Effect of Modified Arabinoxylan from Rice Bran (BioBran/MGN-3) on Experimental Liver Dysfunction in Rats

T. YAMADA, A. DAIZOU and K. POINDOGLUN
Graduate School of Science and Technology, Chiba University, Chiba, Japan
K. EGASHIRA and H. SANADA
Department of Bioproduction Science, Faculty of Horticulture,
Chiba University, Chiba, Japan
H. MAEDA
Daiwa Pharmaceutical Co., Ltd., Tokyo, Japan

Objective

BioBran/MGN-3 is a partial hydrolysis product obtained by treating water-soluble hemicellulose from rice bran with enzymes from a basidiomycete. It has been shown to have a variety of physiological functions. However, its effect on liver dysfunction has not been fully investigated. In this study, the effect of BioBran/MGN-3 on experimental liver dysfunction in rats induced by galactosamine (GalN) and acetaminophen (AAP) was examined.

Method

Male SLC Wistar clean rats (aged 4 weeks) were used. After 4 days of preliminary raising, the animals were divided into groups. During experiments, they received the standard feed AIN-76 *ad libitum*. The feed composition is shown in **Table 1**.

This paper is published upon partial modification of an article in *Journal of Japanese Association for Dietary Fiber Research*, Vol.6 (2), pp.107, 2002

Table 1 Composition of the experimental diet (%)

Casein	20
Gelatinized cornstarch	40
Sucrose	25
Corn oil	5
Vitamin mixture*	1
Mineral mixture*	3.5
Cellulose	5
Choline bitartrate	0.2
DL-Methoinine	0.3

*: Composition of the AIN-76™ diet

To induce experimental liver dysfunction, GalN was administered in experiments 1-3 and AAP in experiments 4 and 5.

In experiment 1, rats received intraperitoneal (i.p.) administration of BioBran/MGN-3 at 20, 40, and 80 mg/kg and GalN at 800 mg/kg, 1 hour after the administration of BioBran/MGN-3.

In experiment 2, rats received either BioBran/MGN-3 orally, or a high or low molecular weight fraction of BioBran/MGN-3 i.p., and were given GalN at 800 mg/kg 1 hour after the administration of BioBran /MGN-3.

(Treatment groups)
Group A: D-galN
Group B: D-galN+MGN-3 i.p.
Group C: D-galN+MGN-3 high molecular weight i.p.
Group D: D-galN+MGN-3 low molecular weight i.p.
Group E: D-galN+MGN-3 p.o.
Group F: AAP
Group G: AAP+MGN-3 i.p.
Group H: AAP+MGN-3 p.o.

Experiment 2 (1)

Fractionation of BioBran/MGN-3 by gel filtration

Sephadex-G 25 (Fine) was swollen with distilled water (d.w.) and packed into a column (15 mm×200 mm). A mixture of riboflavin and blue dextran in aqueous solution was applied as markers and elution detected with UV. BioBran/MGN-3 at 10 mg/m*l* in d.w. was also applied to the column and the soluble part fractionated. On elution, BioBran/MGN-3 was separated into high and low molecular weight frac-

tions using the midpoint between the peaks of the above two markers.

Experiment 2 (2)

Preparation and administration of BioBran/MGN-3 solution

In Groups B and G, BioBran/MGN-3 was administered i.p. to rats at 40 mg/kg body weight 7 days after the start of raising for experiments. Group C received i.p. administration of the high molecular weight fraction at a dose equivalent to 40 mg/kg and Group D with the low molecular weight fraction at a dose equivalent to 40 mg/kg. Groups E and H received BioBran/MGN-3 orally at 120 mg/kg.

Experiment 2 (3)

Preparation and administration of D-galN solution

Groups A to E were given an i.p. injection of D-galN at 400 mg/kg 1 hour after BioBran/MGN-3. Rats were fasted for 4 hours before and after administration. D-galN (Sigma) was dissolved at 160 mg/m*l* in PBS (phosphate buffered saline) and the solution was adjusted to pH 7.0 with 2N NaOH and passed through a sterile filter.

Experiment 2 (4)

Preparation and administration of AAP solution

Groups F to H were given an i.p. injection of AAP at 700 mg/kg 1 hour after administration of BioBran /MGN-3. Rats were fasted for 18 hours before and 24 hours after administration. p-Acetamidophenol (Wako Pure Chemical Industries, Ltd.) was dissolved in a 0.9% NaCl solution at 35 mg/m*l* and autoclaved at 120℃ for 10 mins. The solution was cooled to 40℃ just prior to administration. Where precipitation was observed, the material was redissolved by increasing the temperature, and the solution was again cooled to 40℃ before administration.

In experiment 3, rats were received i.p. administration of BioBran/MGN-3 heated, hydrolyzed, or treated with an ion exchange resin and GalN at 800 mg/kg 1 hour after the administration of BioBran/MGN-3.

(Treatment groups)

Group A: Control

Group B: MGN-3 heated (i.p.)

Group C: MGN-3 hydrolyzed (with HCl) (i.p.)

Group D: MGN-3 treated with cation exchange resin (i.p.)

Group E: MGN-3 treated with anion exchange resin (i.p.)

Experiment 3 (1)

Heating of BioBran/MGN-3

BioBran/MGN-3 was dissolved in d.w. at 10 mg/m*l*, heated in boiling water at 100℃ for 5 min, and freeze-dried.

Experiment 3 (2)

Hydrolysis of BioBran/MGN-3

BioBran/MGN-3 was dissolved at 10 mg/m*l* in 1N HCl and hydrolyzed in boiling water at 100℃ for 60 min. The solution was evaporated to dryness under decreased pressure using a rotary evaporator.

Experiment 3 (3)

Ion exchange resin treatment of BioBran/MGN-3

The cation exchange resin was DOWEX 50W-X8 (Dow Chemical). To the resin, 1N NaOH was added

and the mixture stirred. The resin was washed with d.w. and packed into a column (15 mm×100 mm). After a sufficient amount of 1N HCl had flowed through, the column was washed with d.w. An aqueous solution of BioBran/MGN-3 (10 mg/m*l*) was applied and the non-adsorbed components were eluted with d.w. and freeze-dried.

The anion exchange resin was DOWEX 1-X2 (Dow Chemical). To the resin, 1N HCl was added and the mixture stirred. The resin was washed with d.w. and packed into a column (15 mm×100 mm). When a sufficient amount of 1N NaOH had flowed through, the column was washed with d.w. An aqueous solution of BioBran/MGN-3 (10 mg/m*l*) was applied applied and the non-adsorbed components were eluted with d.w. and freeze-dried.

In experiment 4, 1 hour after i.p. or oral administration of BioBran/MGN-3, AAP was given at 700 mg /kg.

In experiment 5, rats received hydrolyzed BioBran/MGN-3 i.p. and AAP was given at 500 mg/kg 1 hour later.

In all experiments, rats were sacrificed for autopsy 24 hours after the administration of GalN or AAP to determine the activities of serum transaminase (GOT and GPT). The latter was performed using a Transaminase CII-Test Wako (Wako Pure Chemical Industries, Ltd.) and activities expressed in international units (IU/L). Differences were analyzed at a significance level of 5% or lower using the Scheffe test.

Results and Discussion

Experiment 1

The increases in serum GOT and GPT activities due to GalN-induced liver dysfunction were significantly inhibited in all BioBran/MGN-3 treatment groups compared with controls. BioBran/MGN-3 produced a significant inhibitory effect on GalN-induced liver dysfunction at 20 mg/kg and the effect was unchanged at higher doses (**Figures 1** and **2**).

Experiment 2

The increase in serum GPT activity due to GalN-induced liver dysfunction was significantly inhibited in all treatment groups receiving BioBran/MGN-3 i.p. or the high or low molecular weight fractions of BioBran/MGN-3, compared with controls. Similar inhibition was observed in rats given BioBran/MGN-3 orally (**Figures 3** and **4**).

Experiment 3

The increase in serum GOT activity due to GalN-induced liver dysfunction was significantly inhibited in the group receiving hydrolyzed BioBran/MGN-3 compared with the control group (**Figure 5**).

Experiment 4

The increase in serum GOT activity due to AAP-induced liver dysfunction was significantly inhibited in the i.p. and oral BioBran/MGN-3 treatment groups compared with the control group (**Figures 6** and **7**).

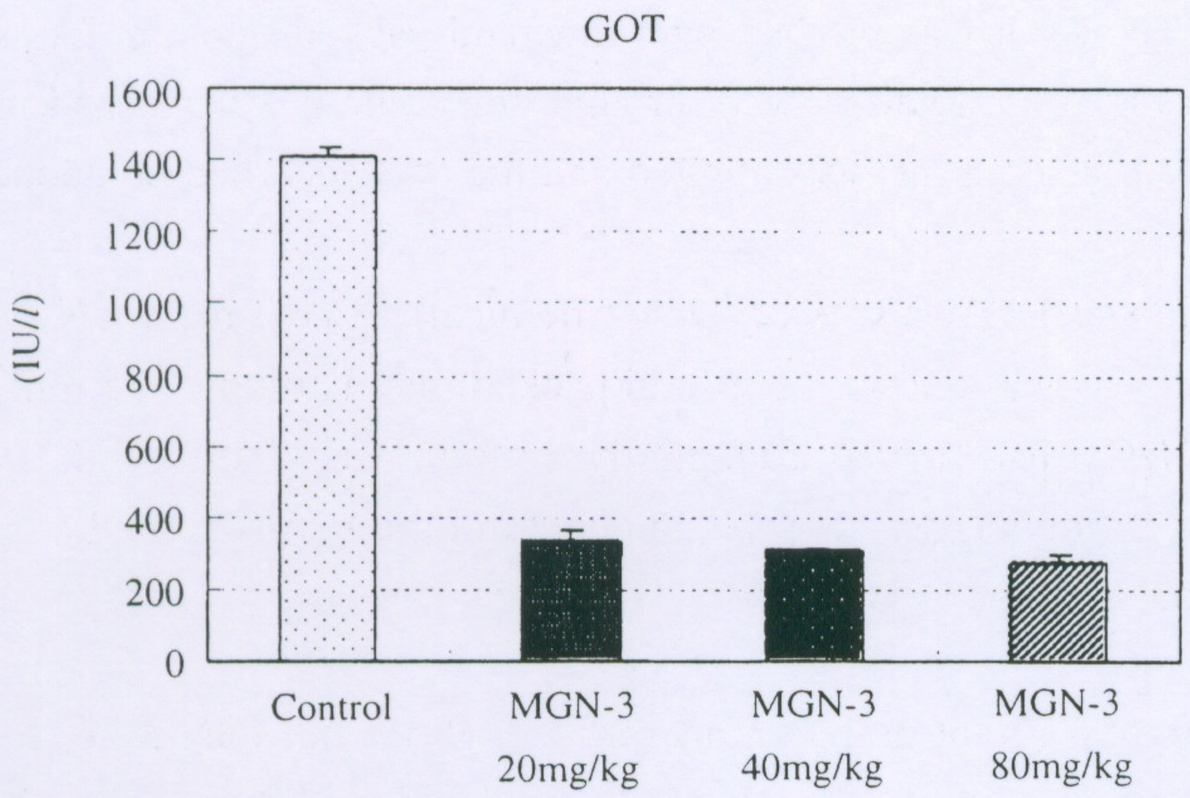

Fig. 1 Effect of MGN-3 on serum GOT activity in galactosamine-induced hepatitis in rats

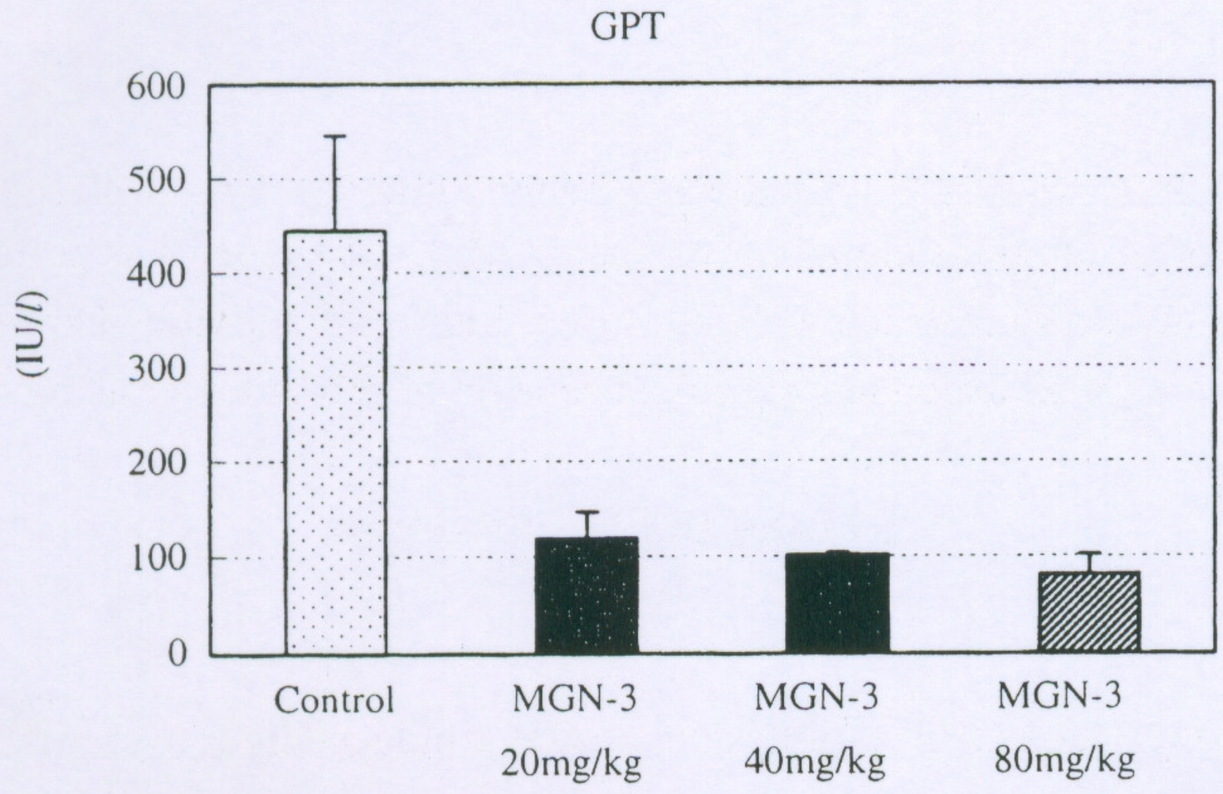

Fig. 2 Effect of MGN-3 on serum GPT activity in galactosamine-induced hepatitis in rats

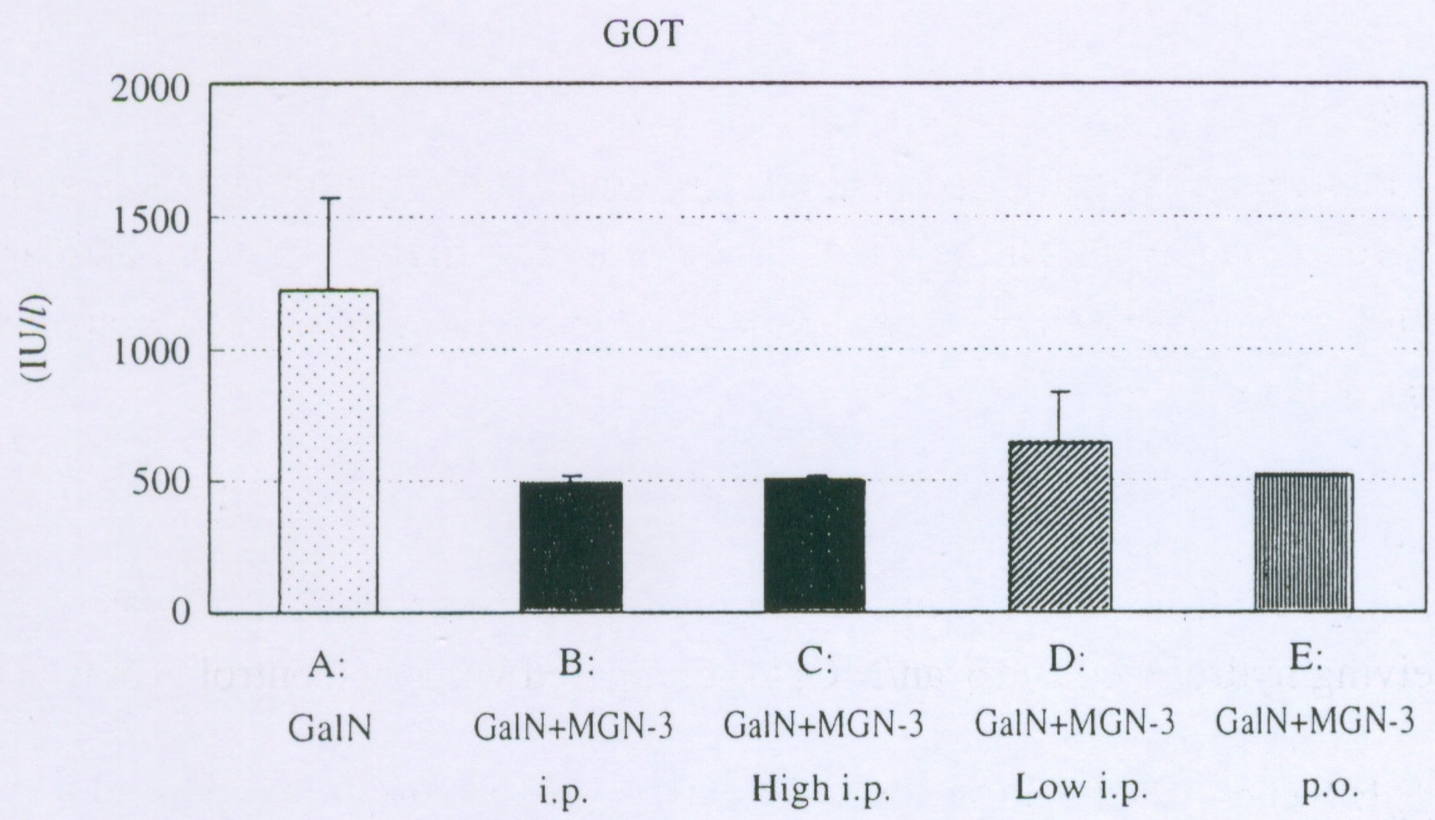

Fig. 3 Effect of MGN-3 on serum GPT activity

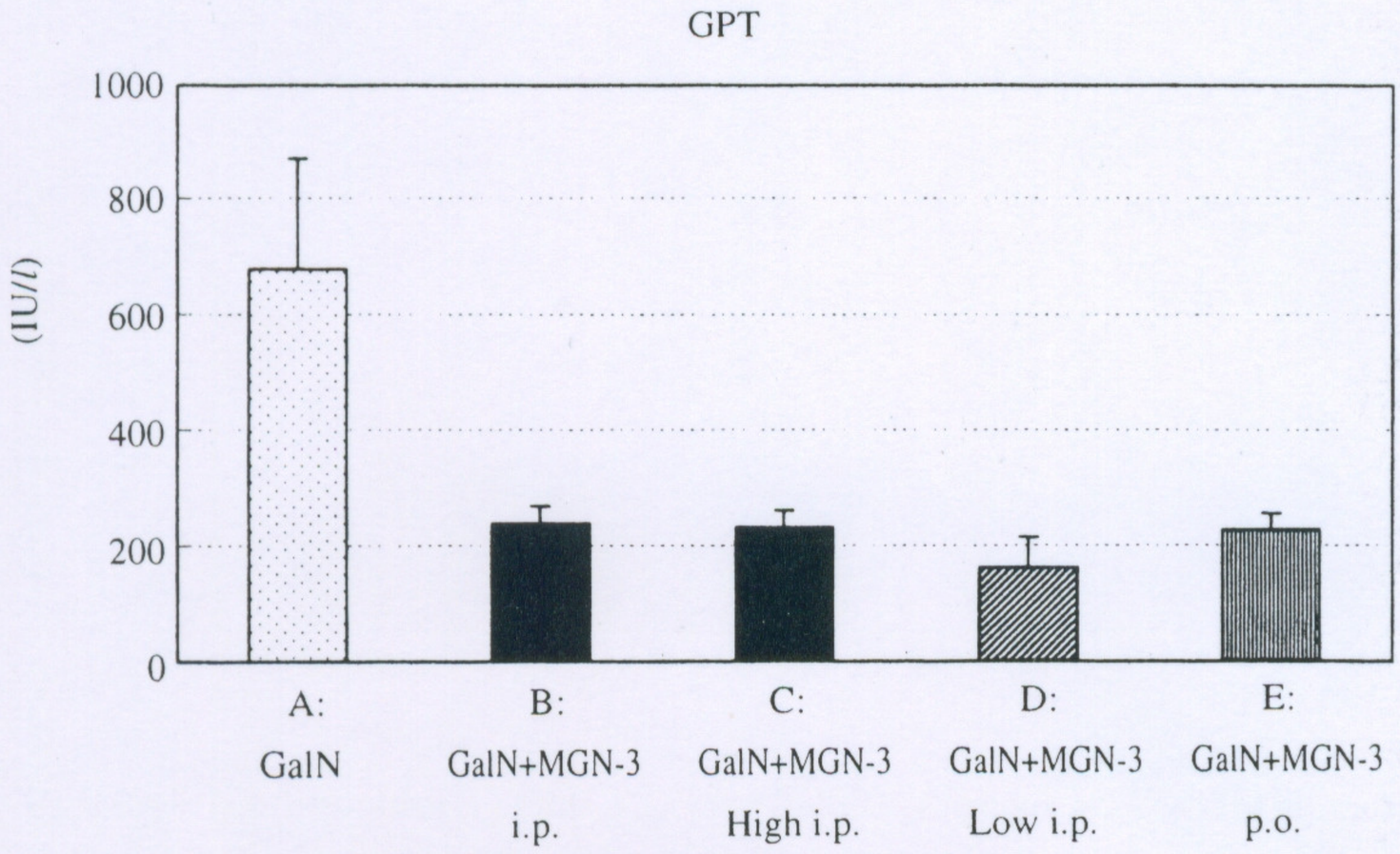

Fig. 4 Effect of MGN-3 on serum GPT activity

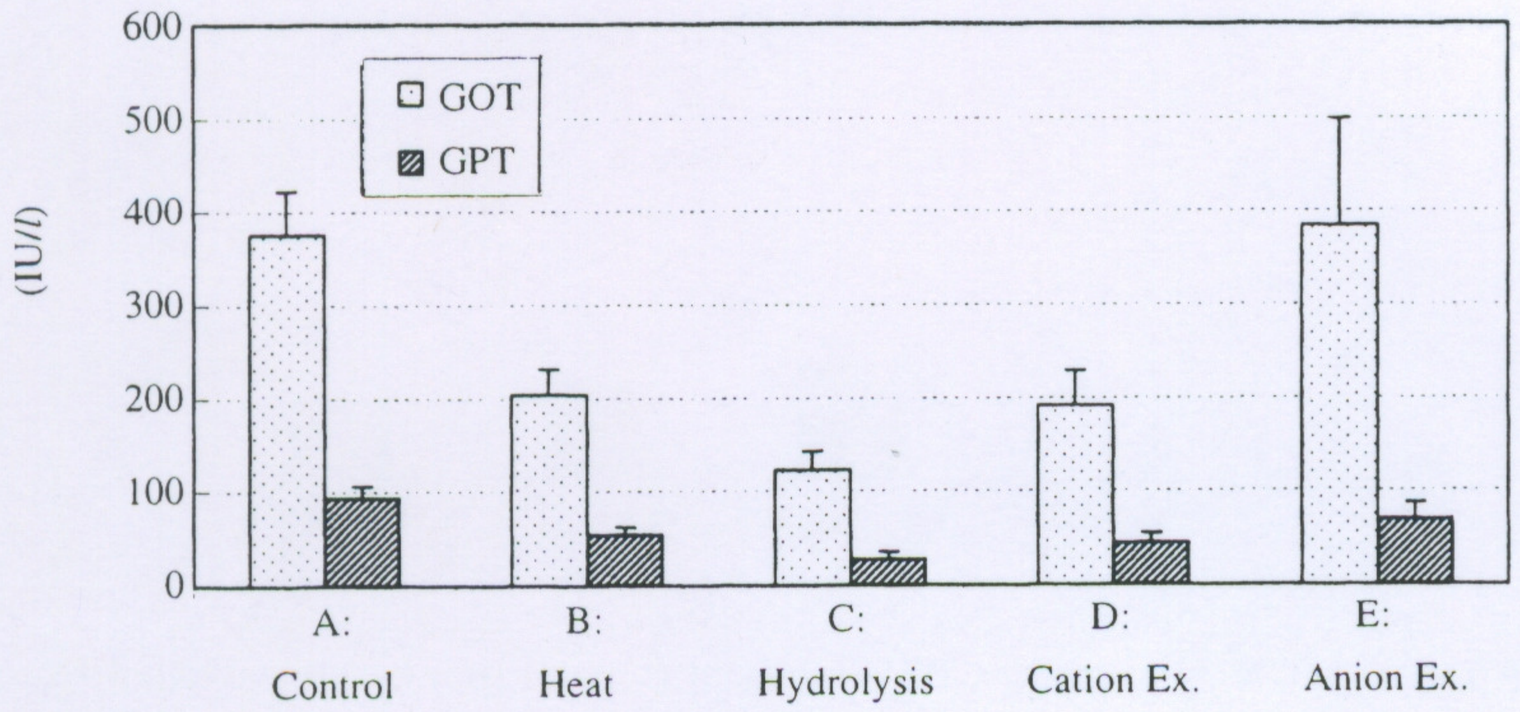

Fig. 5

GOT

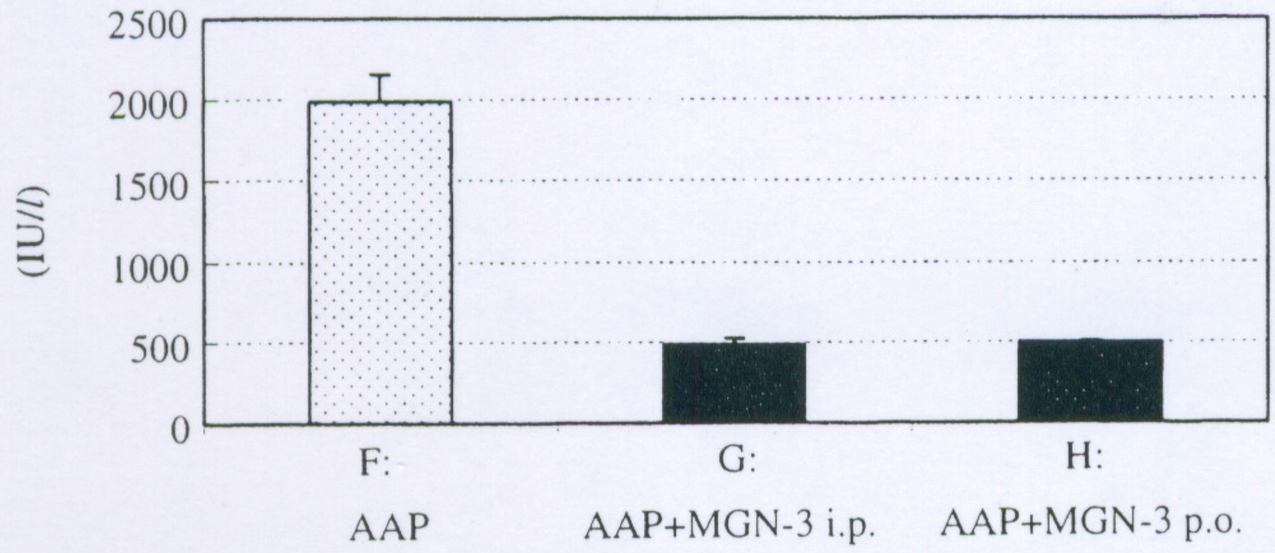

Fig. 6 Effect of MGN-3 on serum GOT activity in galactosamine-induced hepatitis in rats

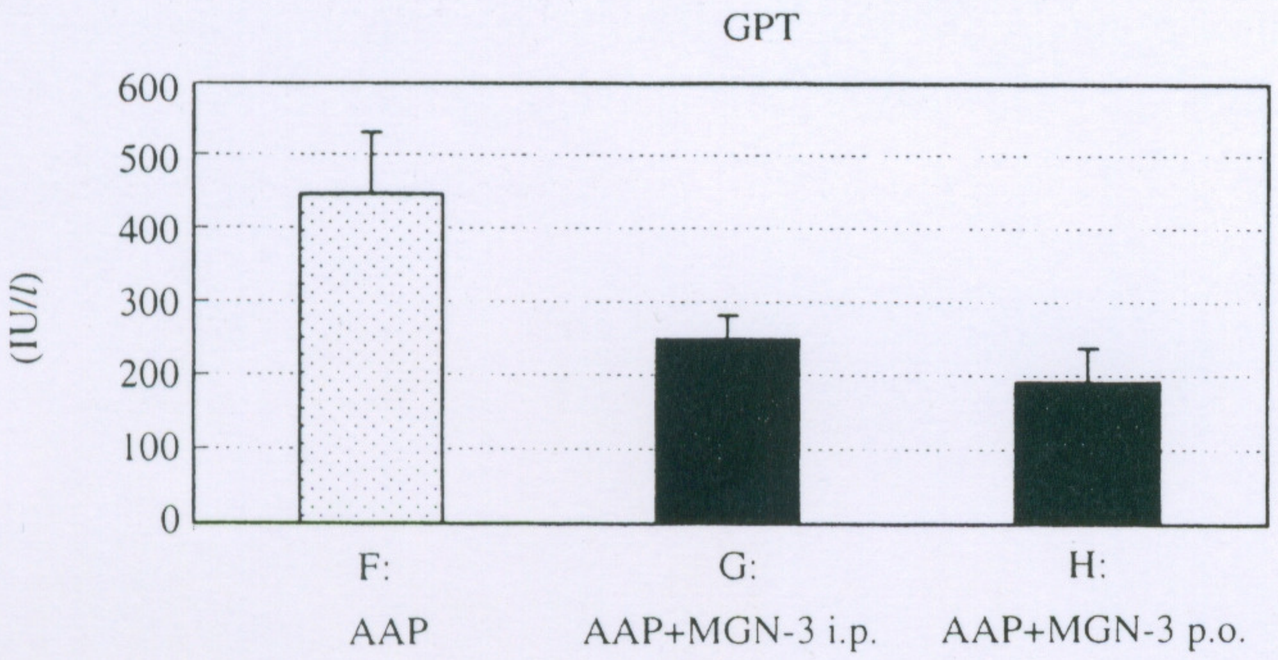

Fig. 7 Effect of MGN-3 on serum GPT activity in galactosamine-induced hepatitis in rats

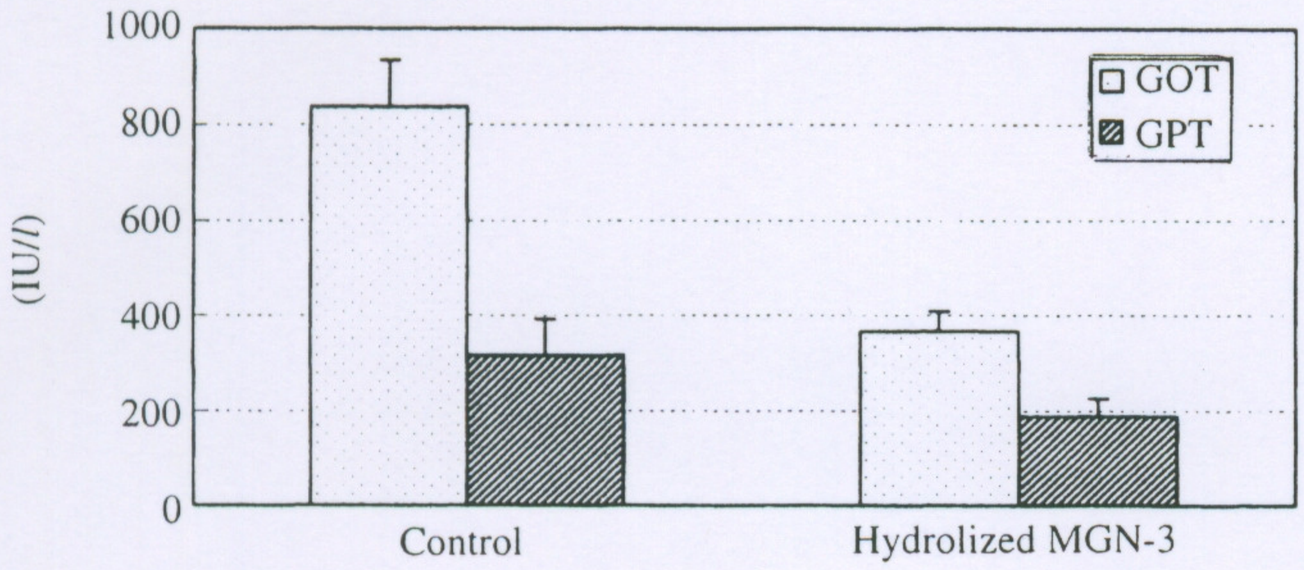

Fig. 8

Experiment 5

Based on the results of experiment 3, the effect of hydrolyzed BioBran/MGN-3 on AAP was studied. Hydrolyzed BioBran/MGN-3 significantly inhibited the increase in serum GOT activity in the treatment group compared with the control group (**Figure 8**).

As stated above, i.p. administration of BioBran/MGN-3 1 hour before treatment with D-galactosamine inhibited D-galN-induced liver dysfunction at an effective dose of 20 mg/kg. This inhibitory effect was obtained with both high and low molecular weight fractions and even after oral administration. Acetaminophen-induced liver dysfunction was also inhibited by i.p. and oral administration of BioBran/MGN-3 1 hour before AAP treatment. The subsequent experiments examined the stability of the active ingredient in BioBran/MGN-3 for inhibition of GalN-induced liver dysfunction during heating and hydrolysis with HCl, and its adsorption to cation and anion exchange resins after acid hydrolysis. The results showed that BioBran/MGN-3 was stable to heating to 100℃ whereas hydrolysis with 1N HCl for 1 hour tended to increase inhibitory activity. The active ingredient in BioBran/MGN-3 could be adsorbed onto an anion exchange resin but not to a cation exchange resin and treatment with either resin slightly decreased its inhibitory action. In addition, the stability to acid hydrolysis of the active component in BioBran/MGN-3 responsible for inhibition of acetaminophen-induced liver dysfunction was studied. As with GalN-induced liver dysfunction, the inhibitory activity on AAP-induced liver dysfunction was stable to hydrolysis by 1N

HCl at 100℃ for 1 hour. These results showed that BioBran/MGN-3 has an inhibitory effect on GalN and AAP induced liver dysfunction and suggested that the active ingredient is not susceptible to hydrolysis with HCl.