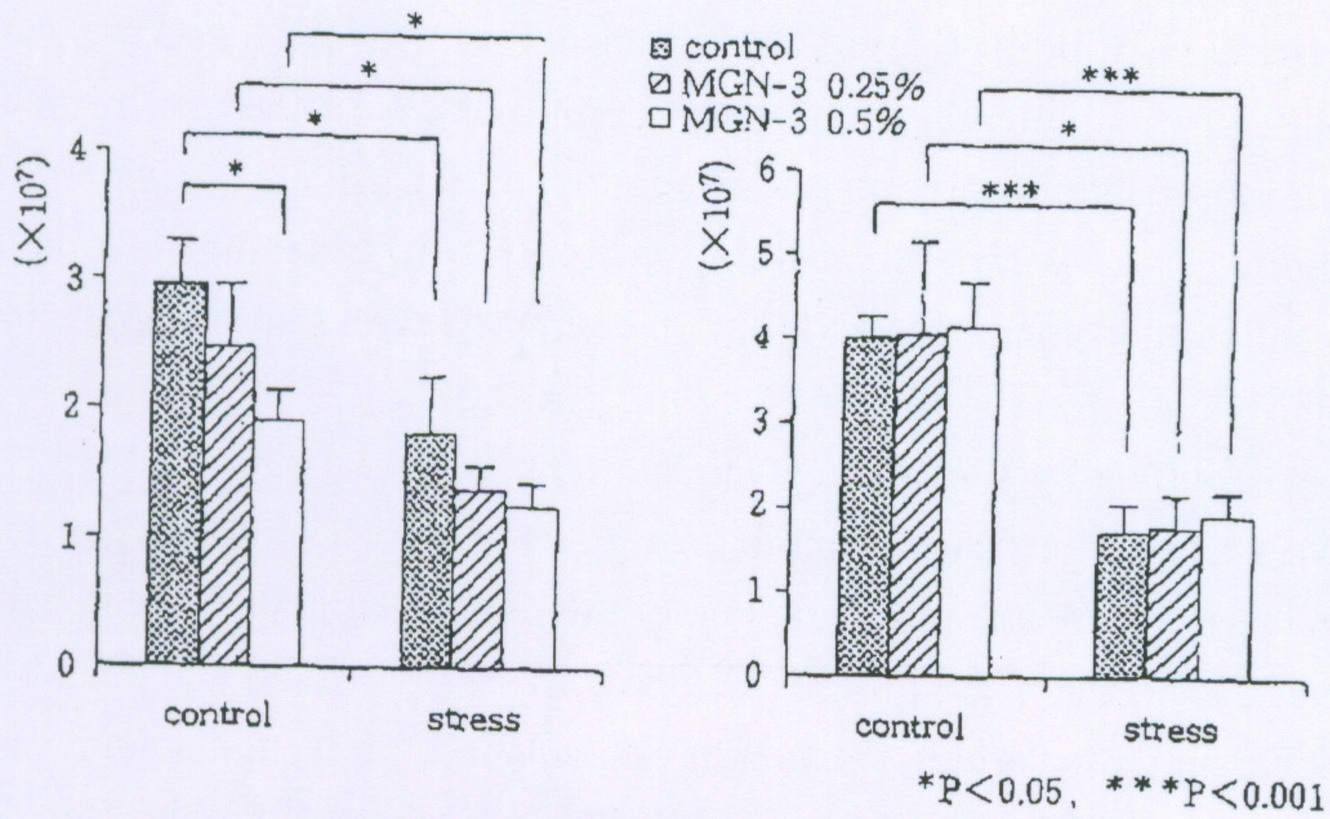

Fig. 3 Changes in numbers of cells in thymus and spleen

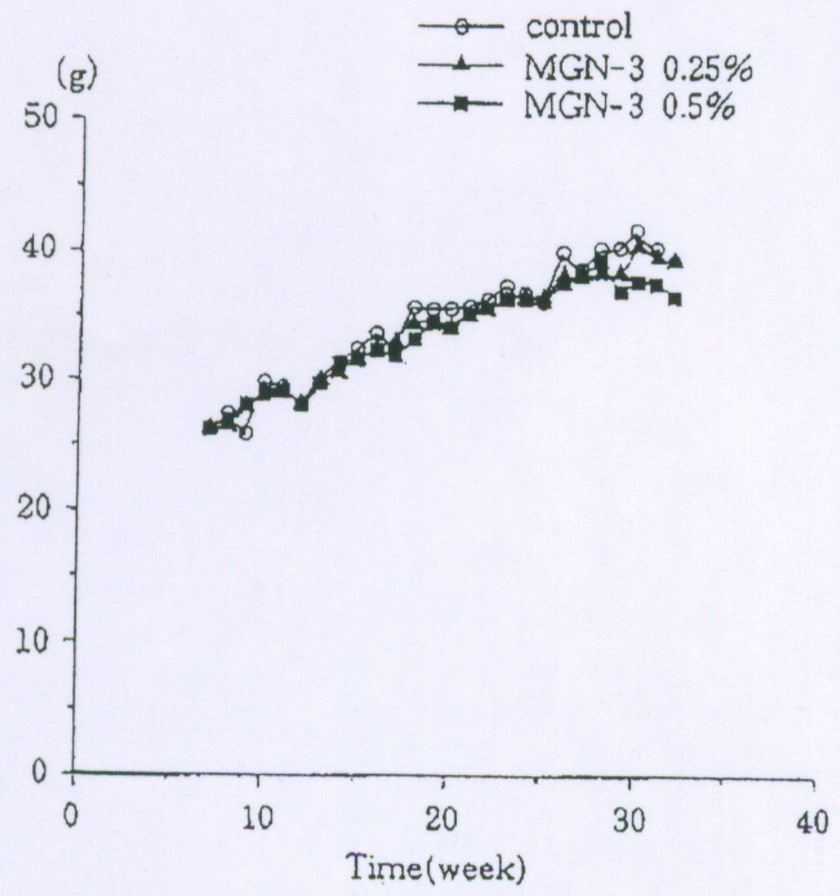

Fig. 4 Changes in body weight

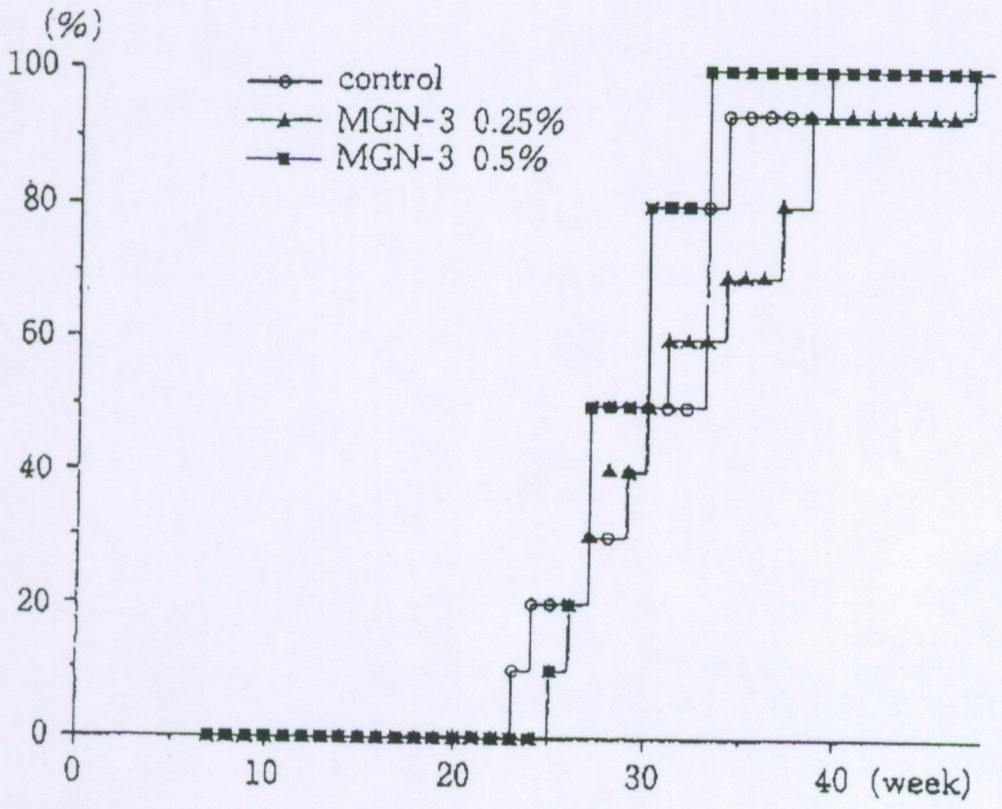

Fig. 5 Time-dependent changes in rate of animals with proteinuria

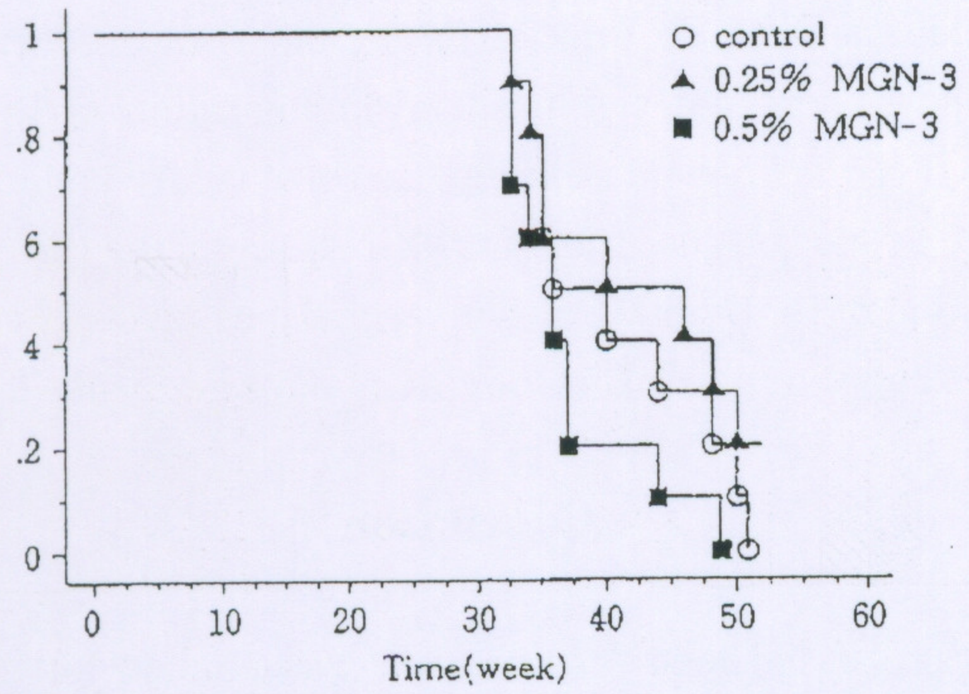

Fig. 6 Effect of MGN-3 on survival rate for (NZB × BZW) F1 mice

3. The effect of BioBran/MGN-3 on clinical signs in mice with collagen vascular diseases

As shown in **Fig. 4**, changes in body weight were similar for the BioBran/MGN-3 and control groups. Proteinuria occurred, in increasing order of frequency, in the 0.5% BioBran/MGN-3, control, and 0.25% BioBran/MGN-3 groups, and was positive in all mice at 46 weeks (**Fig. 5**). There was no significant difference in death rate between the 3 groups, but the 0.5 BioBran/MGN-3 group tended to have a higher death rate than the 0.25 BioBran/MGN-3 group (**Fig. 6**, 0.5% BioBran/MGN-3 vs 0.25% BioBran/MGN-3, $p=0.0769$).

Discussion

In the lipopolysaccharide-induced lethal sepsis model, a large amount of inflammatory cytokines (IL-1, IL-6, and TNF-α) released from the reticuloendothelial system of the whole body induces multiple organ failure, which leads to death of the animal. In this study, BioBran/MGN-3 significantly improved survival rate. One hypothetical explanation for this was that BioBran/MGN-3 may have inhibited the production of tissue-damaging cytokines by macrophages, and blocked reactions leading to damage to target cells. However, although the blood IL-6 level at 2 hours was decreased by BioBran/MGN-3 ingestion, the level of TNF that actually causes tissue damage was significantly increased in the BioBran/MGN-3 groups, suggesting that it is unlikely that BioBran/MGN-3 inhibited the production of inflammatory cytokines. TNF-α is a critical factor determining death or survival in this model. The *in vivo* effect of TNF-α is known to be controlled by competitive soluble TNF-receptors[7]. The mechanism of survival improvement by BioBran/MGN-3 is as yet unknown. Plausible theories for the mechanism are a contribution of cytokines with a competitive effect on TNF and the decreased sensitivity of target cells to TNF (for example, by down-regulation of receptors).

Non-inflammatory stress (restraint stress) decreases the numbers of cells in the thymus and spleen. As similar decreases in the numbers of cells were observed in the BioBran/MGN-3 groups subjected to restraint stress, it can be concluded that BioBran/MGN-3 had no anti-stress effect on the thymus or spleen. However, BioBran/MGN-3 decreased the number of cells in the thymus. This, together with its mechanism of action, remains to be studied.

SLE-prone (NZB × NZW) F1 mice will develop anti-DNA antibody in the blood and proteinuria with the

passage of time, and prematurely die of renal failure. In the present study, proteinuria appeared earlier and the death rate was increased in the 0.5% BioBran/MGN-3 group compared with the 0.25% BioBran/MGN-3 group, although the differences were not significant. Administration of BioBran/MGN-3 at large doses needs careful consideration. IFN-γ is known to exacerbate renal failure and increase the death rate in (NZB × NZW) F1 mice[8]. It has been reported that BioBran/MGN-3 promotes the *in vitro* production of IFN-γ by NK cells[1]. Thus, administration of BioBran/MGN-3 in large doses involves the risk of IFN-γ-induced renal damage. In conclusion, it was suggested that BioBran/MGN-3 may enhance Th1 type immune reactions.

Conclusion

The plant polysaccharide processed food arabinoxylan was studied for its effect on the vital defence activation in animals. The following results were obtained:

1) The food improved survival rates in an LPS-induced lethal sepsis model.
2) It had no anti-stress effect on restraint-decreased cells in the thymus and spleen.
3) It did not prolong the survival in a mouse model of autoimmune disease.

References

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