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STUDIES OF GINSENG SAPONIN FRACTIONS ON CHOLESTEROL ABSORPTION AND BLOOD SERUM LIPIDS IN RATS.

Song Jae Lee, In Cheol Jeong, and Moo Youn Cho

Department of Biochemistry, Kosin Medical College, Pusan, Korea

= Abstract =

It was attempted in this present study to observe the effect of ginseng saponin fractions, one of the active components of ginseng, on cholesterol absorption using the radioactive material and the blood serum lipids in orally long-term administeration of ginseng saponin fraction to rats.

1) The purified ginseng total saponin was identified with High Performance Liquid Chromatography

2) It was demonstrated that the absorption peak pattern of blood serum and hepatic lipid on time course after the 4-^{14}C-cholesterol feeding was similar between two groups However, the radioactivity value of ginseng saponin administered rats was much higher than that of control rats.

3) In protopanaxdiol saponin fed rats, the absorption peak pattern and the radioactivity value of blood serum and hepatic lipid was similar between two groups.

4) The absorption peak pattern of blood serum lipid and hepatic lipid in protopanaxtriol saponin fed rats showed that the highest peak of radioactivity appeared earlier than control, furthermore the radioactivity value of the test group was much higher than that of control group.

These results suggest that the above stimulatory effect of ginseng total saponin on cholesterol absorption might be due to the protopanaxtriol saponin

5) The radioactivity value of the prosapogenin administered rats was also much higher than control group

These results suggest that ginseng saponin, during digestion, would greatly be favorable for the formation of micellar structure of non polar lipids (such as triglycerides and cholesterol) with bile salts, phospholipids and other polar lipids so that fatty materials might be absorbed effectively from small intestine

6) The analysis of blood serum lipids in ginseng saponin and prosapogenin administered rats for 4 weeks showed that ginseng saponin and prosapogenin increased the phospholipid level, while the cholesterol level slightly decreased than that of control group.

These results again suggest that ginseng saponin might be effective on cholesterol absorption and its utilization.

Ⅰ. INTRODUCTION

Ginseng has been used widely as herbal medicine for many centries. Ginseng saponin, one of the active components of ginseng, was first reported by Garriques in 1894. He isolated a saponin component from american ginseng and named it panaquilone.

The study of ginseng saponin on lipid metabolism has been centered for many reseachers during the past two decades

Oura and his coworkers[6] reported that ginseng saponin increased transiently lipid synthesis rates in ^{14}C-acetate administered rats. They also studied the effect of ginseng saponin on lipid synthesis in rats with different diet condition In fasted rats, ginseng saponin didn't affect on ^{14}C-acetate incorporation, but in rats with high carbohydrate, high protein and normal diets, ginseng saponin increased the lipid synthesis. However, in high fat diet groups, ginseng saponin didn't increased the lipid synthesis rate.

Yamamoto and his coworkers[8] demonstrated that ginseng saponin fraction was effective on the improvement of lipid metabolism in liver cancer host; the capacity of fatty acid synthesis, particularly neutral fatty acid was decreased in liver cancer aminals and in cancer victims. They also reported that ginseng saponin fraction improved the hyperlipidemia in rats and in man; with the improvement of blood apoprotein, lipoprotein and prostaglandin in experimental hyperlipidemia animals.

Joo and his coworkers[3] suggested that ginseng saponin are surface active, therefore nonpolar lipids such as triglyceride and cholesterol are more effectively dispersed in the presence of saponin. They also suggested that ginseng saponin might be favorable for the formation of micellar structure of nonpolar lipids such as triglyceride and cholesterol with bile salts, phospholipids and other polar lipids such as partially hydrolyzed glycerides so that the fatty material might be absorbed effectively from small intestine.

In connection with the above results, the present studies aim to study the effect of ginseng saponin fractions on cholesterol absorption and transport in rats. In addition, the effect of ginseng saponin fraction on blood serum lipids in orally long term administrated rats was studied.

Ⅱ. MATERIALS AND METHODS

1. Preparation of purified ginseng saponin.

Crude ginseng saponin mixture was obtained from powered red ginseng roots (6 years old) according to the modified procedure described elsewhere. The crude saponin mixture was applied to active carbon column eluted with methanol. The eluate was then concentrated and lyophilized with LABCONCO Freeze Dryer. The ginsenoside peak of purified total saponin was identified with High Performance

Liquid Chromatography (HPLC) as shown in Figure 1 This purified saponin was used during the experimental period.

The prosapogenin was prepared from total saponin according to the modified procedure of Shibata.[7] The purified total saponin was dissolved in distilled water and adjust to pH at 3.0 with acetic acid and then heated at 90℃ for 6 hours. The precipitant was washed many times with distilled water and then lyophilized with LABCONCO Freeze Dryer.

Protopanax-diol and-triol saponin from total saponin were prepared by modified procedure of Hans.[2]

2. Reagents

Cholesterol, Na-taurocholate were purchased from Sigma Co. 4-^{14}C-cholesterol, POPOP, and PPO were obtained from New England Nuclear. The Kit reagents of blood analysis were purchased from Iatron (Japan) and organic solvents were obtained from Wako Co. (Japan)

3. Animals

Sprague-Dawley rats (male, 60~80g) fed a normal diets were used as experimental animals.

4. Observation of the effect of ginseng saponin fractions on cholesterol absorption using 4-^{14}C-cholesterol in prolonged ginseng saponin fraction fed rats.

Five groups of albino rats (male, 60~80g), each consisting of 10 animals, were given normal feed. Total saponin (10mg/kg body weight/day) was orally administered by stomach tubing to test group 1 for 4 weeks. Protopanaxdiol saponin (10mg/kg body weight/day) was orally administered to test group 2 Protopanaxtriol (10mg/kg body weight/day) saponin was administered to test group 3 and test group 4 was administered prosapogenin (10mg/kg weight/day) which was suspended with 0.1% of carboxy methyl cellulose. The control group was given normal diet and water.

They were then fasted for 24 hours before the start of experiment. Cholesterol (10mg) containing 4-^{14}C-cholesterol (1 μCi), 5mg of Na-taurocholate and 10mg of saponin fractions per rat were administered orally to the test groups. The control group was treated similiary, but no saponin fraction The rats were then killed under sodium thiopental anesthesia on time course (1~10hours) after the feeding. The blood were then taken from the heart and the blood serum separated from the blood were used for analysis.

5. Extraction of total lipids from hepatic tissue and blood serum.

The frozen liver (5g) were sliced and homogenized and 30mℓ of homogenate were prepared. Methanol and chloroform were then added to make the volume ratio of chloroform-methanol-water of the mixture being 1 : 2 : 0.8 so that the mixture might be one phase. The mixture was allowed to stand overnight at room temperature and then centrifuged to remove the insoluble material Chloroform and water were then added to the above supernatant to make the volume ratio of chloroform-methanol-water being 1 : 1 : 0.9 which separated the mixture into two phases, methonol-water phase (upper) and chloroform (bottom) phase. The chloroform extract was concentrated under nitrogen stream. The lipid extraction of blood serum were prepared described above.

6. Observation of the effect of ginseng saponin fraction on blood serum lipids in long-term administered rats

Five groups of albino rats (male, 60~80 g), each consisting of 12 animals, were given normal diet. In test group 1 and test group 2 were orally administered 25mg/kg body weight/day and 50mg/kg body weight/day of total saponin respectively for 4 weeks. Test group 3 and test group 4 were orally administered 25mg/kg body weight/day and 50mg/kg body weight/day of prosapogenin for 4 weeks. The control group was given normal diet only. The animal to be sacrificed were not fed for the last 24 hours, anesthetized with sodium thiopental and the blood was taken from the heart.

7. Radioactivity assay

Aliquot of sample (chloroform phase; 1mℓ) was placed in counting vial and added 10mℓ of scintillation mixture (POP 4g+POPOP 100 mg/L of toluene). The radioactivity was measured using Pakard Liquid Scintillation Counter.

Ⅲ. RESULTS AND DISCUSSION

The digestion of fats and other lipids poses a special problem because of their insolubility in water and the lipolytic enzymes, like other enzyme, are soluble in an aqueous medium.

Additionally, even if ingested lipids are hydrolyzed into simple constituents, the products tend to be aggregate to larger complexes that make poor contact with the cell surface and therefore are not easily absorbed. These problems are overcome by the increase in the interfacial area between the aqueous and the lipid phase, and the solubilization of the hydrolysis products with detergents. Therefore, changes in the physical state of lipids are intimately connected to chemical changes during digestion and absorption

It is well known that the major site of the lipid digestion is the small intestine and the absorption of the digestion products of fats, primarily free fatty acids (70%) and 2-monoglyceride, occurs from the micells in the microvilli of the epithelial cells of the small intestinal mucosa. The two most important secretion for these purpose are pancreatic juice and bile. The ability of bile salt solution to dissolve lipids is merely reflection of their detergent properties. Above a critical micelle concentration (CMC), bile salts aggregate to form micelles Bile salt micelles can solubilized other lipids, such as phospholipids and fatty acids. Within these mixed phospholipid-bile acid micelles, other water-insoluble lipids, such as cholesterol can be accomodate and thereby solubilized. Micelles provide the major vehicle for moving lipids from the intestinal lumen to the cell surface when absorption occurs. Thus, efficient lipid absorption depends on the presence of the sufficient bile acids to "solubilized" the ingested and hydrolyzed in micelles Mechanism of cholesterol absorption from small intestine is known being similar pattern to that of fat absorption.[1,7]

Joo and his coworkers[5] demonstrated that ginseng saponin lowered surface tension of water significantly and the CMC of the pure saponin solution was found being about 2%. When saponin was added to the cholesterol suspension, the formation of micelles seemed to start at the saponin concentration of only 0.1%, which was one twentieth of the CMC of the saponin alone. Furthermore, the CMC (5mℓ) of Na-cholate dispersing cholesterol was significantly lowered when saponin was added to the above mixture From the above results, they suggested that under the physiological condition, a small amount of the saponin would affect sufficiently for lipid micelle formation in

intestinal lumen, therefore lipids might be absorbed easily from small intestine. Joo and his coworkers also reported that the saponin stimulated the absorption and transport of water-insoluble vitamins such as vitamin A and vitamin K.

In the present study, it was observed that the radioactivity of blood serum and hepatic lipid extract of 4-^{14}C-cholesterol fed rats on time course after the ginseng total saponin fed group showed that the highest radioactivity of both blood serum and hepatic lipid extract appeared at 7 hours after the 4-^{14}C-cholesterol feeding. In control group, absorption pattern of 4-^{14}C-cholesterol was similar to that of test group, however the maximum value of the radioactivity in the test group was found much higher than that of the control group as shown in Figure 2,3. It was further studied that any fraction of total saponin was effective on the above stimulation effect on cholesterol absorption. In protopanaxtriol saponin (aglycon of ginseng glycoside) fed group, the cholesterol absorption was significantly stimulated, the highest radioactivity of both blood serum and hepatic lipid extract appeared earlier (at 6 hours) after the radioactive 4-^{14}C-cholesterol feeding than that of control which was observed at 7 hours after the feeding. In addition, the radioactivity value of the test group was much higher than that of control rats shown in Figure 4, 5. In the liver and blood serum lipid of both control and test group, the second highest peak was observed at 4 hours after the feeding. These second highest radioactivity peak might be due to freshly absorbed cholesterol. However, protopanaxdiol saponin (another aglycon of ginseng glycoside) did not affect on cholesterol absorption as shown in Figure 6 (hepatic lipid data was not presented) In addition to these saponin fractions, it was also studied the effect of prosapogenin (hydrolytic product of total saponin) on cholesterol absorption. It was found that prosapogenin significantly stimulated cholesterol absorption in blood serum lipid and hepatic lipid extract as shown in Figure 7. (hepatic lipid data was not presented) Furthermore, the radioactivity value of the test group was much higher than that of the contol group

These results suggest that the stimulatory effect of total saponin in cholesterol absorption might be mainly due to the protopanaxtriol saponin. From the above results, it was suggest that ginseng saponin fractions would be facilitate for the absorption and utilization of cholesterol

It was also examined the variation of blood serum lipid in ginseng saponin and prosapogenin treated rats. The blood serum triglyceride and the albumin level of the test groups were similar to that of the control group as shown in Figure 8. 9 It was found that the level of blood serum cholesterol was slightly decreased in the test groups, however, phospholipid level was increased (Fig 10, 11). It is interesting that the ratios of cholesterol to phospholipid of the blood serum of the test group was lower than that of the control group. It is well known that phospholipid plays a significant role in the transport of lipids including cholesterol, therefore the increase of phospholipid by the ginseng saponin may facilitate the transport and utilization of cholesterol.

These above results suggest that orally administered cholesterol might be absorbed faster in saponin fraction treated rats and the saponin fraction might be stimulate cholesterol transport and its utilization.

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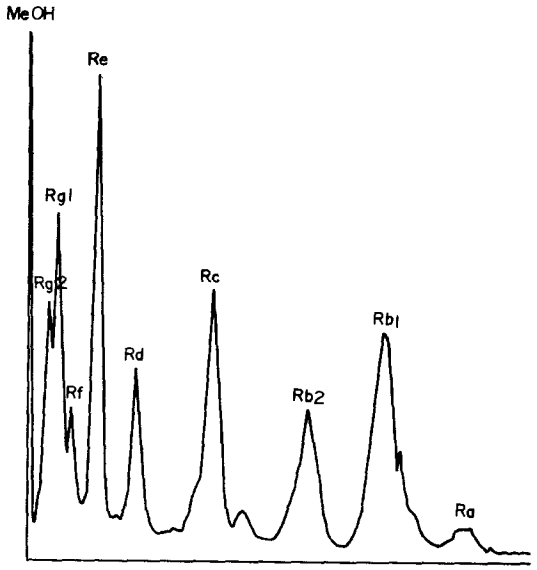

Fig. 1. HPLC-chromatogram of purified total saponin.
Column μBondapak C_{18}, 4 6mm(ID)×20cm; eluent Acetonitrile-H_2O-BuOH(80/20/15), flow rate mℓ/min; Detector, RI-401

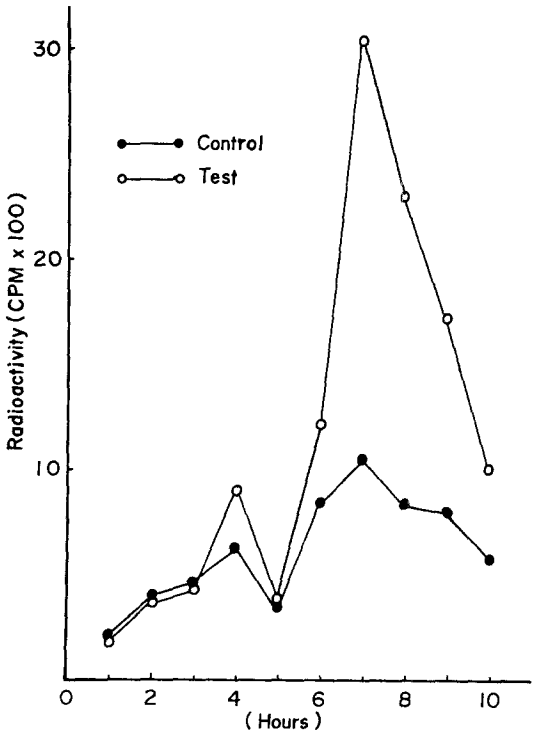

Fig. 2. The radioactivity of blood serum lipid fraction of 4-^{14}C-cholesterol and total saponin administered rat on time course after the feeding

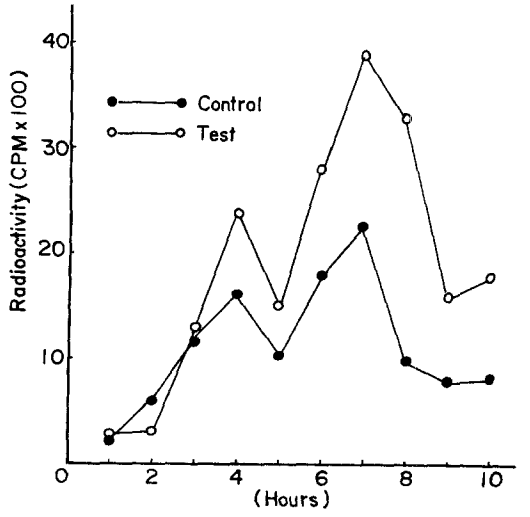

Fig. 3. The radioactivity of hepatic lipid fraction of 4-^{14}-cholesterol and total saponin administered rat on time course after the feeding.

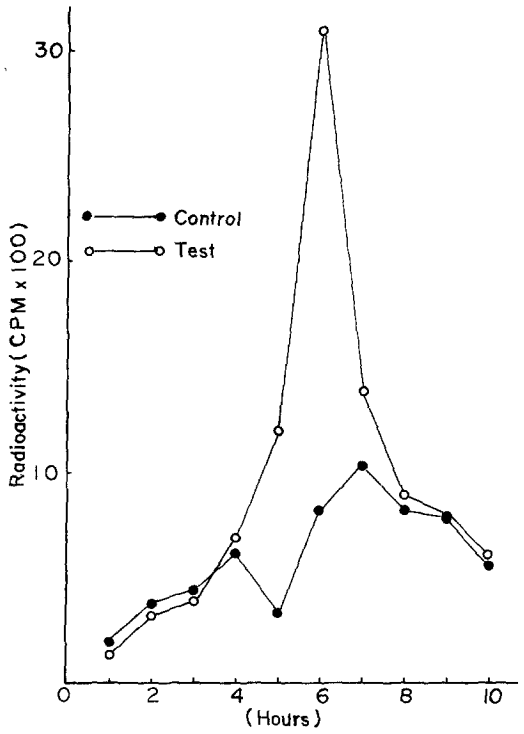

Fig. 4. The radioactivity of blood serum lipid fraction of 4-^{14}C-cholesterol and protopanaxtriol saponin administered rat on time course after the feeding.

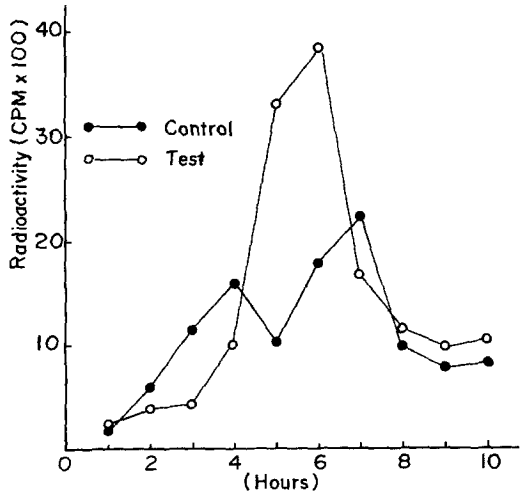

Fig. 5. The radioactivity of hepatic lipid fraction of 4-^{14}C-cholesterol and protopanaxtriol saponin administered rat on time course after the feeding

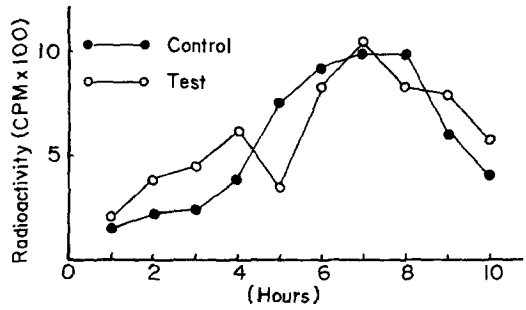

Fig. 6. The radioactivity of blood serum lipid fraction of 4-^{14}C-cholesterol and protopanaxdiol saponin administered rat on time course after the feeding.

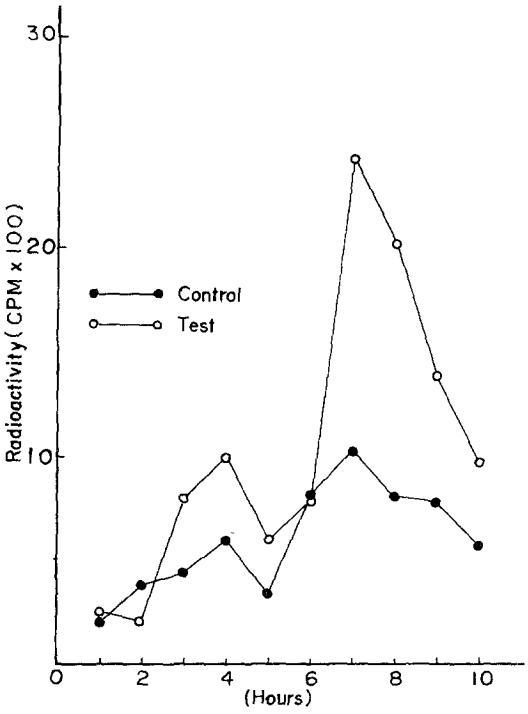

Fig. 7. The radioactivity of blood serum lipid fraction of 4-^{14}C-cholesterol and prosapogenin administered rat on time course after the feeding

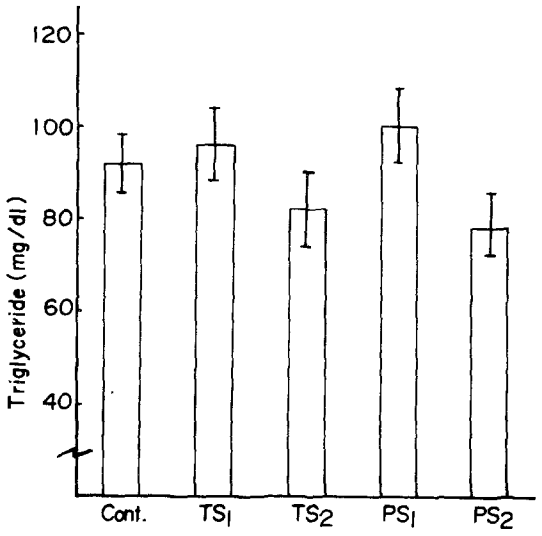

Fig. 8. The triglyceride of blood serum in orally administered with total saponin and prosapogenin for 1 month

TS1, Total saponin 25mg/kg body weight
TS2; Total saponin 50mg/kg body weight
PS1, Prosapogenin 25mg/kg body weight
PS2, Prosapogenin 50mg/kg body weight

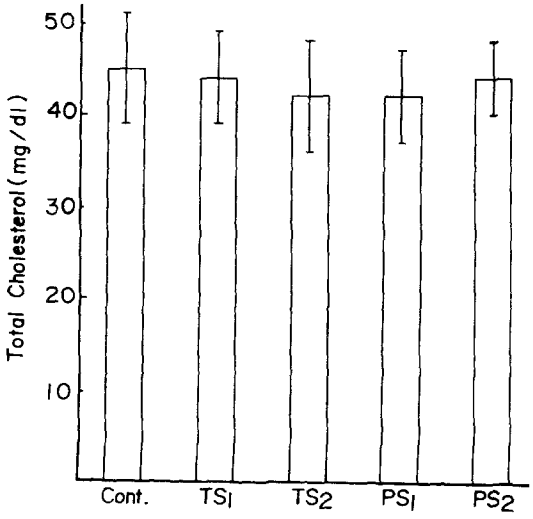

Fig. 10. The total cholesterol of blood serum in orally administered with total saponin and prosapogenin for 1 month

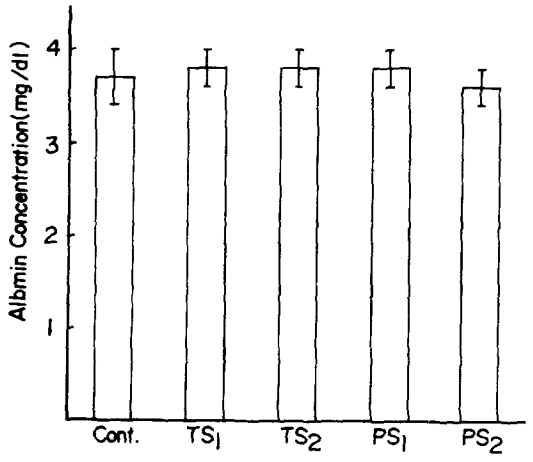

Fig. 9. The albumin of blood serum in orally administered with total saponin and prosapogenin for 1 month.

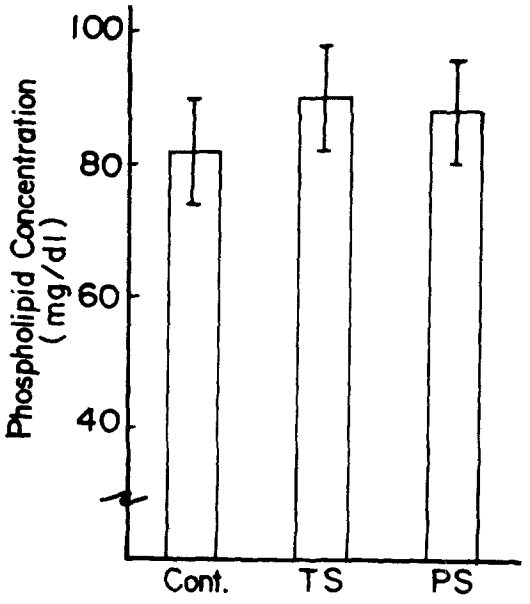

Fig. 11. The Phospholipid of blood serum in orally administered with total saponin and prosapogenin for 1 month.

TS; Total saponin 25mg/kg body weight
PS; Prosapogenin 25mg/kg body weight