

Long-term consumption of polysaccharide gel from durian fruit-hulls in mice

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Abstract

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Consumptive safety studies of durian polysaccharide gel were performed in mice. The polysaccharide gel, DF_{II} and DF_I, were given orally for 60 and 100 days to male and female groups, respectively. The oral doses of 0.25 g/kg/d of DF_{II} or DF_I or standard polysaccharide pectin or a high dose of 0.5 g/kg/d of DF_{II}, were given to test groups. Food and water were given *ad libitum*. The results indicated that no toxic effect was induced in treated mice. Relative body weight gain profile in treated mice was not different ($p>0.05$) from its control. However, the lowest mean values of relative weight gain were obtained at finale in male and female groups treated with 0.5 g/kg/d of DF_{II}. Other pathological effects were examined by clinical analysis of animal blood and serum. Normal hematologic results and clinical data of glucose, cholesterol, creatinine and BUN in treated groups were found and was not significantly different ($p>0.05$) from those of control and/or standard groups. However, mean values of serum cholesterol in DF_{II}- and DF_I- treated groups were rather low in comparison to their control and standard. The serum enzymes ALP, AST (SGOT) and ALT (SGPT) were examined for detection of any pathological changes of the liver. No significant elevation of these enzymes ($p>0.05$) in DF_{II}- and DF_I- treated groups was observed in comparison

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to those of standard and control groups. This finding suggests that liver injury did not occur in treated mice. The relative liver weights of treated mice were not different ($p>0.05$) from those of control male mice, while lower relative liver weights ($p\leq 0.05$) were obtained in female treated and standard groups when compared to their control. Normal number and growth rate of offspring were obtained in DF_{II}- and DF_I- treated and control female groups. Subchronic toxicity in this study suggests that polysaccharide gel from fruit-hulls of durian at oral doses of 0.25 and 0.5 g/kg/d could be consumed safely for a long period of 60-100 days in male and female mice.

Key words : consumptive safety, toxic effects, durian polysaccharide gel, *Durio zibethinus*

บทคัดย่อ

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การบริโภคเป็นเวลานานของโพลีแซคคาไรด์เจล สกัดจากเปลือกผลทุเรียนในหนูถีบจักร

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การศึกษาความปลอดภัยในการบริโภคสารโพลีแซคคาไรด์เจลจากเปลือกทุเรียนกับหนูถีบจักรเพศผู้และเพศเมียโดยให้โพลีแซคคาไรด์เจล DF_{II} และ DF_I ป้อนทางปากเป็นเวลานาน 60 และ 100 วัน กับหนูถีบจักรเพศผู้และเพศเมียตามลำดับ ให้ขนาดของ DF_{II} DF_I หรือสารมาตรฐานโพลีแซคคาไรด์เพคติน 0.25 กรัม/กก./วัน และให้ขนาดสูงของ DF_{II} 0.5 กรัม/กก./วัน กับแต่ละกลุ่มทดลอง ให้อาหารและน้ำกินได้ตลอดเวลา ผลการทดลองแสดงให้เห็นว่าไม่มีพิษเกิดขึ้นกับหนูถีบจักรที่ทดลอง ค่าของน้ำหนักเพิ่มสัมพัทธ์ในกลุ่มทดลองของหนูถีบจักรไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p>0.05$) เทียบกับกลุ่มควบคุม อย่างไรก็ตามในกลุ่มทั้งเพศผู้และเพศเมียที่ให้อาหาร DF_{II} ขนาด 0.5 กรัม/กก./วัน พบมีค่าเฉลี่ยของน้ำหนักเพิ่มสัมพัทธ์อยู่ในระดับต่ำสุดกว่ากลุ่มอื่นเมื่อให้กินระยะยาว ผลทางพยาธิสภาพอื่น ๆ ทดสอบโดยการตรวจวิเคราะห์ทางคลินิกในเลือดและซีรัมของสัตว์ทดลอง พบค่าปกติต่าง ๆ ทางโลหิตวิทยาและการตรวจวิเคราะห์ทางคลินิกของกลูโคส โคเลสเตอรอล ครีโอดีน และ บิยูเอ็น ของกลุ่มทดลองและไม่แตกต่างอย่างมีนัยสำคัญทางสถิติ ($p>0.05$) เทียบกับกลุ่มควบคุมและ/หรือกลุ่มมาตรฐาน อย่างไรก็ตามค่าเฉลี่ยของระดับโคเลสเตอรอล ของกลุ่มที่ให้อาหาร DF_{II} และ DF_I จะค่อนข้างต่ำเมื่อเทียบกับกลุ่มควบคุมและกลุ่มมาตรฐาน การตรวจสอบระดับเอนไซม์ในซีรัมของอัลคาไลน์ฟอสฟาเตส (ALP) แอสพาเทอะมิโนทรานสเฟอเรส (AST หรือ SGOT) และอลานีนอะมิโนทรานสเฟอเรส (ALT หรือ SGPT) เพื่อตรวจการเปลี่ยนแปลงการเกิดพยาธิสภาพของตับ ไม่พบมีการเพิ่มระดับอย่างมีนัยสำคัญทางสถิติ ($p>0.05$) ของเอนไซม์เหล่านี้ของกลุ่มที่ให้อาหาร DF_{II} และ DF_I เปรียบเทียบกับกลุ่มมาตรฐานและกลุ่มควบคุม การพบเช่นนี้เสนอแนะได้ว่าไม่มีการทำลายตับเกิดขึ้นในหนูถีบจักรที่ทดลอง ยังพบว่า ค่าน้ำหนักสัมพัทธ์ของตับไม่มีความแตกต่าง ($p>0.05$) จากกลุ่มทดลองและกลุ่มควบคุมในหนูถีบจักรเพศผู้ ขณะที่กลุ่มทดลองในหนูถีบจักรเพศเมียและกลุ่มมาตรฐานที่ให้อาหารเพคตินมีค่าน้ำหนักสัมพัทธ์ของตับที่ต่ำกว่า ($p<0.05$) กลุ่มควบคุม ได้ผลค่าปกติของจำนวนลูกที่เกิดและอัตราการโตของลูกในกลุ่มทดลองหนูเพศเมียที่ให้อาหาร DF_{II} และ DF_I และกลุ่มควบคุม การทดลองการเกิดพิษกึ่งเรื้อรังในการศึกษาครั้งนี้ให้ข้อเสนอแนะได้ว่า สารโพลีแซคคาไรด์เจลจากเปลือกของผลทุเรียนสามารถให้บริโภคขนาด 0.25 และ 0.5 กรัม/กก./วัน ระยะยาวในช่วง 60-100 วัน ได้ปลอดภัยในหนูถีบจักรทั้งเพศผู้และเพศเมีย

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Polysaccharide gel isolated from fruit-hulls of durian (*Durio zibethinus* L.) has been found to be useful in preparation of jellies and jams, (Pongsamart *et al.*, 1989 a), and as excipient in pharmaceutical preparations such as tablet, suspension and emulsion (Umprayn *et al.*, 1990 abc; Pongsamart, 1989). The chemical composition and sugar components of polysaccharides in form of DF_I (crude) and DF_{II} (partially purified) were previously described (Pongsamart and Panmaung, 1998). Toxic effects of a high oral dose of polysaccharide gel were also investigated in mice and rats, with the results indicating that polysaccharide gel did not induce acute toxicity in mice and rats (Pongsamart *et al.*, 2001). Toxic effects did not occurred in subacute treatment at doses of 0.125, 0.25 and 0.5 g/kg/day for 10 days when compared with water and pectin which were used as a control and a standard polysaccharide, respectively (Pongsamart *et al.*, 1989 b). The purpose of this study was to determine in more detail the consumptive safety after long-term treatment in male and female mice with a dose of 0.25g/kg/day of DF_I, DF_{II} and pectin (a standard polysaccharide) as well as with a high dose of 0.5 g/kg/d of DF_{II}. The pathological changes were characterized by observing the increment of the relative weight of internal organs especially liver weight and high levels of biochemical compositions in blood. The sign of liver damage was also characterized by the high levels of some enzymes in serum such as alkaline phosphatase (ALP) aspartate aminotransferase (AST; SGOT) and alanine aminotransferase (ALT; SGPT) (Fauci *et al.*, 1998).

Materials and Methods

Animals

Female and male Swiss Albino mice, age 30-35 days (20-25 g) were obtained from the National Laboratory Animal Center, Mahidol University at Salaya. All animals were weighed and placed in individual stainless steel cages (8 × 10 in.). The animals were acclimatized for at least 3 days prior to the initiation of experi-

ments in an experimental room with a controlled temperature (25±1°C) and 50-60 % humidity. Animals were maintained on a commercial pellet diet (from F.E. Zuellig Co.) and tap water *ad libitum*. Animal weights were recorded every morning at 8-10 am. throughout the experimental period.

Polysaccharide gel extracts

The polysaccharide gel isolated from fruit-hulls of durian includes DF_I, a crude extract, and DF_{II}, a partially purified form of DF_I. The method of isolation of DF_I and DF_{II}, and its properties were previously described (Pongsamart and Panmaung, 1998). A 5% polysaccharide gel in water was freshly prepared before use. Oral feeding with stainless steel stomach needle was performed at a volume of 10 ml/kg body weight. The dose of 0.25 g/kg of DF_I and DF_{II} was given in test groups. A high dose of 0.5 g/kg of DF_{II} was also performed in test groups of female and male mice.

Experimental protocol

Mice were housed singly in stainless steel cages, and given food and water *ad libitum*. Initial body weight of animals was recorded at day 0, the day on which experiment started. Two preparations of polysaccharide gel, DF_I and DF_{II} (0.25 g/kg/d) were given to the treated groups whereas pectin (0.25 g/kg/d) and water (10 ml/kg/d) were given to the standard and control groups, respectively. A high dose of DF_{II} (0.5g/kg/d) was also given to female and male test groups. Each group of 14-15 male or 8 female mice received polysaccharide gel and pectin everyday for 60 and 100 days, respectively. Each animal was weighed before feeding every morning, food and water intake was also observed through out the experimental period. After 4 weeks of treatment, the treated females were housed with normal untreated male for mating. The pregnancy rate, litter size, number of offspring, offspring growth rate and animal weight were determined for the following 70 days of treatment. Offspring weight was recorded every

day after birth for 4 weeks.

On the final day of treatment, treated animals were weighed and then anesthetized with diethyl ether, and blood withdrawn immediately from the inferior vena cava using a heparinized hematocrit tube and syringe. Serum was obtained following centrifugation at $3,000 \times g$, 5 min, and used for biochemical and clinical analyses. Immediately following collection of the blood sample, liver, heart, lung and kidneys were rapidly removed and weighed. The internal organs were examined grossly.

Assessment of clinical parameters

Hematological parameters of blood samples were analyzed for hematocrit and hemoglobin. Blood count of red blood cells, white blood cells and lymphocytes were examined (Dacie and Lewis, 1975). Clinical pathology was assessed according to biochemical analysis, i.e. the increment of serum concentrations of glucose, cholesterol, creatinine and blood urea nitrogen (BUN) by using spectrophotometric assay. The elevation of enzyme levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST or SGOT), and alanine aminotransferase (ALT or SGPT) was determined to assess toxic liver injury and biological toxicity.

Statistical method

Results are expressed as group mean values $\pm$ SD in tables ; graphs were constructed using SigmaPlot Scientific Graphing Software for Windows, symbols and bars on graphs represent mean $\pm$ SD. Means not having superscripts incommon are statistically different ($p < 0.05$). Multiple means of the treatment, standard and control groups were examined by using SPSS for MS Windows program, and analysis undertaken using analysis of variance followed by Duncan's multiple range test or/and Post Hoc Tests to evaluate significant differences between groups. The limit for statistical significance (α) was set up at 0.05. Relative weight gain mean values were compared cross groups treated with different amounts and types of food at 10-day

intervals for 60 and 100 days in male and female groups, respectively, and at 7-day intervals for 30 days in groups of offspring.

Results and Discussion

In a previous study, investigation of a high oral single dose (2 g/kg) of polysaccharide gel from fruit-hulls of durian indicated that no acute toxic effect was induced in mice and rats. Long-term treatment in mice with an oral dose of 0.25g/kg/d of DF_I and DF_{II} was performed in this study; pectin and water were used as a standard polysaccharide and control, respectively. A high dose of DF_{II} (0.5 g/kg/d) was also assessed. These doses were about 5-10 times or more than 20 times which would be consumed in the recipe of food or drug formula, respectively. Normal behavior of animals was observed. Food and water were taken normally in treated groups as well as in the control group. Soft feces were excreted normally in treated group as well as in the groups of control and standard. However, at the first 1-2 weeks of treatment, some animals of the treated groups excreted very soft feces. This result may suggest a mild laxative property of polysaccharide fiber (Spiller, *et al.*, 1979).

Effect of polysaccharide gel on body weight and internal organ weight

Initial body weight and relative weight gain in test male and female mice are illustrated in Figure 1 and Figure 2, respectively. Final body weights in test male and female are demonstrated in Table 1 and 2, respectively. Average body weights (Table 1) and the average weight gain profile in male groups (Figure 1) were not significantly different ($p > 0.05$) from that of their control. However, the DF_{II} at 0.5 g/kg/d treated group at final period of experiment showed the lowest average body weight. Figure 2 shows the profile of relative weight gain in female mice. The average weight gains of treated and control groups were not significantly different ($p > 0.05$) at day 10 to 30 of treatment before mating period and at day 80 to 100 of treatment after lactation

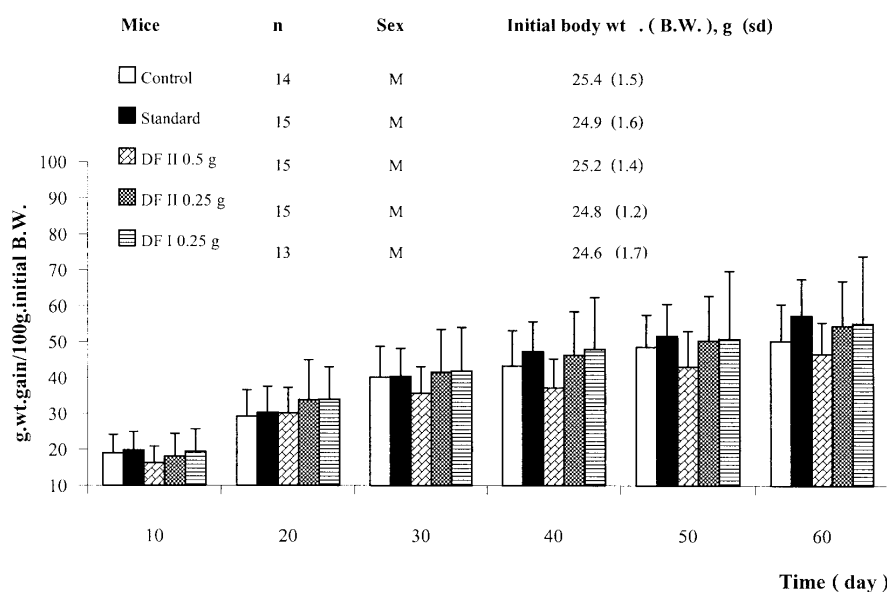


Figure 1. Relative weight gain in male mice during 60 days treatment with DF_I and DF_{II}. Each bar represents mean (sd). Mean values of treated groups at time illustrated are not significantly different from that of control ($p > 0.05$). Control male mice were fed with water 10 ml/kg/d. Standard male mice were fed with pectin 0.25 g/kg/d, M = male, n = number of mice

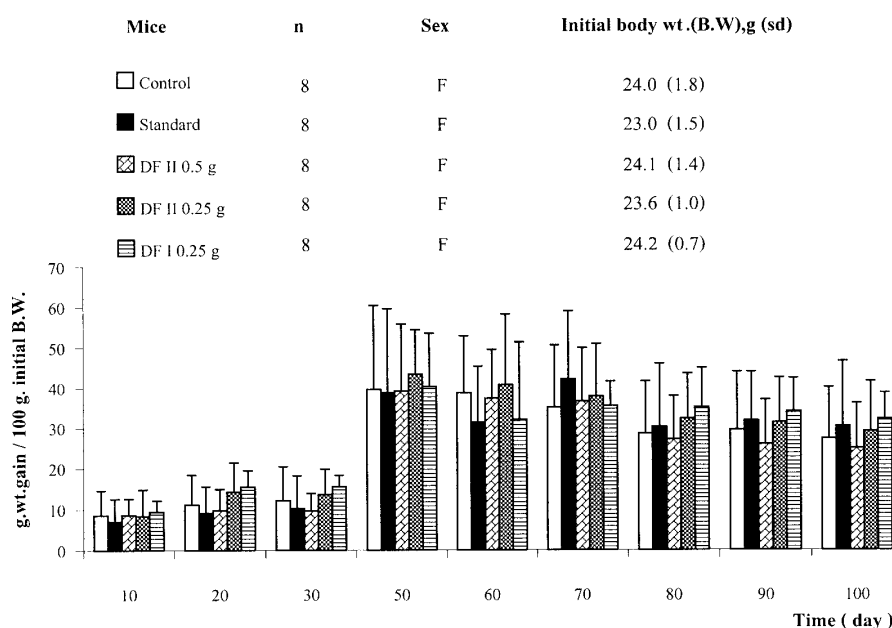


Figure 2. Relative weight gain in female mice during 100 days treatment with DF_I and DF_{II}. Each bar represents mean (sd). Mean values of treated groups at time illustrated are not significantly different from control ($p > 0.05$). Control female mice were fed with water 10 ml/kg/d. Standard female mice were fed with pectin 0.25g/kg/d, F = treated female, n = number of mice.

Table 1. Relative internal organ weight in test male mice after 60 days treatment with DF_I and DF_{II}. NS = no significant difference between groups ($p > 0.05$), a, b = significant difference between groups ($p \leq 0.05$), M = male, n = number of mice.

Test animals (mice)	n, Sex	Weight (g) Mean $\pm$ SD	Internal organ wt. (g)/100 g. body wt. Mean $\pm$ SD				
			Liver NS	Heart NS	Lung	Kidney	
						Right NS	Left
Control group water 10 ml/kg/d	14,M	37.4 $\pm$ 2.9	5.07 $\pm$ 0.52	0.45 $\pm$ 0.04	b 0.62 $\pm$ 0.05	0.95 $\pm$ 0.30	a 0.86 $\pm$ 0.09
Standard group pectin 0.25g/kg/d	15,M	38.4 $\pm$ 2.9	4.72 $\pm$ 0.31	0.45 $\pm$ 0.81	ab 0.66 $\pm$ 0.10	0.91 $\pm$ 0.15	ab 0.82 $\pm$ 0.10
Test group DF _{II} DF _{II} 0.5g/kg/d	15,M	36.6 $\pm$ 1.8	4.39 $\pm$ 0.21	0.46 $\pm$ 0.05	ab 0.67 $\pm$ 0.10	0.93 $\pm$ 0.19	ab 0.83 $\pm$ 0.06
Test group DF _{II} DF _{II} 0.25g/kg/d	14,M	38.2 $\pm$ 3.1	4.89 $\pm$ 0.76	0.40 $\pm$ 0.13	a 0.73 $\pm$ 0.09	0.94 $\pm$ 0.18	b 0.78 $\pm$ 0.10
Test group DF _I DF _I 0.25g/kg/d	13,M	38.8 $\pm$ 2.8	5.15 $\pm$ 0.45	0.44 $\pm$ 0.06	a 0.73 $\pm$ 0.09	0.88 $\pm$ 0.10	ab 0.81 $\pm$ 0.09

Table 2. Relative internal organ weight in test female mice after 100 days treatment with DF_I and DF_{II}. NS = no significant difference between groups ($p > 0.05$), a, b = significant difference between groups ($p \leq 0.05$), F = female, n = number of mice.

Test animals (mice)	n, Sex	Weight (g) Mean $\pm$ SD	Internal organ wt. (g)/100 g. body wt. Mean $\pm$ SD				
			Liver	Heart NS	Lung NS	Kidney	
						Right	Left NS
Control group water 10 ml/kg/d	8,F	31.4 $\pm$ 2.7	a 5.99 $\pm$ 0.70	0.57 $\pm$ 0.12	0.78 $\pm$ 0.12	ab 0.71 $\pm$ 0.08	0.69 $\pm$ 0.03
Standard group pectin 0.25g/kg/d	8,F	29.7 $\pm$ 2.8	abc 5.73 $\pm$ 0.49	0.52 $\pm$ 0.07	0.88 $\pm$ 0.11	a 0.72 $\pm$ 0.05	0.70 $\pm$ 0.06
Test group DF _{II} DF _{II} 0.5g/kg/d	8,F	29.7 $\pm$ 5.6	cd 5.29 $\pm$ 0.58	0.62 $\pm$ 0.02	0.83 $\pm$ 0.14	a 0.73 $\pm$ 0.05	0.71 $\pm$ 0.07
Test group DF _{II} DF _{II} 0.25g/kg/d	8,F	31.1 $\pm$ 2.6	bcd 5.31 $\pm$ 0.41	0.53 $\pm$ 0.09	0.85 $\pm$ 0.13	a 0.71 $\pm$ 0.07	0.70 $\pm$ 0.08
Test group DF _I DF _I 0.25g/kg/d	8,F	33.3 $\pm$ 1.6	d 5.09 $\pm$ 0.48	0.52 $\pm$ 0.12	0.83 $\pm$ 0.12	b 0.65 $\pm$ 0.03	0.68 $\pm$ 0.07

ended. In this experiment, normal untreated male mice was housed with test females on day 28-42 (14 days). The average relative weight gain

during pregnancy period is not shown. Most of test female mice in treated and control groups became pregnant and gave birth by day 50-56.

The high relative weight gain profile on day 50-70 as shown in Figure 2 was observed during the lactation period. However, the average relative weight gain appeared to be the lowest in the DF_{II} (0.5 g/kg/d) treated female group (Figure 2) on days 80-100 towards the end of the experiment and the lowest final body weight was also appeared in the DF_{II} (0.5 g/kg) treated group as well as standard group (Table 2). These results suggest that long term feeding of DF_{II} at an oral dose of 0.5 g/kg/d has potential to reduce weight gain in male and female mice.

The relative weights of liver, heart, lung and kidneys was determined at the end of the experiment in male and female mice. The results are shown in Table 1 and 2, respectively. Final body weight of the animals is also demonstrated. In the male groups (Table 1) the relative weights of internal organs were not significantly different ($p>0.05$) from those of control groups, except for the lung and the left kidney; but these relative weights were not different ($p>0.05$) from that of the standard group. Among females (Table 2) the relative weight of liver in treated groups was lower ($p\leq 0.05$) than that of the control group; but not significantly different ($p>0.05$) from that of

the standard group. Enlargement of liver was not observed in treated groups.

Effect of polysaccharide gel on pregnancy and growth of offspring

The polysaccharide gel from fruit-hulls of durian did not affect pregnancy and offspring in treated female mice. The results are shown in Table 3 and Figure 3. Every female mouse in treated groups became pregnant normally after being housed with an untreated male mouse (within 14 days). Most females gave birth 22-28 days later. Table 3 shows that the number of offspring which was 10-11, the ratio of male : female of offspring was 1 : 1. The average weight at birth (day 1) of offsprings in treated groups was not different from that of control group. The average weight of offspring during 1-4 weeks is also demonstrated in Table 3. The relative weight gain profile of offspring is illustrated in Figure 3. The average relative weight gain of offspring was determined every week during the suckling period (about 3 weeks), and also the following week. Figure 3 shows that the relative weight gain of offspring from treated mother was not significantly different ($p>0.05$) from their

Table 3. Body weight, sex and number of offspring from test female mice treated with DF_I and DF_{II}. Mean values of treated groups are not significantly different from control ($p>0.05$). F = female, M = male, n = number of test female mice.

Test animals (Mice)	n, Sex	No. of offspring Mean $\pm$ SD	F/M	Offspring average body weight (g),			Mean $\pm$ SD	
				Day 1	Day 7	Day 14	Day 21	Day 28
Control group water 10 ml/kg/d	8,F	10 $\pm$ 1	$\frac{4\pm1}{6\pm1}$	1.76 $\pm$ 0.20	4.47 $\pm$ 0.59	6.88 $\pm$ 1.13	10.71 $\pm$ 1.22	19.30 $\pm$ 2.94
Standard group pectin 0.25g/kg/d	8,F	11 $\pm$ 1	$\frac{4\pm1}{7\pm1}$	1.66 $\pm$ 0.07	4.07 $\pm$ 0.49	6.31 $\pm$ 0.71	9.07 $\pm$ 1.05	17.08 $\pm$ 1.78
Test group DF _{II} DF _{II} 0.5g/kg/d	8,F	10 $\pm$ 1	$\frac{5\pm1}{5\pm1}$	1.69 $\pm$ 0.17	4.61 $\pm$ 0.31	7.00 $\pm$ 0.72	10.95 $\pm$ 1.18	19.81 $\pm$ 1.71
Test group DF _{II} DF _{II} 0.25g/kg/d	8,F	11 $\pm$ 1	$\frac{5\pm1}{6\pm1}$	1.63 $\pm$ 0.11	4.06 $\pm$ 0.93	6.57 $\pm$ 1.13	9.44 $\pm$ 2.39	18.16 $\pm$ 2.11
Test group DF _I DF _I 0.25g/kg/d	8,F	10 $\pm$ 1	$\frac{5\pm2}{5\pm1}$	1.54 $\pm$ 0.22	4.32 $\pm$ 1.01	6.71 $\pm$ 1.59	9.82 $\pm$ 3.51	16.99 $\pm$ 3.27

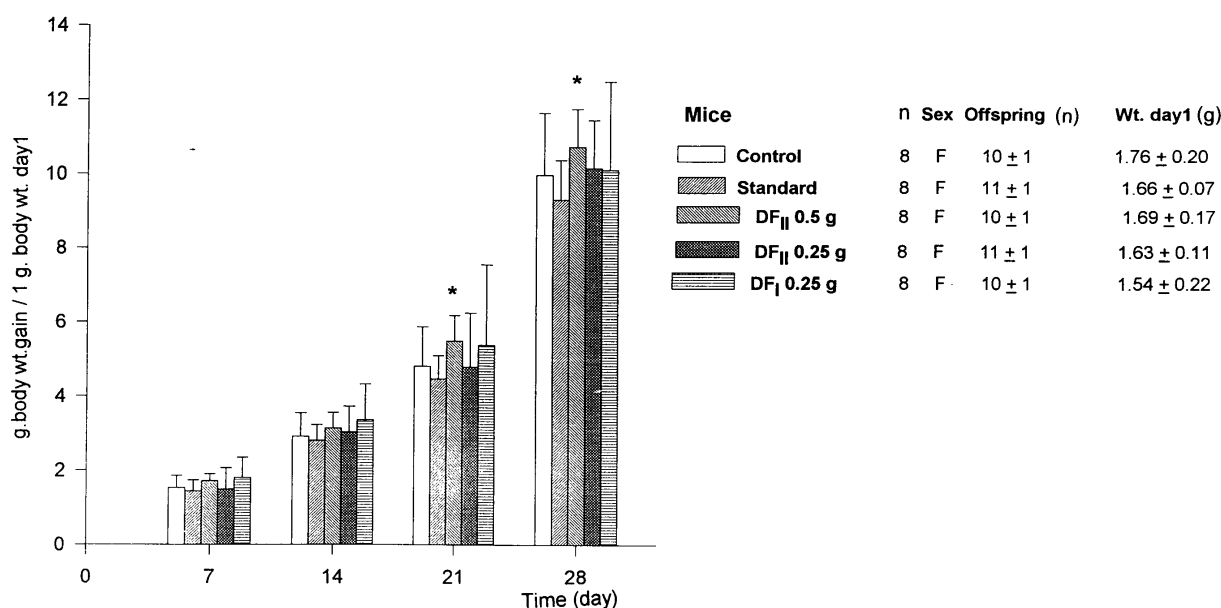


Figure 3. Relative weight gain of offspring during 4 weeks after birth from mother treated with DF_I and DF_{II} for 4 weeks before mating and during lactation. Each bar represents mean $\pm$ SD * = significantly different from control ($p \leq 0.05$). Control = offspring from mother receiving water 10 ml/kg/d. Standard = offspring from mother receiving pectin 0.25 g/kg/d. F = treated female mice. n = number of mice.

control and standard groups, except that of offspring at day 21 and day 28 in group of DF_{II} (0.5 g/kg/d) treated mother, which showed higher relative weight gain profile ($p < 0.05$) than that of offspring from control group. The hematological data of offspring of treated and control groups (Table 4) showed normal hematological parameters. These results suggest that polysaccharide gel did not affect the pregnancy and growth of offspring in mice. The growth rate of offspring from treated mothers was not low in comparison to controls.

Biochemical and clinical analyses

Hematological data in Tables 5 and 6 demonstrate normal hematological parameters in treated male and female mice. The percentages of hematocrit, hemoglobin, lymphocytes, red blood cells and white blood cells count between treated and control male and female mice were not significantly different ($p > 0.05$). However, white blood cells count in DF_I and DF_{II} treated (at a

dose 0.25 g/kg/d) male mice was significantly higher ($p < 0.05$) than their controls but was not significantly different ($p > 0.05$) from that of their standard group. The results indicate that polysaccharide gel DF_I and DF_{II} at an oral dose of 0.25 g/kg/d and DF_{II} at a high dose of 0.5 g/kg/d did not induce hematological changes in treated male and female mice. However, WBC count in treated male (Table 5) was higher than its control ($p \leq 0.05$) but not significantly different ($p > 0.05$) from standard group.

Serum concentrations of glucose, cholesterol, creatinine and blood urea nitrogen (BUN) in male and female mice are shown in Figures 4 and 6, respectively. The results of serum glucose, cholesterol, creatinine and BUN in treated male and female mice were not significantly different ($p > 0.05$) from those of their standard and control groups. Data were compared among the test, standard and control groups by test for homogeneity of variance (Levene test) using ANOVA followed by Duncan's multiple range test, with

Table 4. Hematological parameters in offspring from test female mice treated with DF_I and DF_{II}. Data are expressed as mean $\pm$ SD, M = male, F = female, n = number of test female mice

Test animals (Mice)	n, Sex	No. of offspring Mean $\pm$ SD	Hct (%)		Hb (%)		RBC ($\times 10^6/\text{mm}^3$)		WBC ($\times 10^3/\text{mm}^3$)	
			F	M	F	M	F	M	F	M
Control group water 10 ml/kg/d	8,F	10 $\pm$ 1 4 $\pm$ 1 6 $\pm$ 1	45.9 $\pm$ 2.2	43.6 $\pm$ 2.6	13.5 $\pm$ 1.0	12.8 $\pm$ 1.0	7.9 $\pm$ 0.5	7.6 $\pm$ 0.6	2.6 $\pm$ 1.1	2.3 $\pm$ 0.8
Standard group pectin 0.25g/kg/d	8,F	11 $\pm$ 1 4 $\pm$ 1 7 $\pm$ 1	43.7 $\pm$ 2.1	44.1 $\pm$ 1.9	13.1 $\pm$ 1.0	13.0 $\pm$ 1.2	7.6 $\pm$ 0.6	7.6 $\pm$ 0.7	2.2 $\pm$ 0.9	2.2 $\pm$ 1.3
Test group DF _{II} DF _{II} 0.5g/kg/d	8,F	10 $\pm$ 1 5 $\pm$ 1 5 $\pm$ 1	45.4 $\pm$ 2.8	44.3 $\pm$ 1.5	13.1 $\pm$ 1.2	12.7 $\pm$ 1.3	7.8 $\pm$ 0.7	7.4 $\pm$ 0.9	3.8 $\pm$ 1.6	3.5 $\pm$ 1.1
Test group DF _{II} DF _{II} 0.25g/kg/d	8,F	11 $\pm$ 1 5 $\pm$ 1 6 $\pm$ 1	44.8 $\pm$ 2.5	44.3 $\pm$ 2.6	13.2 $\pm$ 0.9	12.9 $\pm$ 1.1	7.9 $\pm$ 0.6	7.6 $\pm$ 0.6	2.9 $\pm$ 0.9	3.4 $\pm$ 1.5
Test group DF _I DF _I 0.25g/kg/d	8,F	10 $\pm$ 1 5 $\pm$ 2 5 $\pm$ 1	44.3 $\pm$ 3.5	44.4 $\pm$ 1.8	13.2 $\pm$ 1.4	13.1 $\pm$ 1.1	7.8 $\pm$ 0.8	7.7 $\pm$ 0.7	2.5 $\pm$ 1.1	2.4 $\pm$ 1.0

Table 5. Hematological parameters in male mice after 60 days treatment with DF_I and DF_{II}. Data are expressed as mean $\pm$ SD, NS = no significant difference between groups ($p > 0.05$), a, b = significant difference between groups ($p \leq 0.05$), M = male, n = number of mice.

Test animals (mice)	n, Sex	Weight (g) Mean $\pm$ SD	Hct %	Hb %	RBC $\times 10^6/\text{mm}^3$	WBC $\times 10^6/\text{mm}^3$	PMN %	Band %	Lymph %	Mon %	Eos %	Baso %
			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Control group water 10 ml/kg/d	14,M	37.4 $\pm$ 2.9	43.6 $\pm$ 5.3	14.2 $\pm$ 1.9	9.1 $\pm$ 1.1	1.9 $\pm$ 1.2	20 $\pm$ 12	-	79 $\pm$ 11	0-1	0-1	-
Standard group pectin 0.25 g/kg/d	15,M	38.4 $\pm$ 2.9	41.8 $\pm$ 1.5	14.7 $\pm$ 0.6	9.1 $\pm$ 0.2	2.9 $\pm$ 1.8	28 $\pm$ 10	-	70 $\pm$ 10	0-1	0-2	-
Test group DF _{II} DF _{II} 0.5 g/kg/d	15,M	36.6 $\pm$ 1.8	44.2 $\pm$ 1.3	15.7 $\pm$ 1.5	10.1 $\pm$ 1.2	3.5 $\pm$ 1.3	36 $\pm$ 15	-	62 $\pm$ 16	0-2	0-2	-
Test group DF _{II} DF _{II} 0.25 g/kg/d	14,M	38.2 $\pm$ 3.1	45.4 $\pm$ 4.2	15.2 $\pm$ 1.4	9.4 $\pm$ 0.7	4.7 $\pm$ 1.1	25 $\pm$ 4	-	74 $\pm$ 4	0-1	0-1	-
Test group DF _I DF _I 0.25 g/kg/d	13,M	38.8 $\pm$ 2.8	44.0 $\pm$ 4.1	14.6 $\pm$ 1.2	9.5 $\pm$ 0.8	4.3 $\pm$ 1.8	23 $\pm$ 5	-	76 $\pm$ 6	0-1	0-2	-

Table 6. Hematological parameters in female mice after 100 days treatment with DF_I and DF_{II}. Data are expressed as mean $\pm$ SD, NS = no significant difference between groups ($p > 0.05$), a, b = significant difference between groups ($p \leq 0.05$), F = female, n = number of mice.

Test animals (mice)	n, Sex	Weight (g) Mean $\pm$ SD	Hct % NS	Hb % NS	RBC $\times 10^6/\text{mm}^3$ NS	WBC $\times 10^6/\text{mm}^3$ NS	PMN % NS	Band %	Lymph %	Mon %	Eos %	Baso %
Control group water 10 ml/kg/d	8, F	31.4 $\pm$ 2.7	45.2 $\pm$ 2.4	13.9 $\pm$ 1.6	8.8 $\pm$ 0.9	2.0 $\pm$ 0.9	12.5 $\pm$ 3.9	-	ab 86.2 $\pm$ 4.6	0-1	0-2	-
Standard group pectin 0.25 g/kg/d	8, F	29.7 $\pm$ 2.8	42.8 $\pm$ 4.5	13.5 $\pm$ 2.1	8.4 $\pm$ 1.4	1.5 $\pm$ 0.9	13.0 $\pm$ 6.0	-	ab 86.2 $\pm$ 5.9	0-1	0-2	-
Test group DF _{II} DF _{II} 0.5 g/kg/d	8, F	29.7 $\pm$ 5.6	43.2 $\pm$ 5.2	11.3 $\pm$ 2.9	7.2 $\pm$ 1.7	1.3 $\pm$ 0.8	11.2 $\pm$ 3.9	-	ab 88.2 $\pm$ 4.0	-	0-2	-
Test group DF _I DF _I 0.25 g/kg/d	8, F	31.1 $\pm$ 2.6	43.8 $\pm$ 4.8	14.2 $\pm$ 4.2	7.8 $\pm$ 0.9	1.8 $\pm$ 1.3	8.7 $\pm$ 4.9	-	a 90.6 $\pm$ 4.7	-	0-1	-
Test group DF _I DF _I 0.25 g/kg/d	8, F	33.3 $\pm$ 1.6	46.0 $\pm$ 2.0	14.3 $\pm$ 0.7	8.8 $\pm$ 0.5	3.3 $\pm$ 1.9	15.8 $\pm$ 6.1	-	a 81.0 $\pm$ 5.7	0-3	0-4	-

$p \leq 0.05$ as the level of significance. Interestingly, average serum levels of cholesterol in DF_{II} at 0.25 mg/kg/d treated male as well as female mice showed the lowest value in comparison to those of their control and standard groups (Figures 4 and 6). The results of normal values of BUN and creatinine obtained seem to indicate that polysaccharide gel did not produce kidney damage in treated animals (Wildman, 1984).

The pathological change and toxic effects are characterized by an increase in liver and kidney weights, as well as the high levels of biochemical substances especially some specific enzymes in serum, such as alkaline phosphatase (ALP), aspartate aminotransferase (AST; SGOT) and alanine aminotransferase (ALT; SGPT) (Fauci *et al.*, 1998; Wildman, 1984). In the present study, the serum levels of these enzymes in treated male and female mice were not markedly increased in comparison to their controls as shown in Figures 5 and 7, respectively. It is illustrated in Figure 5 that serum levels of ALP in treated male groups were lower than in the control group. The serum levels of AST (SGOT) < 100 U/L and ALT (SGPT) < 20 U/ were not significantly different ($p > 0.05$) from those of their control group. The result of no increment of serum ALP in this study suggests that pathological changes of liver function had not occurred (Fauci *et al.*, 1998). The results of normal levels of AST (SGOT) and ALT (SGPT) in treated male mice also confirmed that pathological changes of liver, heart and muscle had not occurred (Fauci *et al.*, 1998). The serum levels of AST (SGOT) < 100 U/L and ALT (SGPT) < 35 U/L in treated female mice (Figure 7) were not markedly high and not significantly different ($p > 0.05$) from their control. The results clearly indicate that pathological changes of liver, heart and muscle function had not occurred (Fauci *et al.*, 1998). The serum levels of ALP in DF_{II} treated female group was not different ($p > 0.05$) from those of control group and the serum ALP in DF_I treated group was not different ($p > 0.05$) from that of standard group. ALP levels in treated and control mice (< 90 U/L) were not markedly

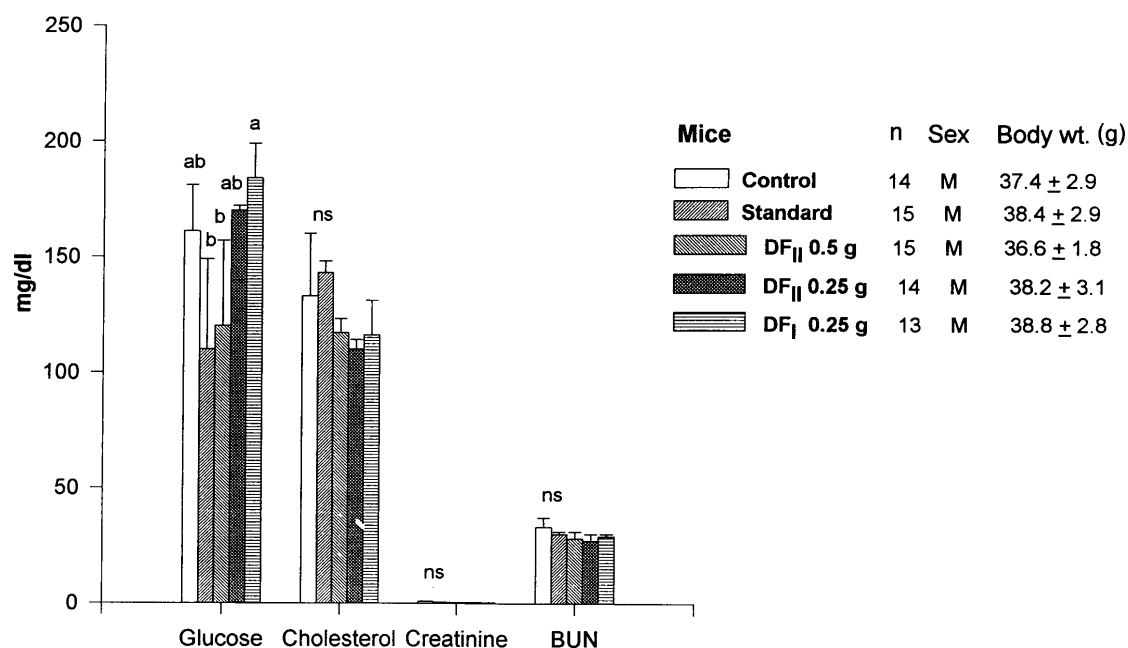


Figure 4. Biochemical analysis of serum in male mice after 60 days treatment with DF_I and DF_{II}. Each bar represents mean ± SD, ns = no significant difference between groups ($p > 0.05$), a, b = significant difference between groups ($p \leq 0.05$). Control male mice were fed with water 10 ml/kg/d. Standard male mice were fed with pectin 0.25 g/kg/d. M = male, n = number of mice.

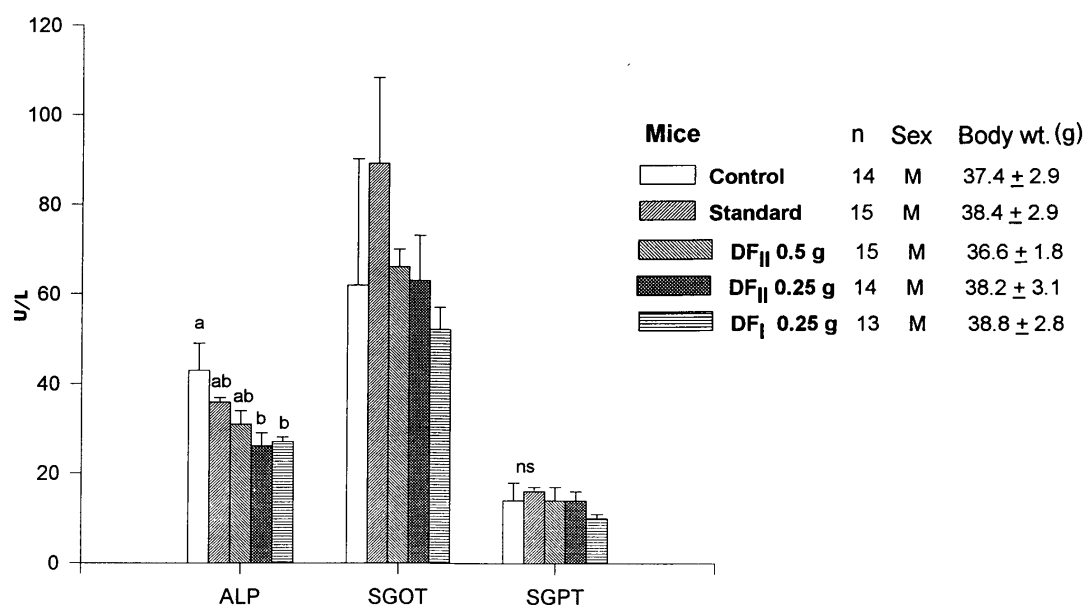


Figure 5. Levels of enzymes in serum in male mice after 60 days treatment with DF_I and DF_{II}. Each bar represents mean ± SD, ns = no significant difference between groups ($p > 0.05$), a, b = significant difference between groups ($p \leq 0.05$). Control male mice were fed with water 10 ml/kg/d. Standard male mice were fed with pectin 0.25 g/kg/d. M = male, n = number of mice.

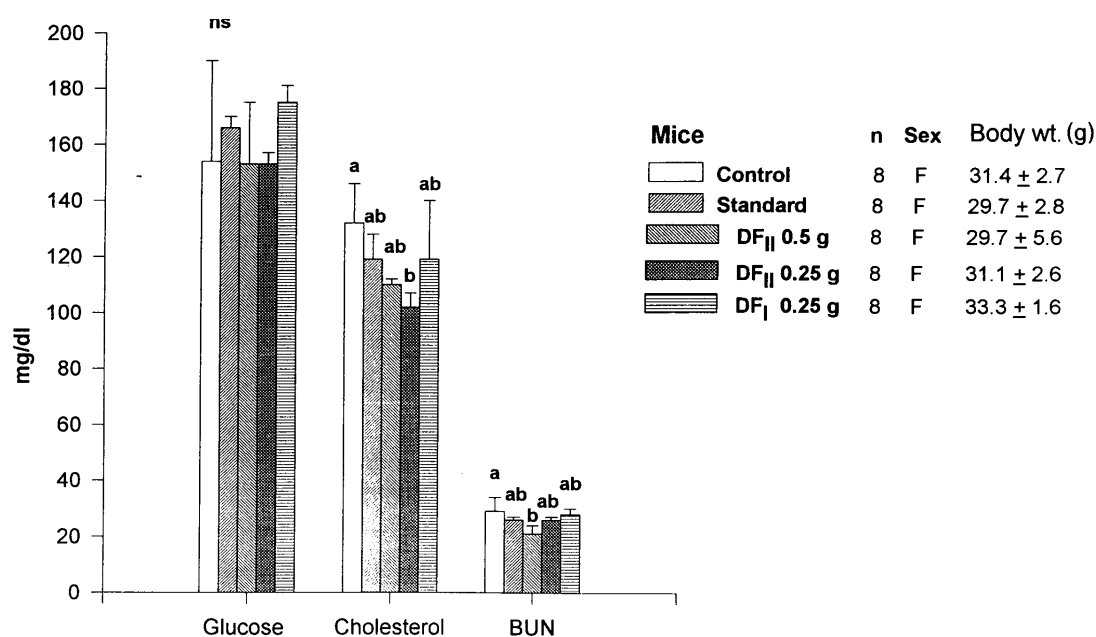


Figure 6. Biochemical analysis of serum in female mice after 100 days treatment with DF_I and DF_{II}. Each bar represents mean ± SD, ns = no significant difference between groups ($p > 0.05$), a, b = significant difference between groups ($p \leq 0.05$). Control female mice were fed with water 10 ml/kg/d. Standard female mice were fed pectin 0.25 g/kg/d. F = female, n = number of mice.

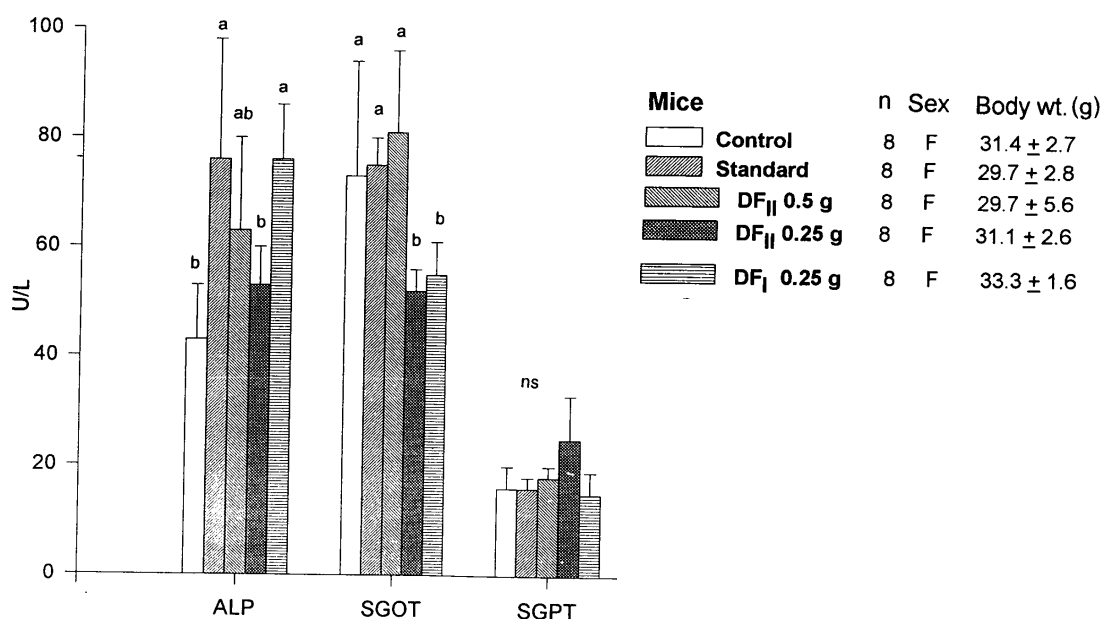


Figure 7. Levels of enzymes in serum in female mice after 100 days treatment with DF_I and DF_{II}. Each bar represents mean ± SD, ns = no significant difference between groups ($p > 0.05$), a, b = significant difference between groups ($p \leq 0.05$). Control female mice were fed with water 10 ml/kg/d. Standard female mice were fed pectin 0.25 g/kg/d. F = female, n = number of mice.

higher than normal ALP level in female mice. The results of the present study suggest that polysaccharide gel, DF_I and DF_{II} at a dose 0.25g/kg/d and DF_{II} at a high dose of 0.5 g/kg/d, could be consumed safely for long periods of 60 and 100 days in male and female mice, respectively. Interestingly, at a high oral dose (0.5 g/kg/d), DF_{II} seems to induce low level of serum cholesterol and low body weight gain in male and female mice after long term treatment. These properties have also been observed with some other dietary fiber (Chandalia, *et al.*, 2000; Vahouny, *et al.*, 1988).

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