Anti-HIV Activity *In Vitro* of Modified Arabinoxylan from Rice Bran (BioBran/MGN-3)

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Abstract

BioBran/MGN-3, an arabinoxylan from rice bran that has been enzymatically modified with extract from *Hyphomycetes mycelia*, was tested for anti-HIV activity *in vitro*. BioBran/MGN-3 activity against HIV-1 (SF strain) was examined in primary cultures of peripheral blood mononuclear cells. BioBran/MGN-3 inhibited HIV-I replication by: (1) inhibition of HIV-1 p24 antigen production in a dose dependent manner —BioBran/MGN-3 at concentrations of 12.5, 25, 50, and 100 μg/m*l* showed 18.3, 42.8, 59, and 75% reduction in p24 antigen, respectively; and (2) inhibition of syncytia formation maximized (75%) at concentrations of 100 μg/m*l*. Further studies showed that ingestion of BioBran/MGN-3 at concentration of 15 mg/kg/day resulted in a significant increase in T and B cell mitogen response at 2 months after treatment: 146% for PHA, 140% for Con A, and 136.6% for PWM mitogen. We conclude that BioBran/MGN-3 possesses potent anti-HIV activity and in the absence of any notable side effects, BioBran/MGN-3 shows promise as an agent for treating patients with AIDS.

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Purpose

Human immunodeficiency virus (HIV) is the causative agent of the acquired immunodeficiency syndrome (AIDS). HIV is one of the principal threats to human life worldwide. According to several sources (Center for Disease Control, American Red Cross, and other public health agencies) there are approximately 1.5 million HIV infected people in the United States and as many as 50 million worldwide. It has been estimated that by the year 2000, 110 million will be infected with HIV (2% of the world population). Drugs such as AZT and other nucleoside analogues pose as major problems in slowing the progression of the disease. At the last AIDS International Conference held in Vancouver, Canada, there was very little promise of a vaccine. The lack of vaccination or effective treatment send alarming singnals. Therefore, there is great interest and need to identify anti-HIV agents that are not only active against the virus, but also can potentiate the host immune system without having deleterious side effects. Recently we demonstrated that BioBran/MGN-3, a modified arabinoxylan from rice bran, is a potent biological response modifier (BRM) that is able to enhance natural killer (NK) cell activity in cancer patients[1, 2]. In this study we showed that BioBran/MGN-3 inhibited HIV replication in patients' peripheral blood mononuclear cells (MNC) as well as syncytia formation. BioBran/MGN-3 also increases T and B cell mitogen response upon ingestion. These studies demonstrated BioBran/MGN-3 has strong anti-HIV activity and may be of value in combination therapy in the treatment of HIV-1 infected patients.

Materials and Methods

1. BioBran/MGN-3

BioBran/MGN-3 is an arabinoxylan extracted from rice bran that is treated enzymatically with an extract from Basidiomycetes mycelia. It is a polysaccharide that contains β-1, 4 xylopyronase hemicellulose (**Fig.1**). MGN-3 is commercially known as Biobran (Daiwa Pharm., Co., Ltd., Tokyo, Japan).

2. Complete medium (CM)

RPMI-1640 (Sigma) was supplemented with 1% antibiotics (v/v) and 20% (v/v) fetal bovine serum and recombinant IL-2.

3. Production of HIV-1 p24 antigen

MNCs from 3 healthy individuals were incubated (37℃) with PHA (5 μg/m*l*) for 3days and then washed before incubation (37℃, 1 hr) with HIV-1 SF strain (HIV-1 p24 of 3000 pg/10^6 cells). MNCs were then washed 3× with PBS to remove unbound virus. Infected cells were incubated (37℃, 7 days) either with or without BioBran/MGN-3 at various concentrations (0-100 μg/m*l*), in CM. Half of the medium was changed twice per week with corresponding BioBran/MGN-3 concentrations. At the end of the incubation period, culture supernatants of HIV-1 infected cells were collected and analyzed for viral production. HIV-1 p24 was measured by antigen capturing ELISA using a commercially available kit (DuPont NEN, Boston, MA) according to the protocol provided by the manufacturer.

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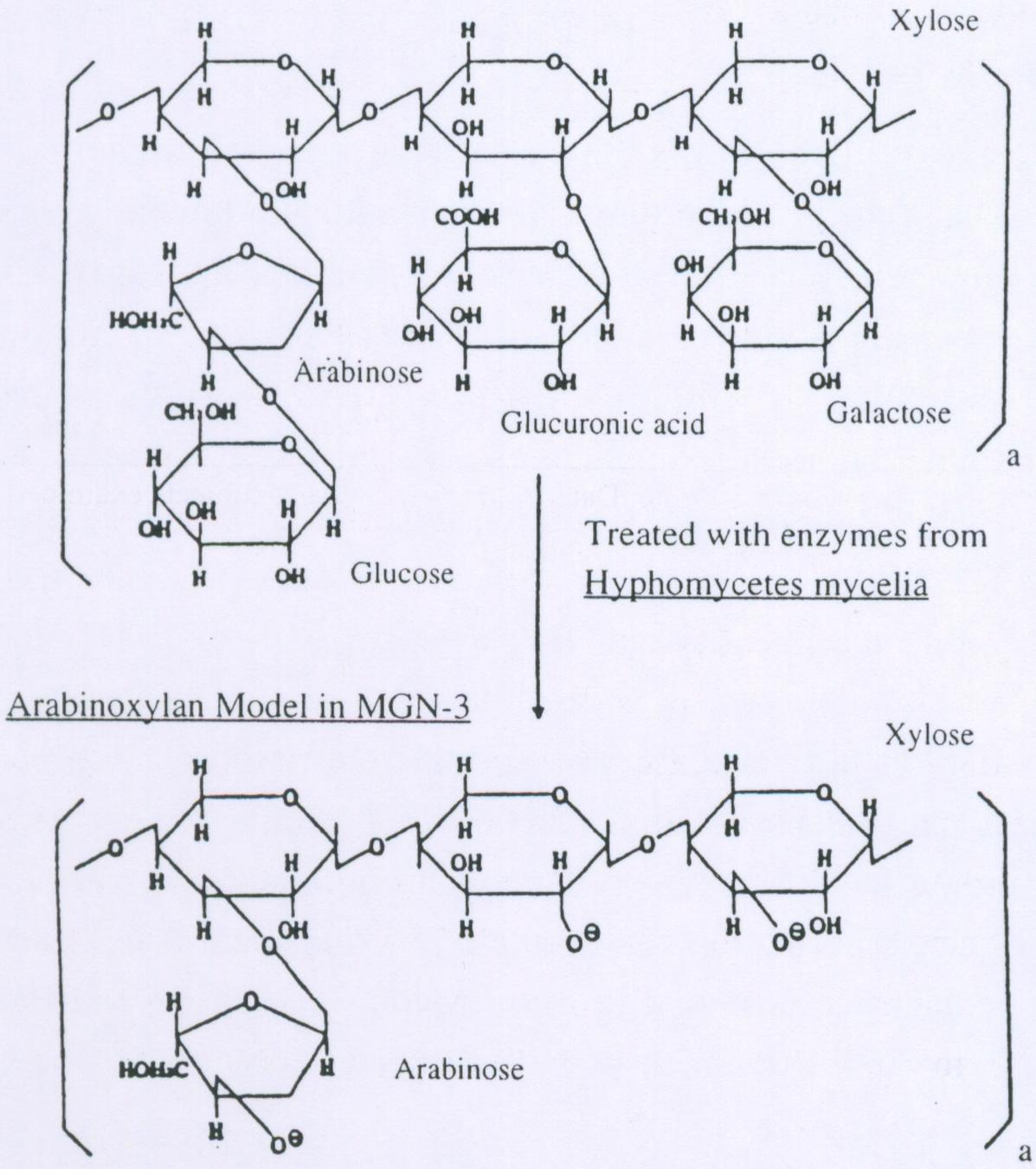

Fig. 1 Crude hemicellulose model in extract of rice bran treated enzymatically by glycosidases from *Hyphomycetes mycelia*

Main chemical structure of MGN-3.

It is an arabinoxylan with a xylose in its main chain and an arabinose polymer in its side chain.

4. Syncytia formation

A slight modification of Johnson and Walker[3] cell fusion assay was used. Briefly, MNC from 5 AIDS patients were cultured with PHA and in the presence or absence of BioBran/MGN-3 at various concentrations (0-100 μg/m*l*). HIV-infected MNC cells were incubated (37℃) in flat bottom 96-well plates (2×10^4 /well). Cultures were then examined after 7 days. The total number of syncytia were counted per well and are reported as number of syncytia/well.

5. *In vivo* T and B lymphocyte proliferation

We investigated the *in vivo* effects of BioBran/MGN-3 on T and B cell proliferation using ^{3}H-thymidine uptake. Five healthy control subjects were given BioBran/MGN-3 at concentrations of 15 mg/kg/d orally for two months. MNCs were prepared from peripheral blood of these individuals before treatment (base line) and at two months after treatment. MNCs were incubated with or without 10mg/m*l* of phytohemagglutinin (PHA), Concavalin A (Con A), or pokeweed mitogen (PWM) for three days. One mCi of ^{3}H-thymidine was added to the cell cultures for the last 8 hours. DNA was harvested and ^{3}H-thymidine uptake was determined by scintillation counter.

Table 1 Dose deependent inhibition of HIV-1 replication by MGN-3

MGN-3 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. Date from three different subjects examined at 7 days.

6. Cell viability

Viability was measured by calorimetric method using the tetrazolium salt MTT assay. A mitochondrial dehydrogenase catalyzes the formation of blue formazan crystals from tetrazolium salt. The amount of formazan produced is proportional to the number of living cells. Briefly, HIV-1 infected cells at 4, 7 and 11 days post-infection were dispensed in triplicate into 96 well round bottom tissue culture plates. MTT (50 μg) was added to each well and the plates were incubated for 4 hrs at 37℃. The formazan crystals were solubilized with 40 mM HCI/isopropanol and the optical density at 590 nm was measured using an ELISA plate reader (Molecular Devices, Menlo Park, CA).

7. Statistical analysis

A student's *t*-test was used to examine the significance of the differences between control and BioBran /MGN-3 treated cells *in vitro* as well as differences in T and B cell mitogen response before and after treatment *in vivo*.

Results

1. Production of HIV-1 p24 antigen

BioBran/MGN-3 inhibited HIV-1 replication in MNC in dose dependent manner. As shown in **Table 1**, BioBran/MGN-3 caused inhibition in HIV p24 antigen production in all subjects, however, there was a clear differential response among different individuals towards BioBran/MGN-3, inhibitory effect by BioBran/MGN-3. The effect of BioBran/MGN-3 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/MGN-3, the percentage of p24 antigen inhibition varied greatly among the three subjects (59%-90%). Data in **Fig.2** summarizes the mean and SD of the results depicted in **Table 1**. At concentrations of 25, 50, and 100 μg/ml, BioBran/MGN-3 demonstrated 18.3, 42.8, 59 and 75% inhibition in the production of HIV-1 p24 antigen, respectively.

2. Effect on syncytia formation

We conducted studies on the effect of BioBran/MGN-3 on HIV induced syncytia formation *in vitro*. Results in **Table 2** showed that BioBran/MGN-3 siginificantly inhibited syncytia formation. The effect

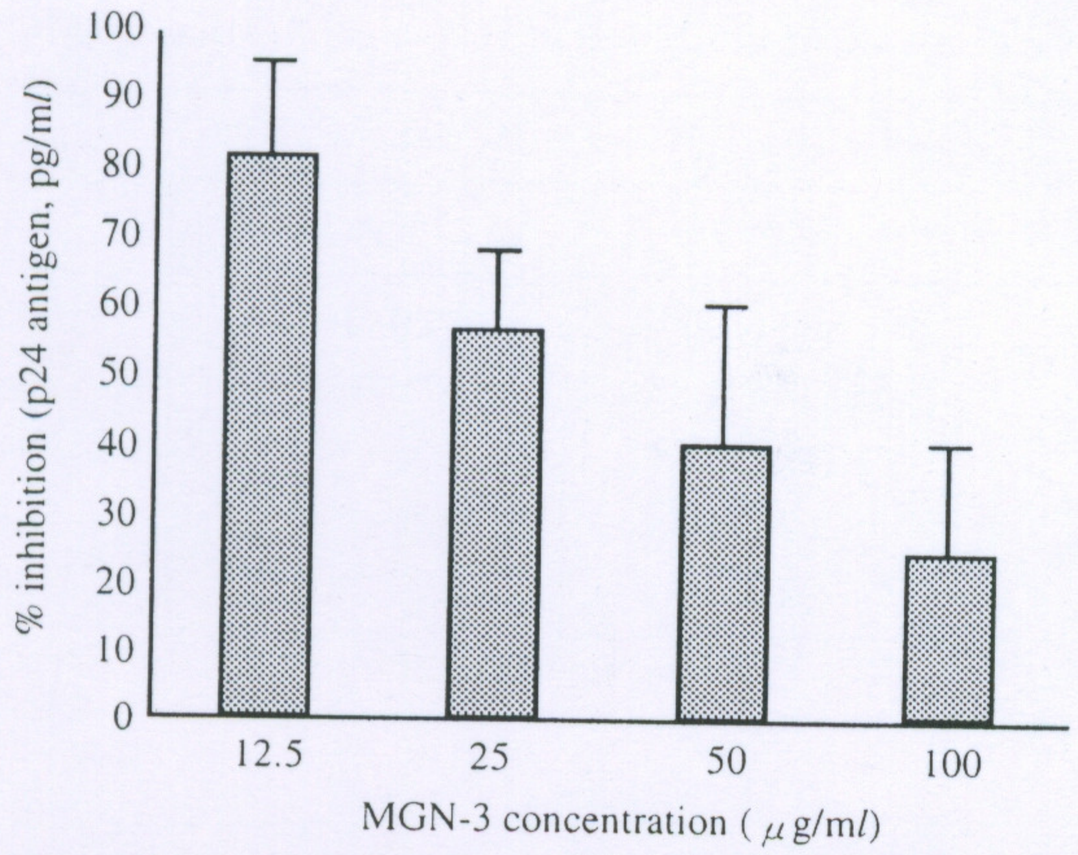

Fig. 2 Effect of MGN-3 on production of HIV-1 p24 antigen

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

Table 2 Inhibition of syncytia formation by MGN-3

MGN-3 dosage (μg/ml)	Syncytia formation (SF)	
	No. of SF	% Inhibition
0	42±8	0
12.5	25.8±7	38.5
25	21.5±5	50
50	15.8±4	62.5
100	10.5±3	75

Note. Data represent means ±S.D. of five individuals examined at 7 days.

was dose dependent and maximum inhibition (75%) was observed at a concentration of 100 μg/ml.

3. *In vivo* effect of BioBran/MGN-3 on T and B cell proliferation

The *in vivo* effect of BioBran/MGN-3 on cell proliferation was studied using 3H uptake. MNC were prepared from peripheral blood of five healthy individuals who were given BioBran/MGN-3 at concentration of 15 mg/kg daily for two months. **Fig.3** showed that treatment with BioBran/MGN-3 resulted in significant changes in MNC proliferation. MNC is the presence of PHA (T cell mitogen) exhibited signicant increase in cell proliferation (146%) as compared with baseline value ($P<0.001$). Similar results were observed when Con A mitogen was used (140%, $P<0.001$). MNC showed 136.6% increase in their proliferative response to PWM, a B cell mitogen as compared to base line value ($P<0.05$).

4. Cell viability

The effect of BioBran/MGN-3 on the viability of HIV-1 infected cells was examined. MTT assay detected no significant differences between treated cells and controls examined at 4, 7, and 11 days post infection.

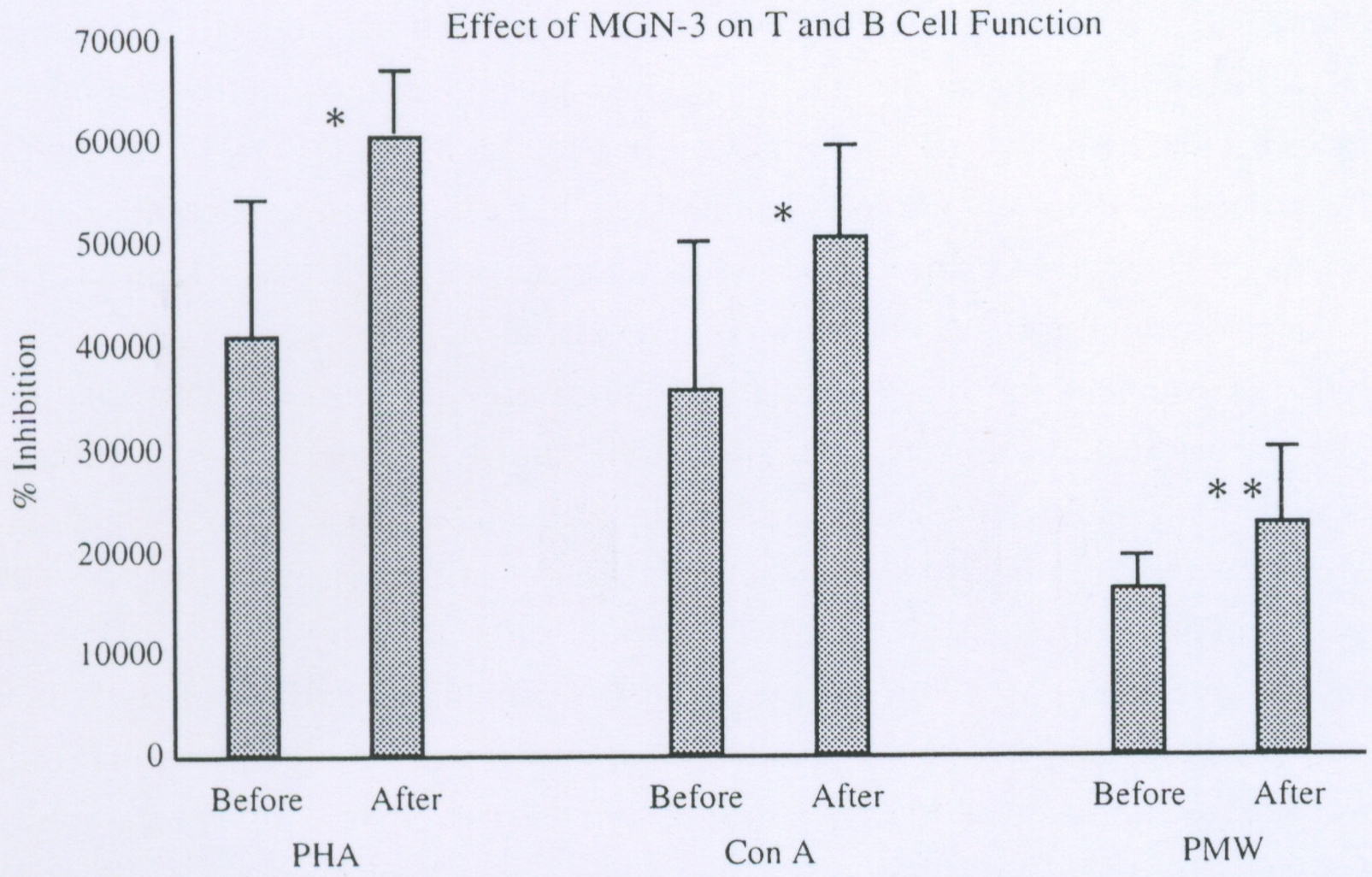

Fig. 3 ***In vivo*** **action of MGN-3 on T and B cell mitogen response at 2 months after treatment**
MNC were cultured for three days in the presence or absence of PHA, Con A and PWM.
^{3}H incorporation was examined.
Data represent mean ±S.D. of five different individuals.
*P<0.001, **P<0.05.

Discussion

In this study we demonstrated that BioBran/MGN-3 possesses an inhibitory effect on HIV replication *in vitro* without cytotoxicity. BioBran/MGN-3 is composed of denatured hemicellulose that is obtained by reacting rice bran hemicellulose with multiple carbohydrate hydrolyzing enzymes from Hyphomycetes, mycelia. The main chemical structure of BioBran/MGN-3 is an arabinoxylan with a xylose in its main chain and an arabinose polymer in its side chain (**Fig.1**). BioBran/MGN-3 has proven to be a potent biological response modifier(BRM) that activates human natural killer(NK) cell activity *in vivo* and *in vitro*[1, 2]. The results of this study also show that BioBran/MGN-3 acts as an anti-viral agent; it inhibited HIV-1 production in peripheral blood mononuclear (MNC) in vitro as manifested by: 1) inhibition of HIV-1 24 antigen production, and 2) inhibition of syncytia formation.

Side effects are one of the problems of using anti-HIV agents for treatment. The prolonged use of several drugs such as PI, azidothymidine, dideoxycytidine, dideoxyinosine and D4T are associated with severe toxicitity and development of drug resistance[4-6]. Therefore, many attempts have been made recently to develop new products that possess anti-HIV activity without the side effects. A number of plants belonging to the mint family (Labiatae) have been reported to have anti-viral activity against different viruses, including HIV[7-10]. Hyssop officialis contains several active ingredients that exhibit anti-HIV activity, for example, tannins[11], and polysaccharide (MAR-10) that inhibits production of HIV-1 antigen in HIV-1 infected MNC and in HUT78 T cell line[12]. Another polysaccharide from pine cones (Pinus parvifloria Sieb Zucc) has also been reported to inhibit HIV activity[13]. With respect to polysaccharide from rice bran.

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Earlier studies demonstrated that extracted hemicellulose from rice bran fiber (RBF) has known unique biological effects; for example, a-glucan from rice bran show potent antitumor activity in mice[14], arabinose and xylose from RBF show defensive effects against bis (n-tributyltin) oxide (TBTO) induced thymic atrophy in rats[15]. Unprocessed RBF and cholestyramine have been observed to increase peripheral blood leukocyte in humans[16]. The polysaccharide used in this study acts as an interferon inducer[17] and has been tested as an anti-cancer agent in patients with different types of malignancy[2].

BioBran/MGN-3 was examined for toxicity using blood chemistry analysis for SMAC and liver enzymes (SGOT and SGPT). Five healthy subjects were given BioBran/MGN-3 orally at concentration of 45 mg /kg/d. After one month, no significant changes were detected in all parameters investigated. *In vivo* studies showed BioBran/MGN-3 has highly significant augmentory effects on lymphocyte proliferation as shown by mitogen response with PHA ($P<0.001$), Con A ($P<0.001$) and PWM ($P<0.05$). Moreover, cell viability was not affected in MNC up to 11 days post-treatment. Clearly BioBran/MGN-3 inhibits HIV-replication in a dose dependent manner and maximum effect was observed at a concentration of 100 μg/m*l*. The results also showed differential response among participants toward the inhibitory effect against HIV replication by BioBran/MGN-3. The mechanism by which BioBran/MGN-3 inhibits HIV replication is not fully understood. HIV infects CD4+ cells, primary T lymphocytes and macrophages by binding the CD4 receptors of the host cells. The inhibitory effect on HIV replication by BioBran/MGN-3 may be through the drug's interference with HIV replication post-entrance, alteration of chemokine receptors or chemokine production.

We conclude that the results generated in this investigation may represent the basis of future studies on clinical trials of BioBran/MGN-3 as an anti-HIV agent.

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