Study on the Effect of Concentrated Extracts of *Pteridium* sp. Tube Feeding on the Serum Lipid Peroxides and Hepatic Homogenate Lipid Peroxides Value

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Pteridium sp. 의 濃縮抽出液이 動物血清 및 肝組織의 脂質過酸化에 미치는 影響에 關한 研究

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ABSTRACT

This experiment was carried out to investigate variation of plasma lipid level and liver peroxide level (Thiobarbituric acid, T.B.A) caused by tube feeding of Pteridium sp. extracts for seven weeks in rats.

A seven week rat's plasma triacylglyceride (T.G.) level showed remarkably higher than that of the control group and that of the three week rats. On the other hand, no remarkable differences of plasma phospholipid, free fatty acid and thiobarbituric acid level have been recognized.

Liver homogenate lipid peroxides value of seven week feeding rats showed remarkablly higher level compared with that of the control group and the three weeks rats.

So it could not be agreed that short time feeding of Pteridium sp. plant or even it's concentrated extracts influenced to the levels of liver homogenate lipid peroxides, plasma lipids and its peroxides.

INTRODUCTION

Pteridium sp. is one of the widely distributed plant in the korea peninsula and it is the favourite food as a substitute food and as a greens among the korean falks. But in recent years, attention has been drawn to the relation of toxicants of the food to various diseases associated with carcinogenesis and so on. Since the report of Braid(1) in 1936, numerous studies have appeared in the literature.

The toxicant which is contained in the Pteridium sp. is one of the delivatives of 1-indanone nucleus and pterosides is a compounded chemical component of p-D-glucopyranose which has an aglycone, they made clear (27, 28, 29).

Previous reports indicate that some of the grazing cow show sudden high fever and bleeding condition and lead to the death(1,2). It was reported that Pteridium, sp. contained certain carcinogenic substances and animal may be occured carcinogenesis by long term intake of Pteridium sp. (3,4,5,10). A calf

milked by grazing cow got cancer and certain mutagenic disorders in organs(31).

Alopecia phenomena of skin tissue and other tissue pathological lesions were confirmed not only on the circulatory system but also on the digestive and excretory system by long term intakes of Pteridium, sp. plants or shikimic acid which is one of the toxicant of Pteridium sp's as it is now known(30,6, 7,32,4,36). Author's previous report indicated the histological disorders that is, cell infiltration of blood vessels, glomerulus area in kidney, enlargement of blood capilary of organs and even necrosis of hair root, could be microscopically found. Furthermore, quercetin which is also one of the component of Pteridium sp. induce cancer in the intestine(40). In particular, the recent development of microassay technique for pterosides succeeded in isolating a single specific component "ptaquiloside" which is very unstable under both acidic and basic condition, water soluble, colorless and is in the form of noncristalized powder (8,38).

The ptaquiloside is a compound of illudance type glucoside has dienone as an aglycone and the aglycone is easily released and make an alkyl compound with biomolecule in vivo when the ptaquiloside is administered and the substances can easily induce cancer of certain organ, platelet decrement and bone marrow disease(8).

Phospholipid which is one of the composite lipids of animal tissue cell membrane, the phospholipid has polyunsaturated fatty acids, can be oxidized and make peroxides by certain stimulus or factors. Then the tissue cell can easily be induced to have disease as histological damage and cell mem brane permeability disorder follows.

It is assumable studying whether the extrects of pteridium sp. affect to the animal cell composite phospholipid and produce lipid peroxide is valuable when we agree with above fact.

The present study was conducted to investigate variations of plasma and tissue lipid peroxides level caused by Pteridium sp. extracts tube feeding in the rats and to detect whether any toxicants still remained in the food which is boiled and soaked in water.

MATERIALS and METHODS

1. Materials

Fresh and natural Pteridium sp., grown in 1985, was gathered from all parts of the Korea(11 markets) and prepared as experimental materials A(E.A) and B(E.B) as following process.

E.A - the materials were subcutted and boiled for twenty minutes with three times the volume of water, and soaked in water for twelve hours to extracts substances. The water was concentrated to one tenth volume with rotary pump.

E.B - the materials were subcutted and boiled for twenty minutes with three times the volume of water and the water was immediately concentrated to one tenth volume with rotary pump.

Propriety of the E.A and E.B - Pteridium, sp. contains 0.17% of shikimic acid, 0.1% of Ptaquiloside and others, these substances are all well water soluble and above methods can fully extracts them.

2. Animals and Diets

Eighteen male rats of Sprague-Dawley, weighing 150±6g, were separated into three groups of six animals each and fed with commercial diets for seven days adapting to the environment. After seven days prefeeding, Group 1(G.1) was fed with commercial diets and twenty ml of the extarcts (E.A) was administered by tube feeding each day for seven weeks. Group 2(G.2) was fed with commercial diets and twenty ml of the extracts(E.B) administered by tube feeding each day for seven weeks. And, control Group(C.) was fed with commercial diet only for seven weeks. Each group was fully supplied with tap-water, and diet was provided ad. libitum. The room was kept at costant temperature and humidity of 19-22°C and 56±1% respectively, and was kept light for twelve hours and dark for twelve hours a day.

Principal diet components are given in Table 1.

Table 1. Composition of Commercial foods(%)

Component	
Protein	25.8
Fat	6.2
Carbohydrate	57.1
Cellulose	4.4
Salt mixture	6.4*
Vitamins	**

* Mc Collums

** thiamin 900 ug, riboflavin 800 ug. pyridoxin 800 ug, nicotinic acid 4mg, pantothenic acid 2.5mg, ascorbic acid 50mg, V.A 2000 I.U., V.D. 400 I.U.

3. Analytical Procedures

1) Serum lipids and lipid peroxides - After three and seven weeks of experimental feeding, following analytical procedures were done.

The animals were fasted for twelve hours and under light anesthesia, specimens were taken at two p.m. immediately after decapitation.

Blood samples were immediately taken and serum was seperated, and assayed for serum peroxides(serum TBA) by the method of Yagi et al. (33)(Sulfate-Kento co., Phosphoryltangusnate-Wako Zunka co., 2-thio barbiturate-Tokyo Kasei co., Acetate-Wako Zunka co., and 1,1,3,3-tetramethoxypropane-Tokyo Kazei co.), and serum triacylglyceride(T.G) by the T.G analytical enzyme method Kit(Nippon Kokusai siyaku co.), serum free fatty acids(S.F F.A) by the serum free fatty acid analytical enzyme method Kit(Nippon Shoshya), and serum phospholipids(P.L) by the serum phospholipid analytical enzyme method Kit(Nippon daiayatron co.) and optical density were measured by wave length of 532nm. 505 nm and 500nm respectively.

2) Tissue (liver) lipid peroxides(TBA)

Tissues were dissected, minced and homogenized with a bio-mixer(Nippon Seiki co.). Homogenate was centrifuged and supernatant was removed for the measurement of the lipid peroxide value. The method of measurement was Mastushita modified method(13), and the optical density was measured with wave length of 532nm.

RESULTS

1. Serum lipids

The serum lipids values of each group are summerized in Table 2. Serum triacylglycerides value of G.1 and G.2 did not show any change in three weeks feeding group compare with control group, but in seven weeks feeding it was increased in significant amount, that is, the triacylglycerides value of G.1 and G.2 in three weeks feeding were 184.1 mg/100ml and 182.1mg/100ml, but in seven weeks feeding it increased greatly up to 249.3mg/100ml and 233.1mg/100ml repectively. It means approximately 80% of increment by seven week feeding (age increasing).

The phospholipid values showed also sharp increment in seven weeks feeding in both groups, but

Table 2. Effect on the Serum Triacylglyceride, Phospholipid, Free Fatty Acid and Peroxides Level of rats Tube Feeded with Pteridium sp's Extracts

Group	Age (week)	Triacylglyceride (mg/100ml) (TG)	Phospholipid (mg/100ml) (PL)	Free Fatty Acid (μEq/1) (FFA)	Peroxides (nmol/ml) (TBA)
Control	3	181.0±10.8	116.2±12.2	468.3±18.4	2.21±0.17
	7	181.1±10.8	118.8±11.8	467.4±18.3	2.38±0.18
1	3	184.1± 7.2	121.8±11.4	466.6± 9.8	2.42±0.21
	7	249.3±12.2	130.9±11.5	465.6±10.1	2.38±0.18
2	3	182.1± 7.7	125.8±10.0	459.0±12.7	2.43±0.21
	7	233.1±12.0	130.8±11.2	462.1±10.4	2.36±0.19

there is no significant change.

Serum peroxide values(TBA) showed no difference between control, G.1 and G.2.

2. Hepatic homogenates 2-thiobrabituric acid

Differences of hepatic homogenates 2-thiobarbituric acid values are shown in Table 3. The 2-thiobarbituric acid value of control group and G.1 and G.2 showed all increment inclination with aging.

And the thiobarbituric acid value of G.1 and G.2 showed uniformly higher level compared with that of control group, in particular the G.1 and G.2 exhibits marked change from 0.082 and 0.059 up to 0.200 and 0.163 of absorbances in hepatic homogenate thiobarbituric acid value with aging respectively.

Table 3. Effects on the Liver Homogenates TBA Values of rats Tube Feeded with Pteridium sp.'s Extracts

Group	Age(week)	
	3	7
Control	0.038±0.019	0.066±0.022
1	0.082±0.056	0.200±0.061
2	0.059±0.017	0.163±0.039

* Optical Density = 532 nm

DISCUSSION

Serum lipids –

There was no marked differences of serum triacyglycerides value between group 1 and 2 for seven weeks feeding, this indicates that we could not expect any toxic influences or reacts in vivo in short duration intakes of the extracts which contains approximately 0.17% of shikimic acid and 0.1% of ptaquiloside and others so called carcinogenic toxic sustances. In another words, the extracts should be intaken for longer duration to expect any toxicity in vivo. Ito et al. reported that(39) average value of serum lipids id est, serum triacygly-ceride, serum phospholipid and serum cholesterol so on, increased with aging and with toxic substances in rats.

And not only plasma thiobarbituric acid but also tissue homogenate thiobarbituric acid value may be affected by the toxicants and with aging(17,18, 19,39).

Sagai et al.(35) attempted to confirm the changes of serum lipid peroxide level by the method of measuring the amount of methane and ethane in the breath, and they indicated that the tending up level of the gas showed positive relation with aging. Kato et al.(21,24) insisted that certain toxicant causes various physiological bad exasperation, for

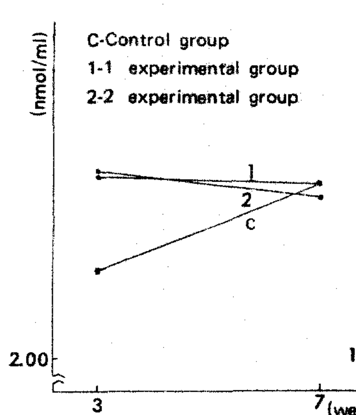

Fig. 1 Changes of Serum TBA Value of rat Tube Feeded with Pteridium sp.'s extracts.

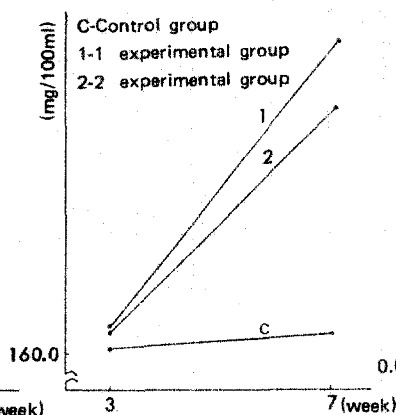

Fig. 2 Changes of Serum Triacylglyceride level of rats Tube Feeded with Pteridium sp.'s extracts.

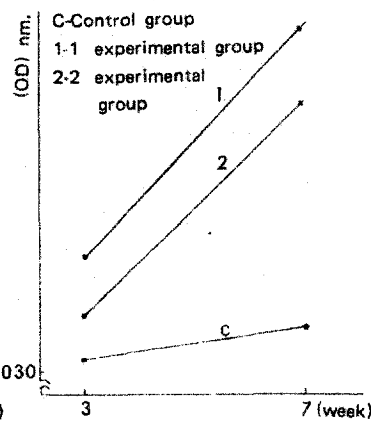

Fig. 3 Effect on the liver homogenate TBA Values of rat Tube Feeded with Pteridium sp.'s extracts. (by Matsushida method)

example, the hepatic lipid being greatly decomposed by a dose with certain toxicant, and triacyiglyceride level increaseed simultaneously in rats, and so do on the serum phospholipid level. On the contrary, Kato et al.(21) denied that the increment of serum lipid level by dose with certain toxicant.

While, in the present study, the serum thiobarbituric acid showed increased level compared with that of control by the extracts tube feeding in both groups. But no significant declined result was recognized between three weeks feeding and seven weeks feeding. This is assented to the report of Hiroshi et al.(34). In the report they noted that not only dose of toxicant induce an increasing tendency of serum lipid peroxide level but also the aging and sex do so. The present study could not nod to the notes, and furthermore study will be available.

Even if, these instances are not fully quoted, it should be agreed that the serum lipids and serum thiobarbituric acid level is tending up with aging and by the extract tube feeding in the present study.

Hepatic homogenate thiobarbituric acid

The hepatic thiobarbituric acid value of the rat fed for seven weeks with Pteridium sp. extracts indicate significantly higher level approximately three times than that of the rat fed for three weekes. On the other hand there is no distinctively distinguishable differences between both experimental group of thiobarbituric acid value. This is presumably because of the toxicant, so called, can fully be extracted only in three minutes of boiling(26). Namely, the extracts of both experimental groups are contained almost equal toxicants in amount.

The principal characteristic difference of Matsushita modified method for the detection of tissue lipid peroxides quantity from Uchiyama(11) is that, all the process were operated in anaerobic conditions.

Kenston(14) tried to measure the thiobarbituric acid value of oleic acid, linoleic acid and linolenic acid under the equal conditions and he got the following result that the oleic acid shows no react, linoleic acid appears weak red colour react and linolenic acid shows perfect red colour reaction(this is almost 100 times redness of linoleic acid colour react).

Related to the report, Terao et al.(15) indicate that animal tissue phospholipids are mostly composed with polyunsaturated fatty acid of linolenic acid (18:2), arachidonic acid(20:4) and docosahexaenoic acid(22:6), and so on. And Dahle et al.(16) insists that there is a positive co-relation between number of double bonds and the malondialdehyde reaction. Malondialdehyde indicate the red colour zone, of which the optical density wave length is 532 nm. And in the present study we used the 532 nm wave length for optical density.

Certain stimulatives can stimulate certain tissues and organ's composed polyunsaturated fatty acids and induces some malondialdehyde or it's precursors(17,40,18,19,26). Matsuhsita et al.(15) reported that rat's hepatic phospholipid possesses approximately 60% of unsaturated fatty acid from monoenoic to hexaenoic, and when certain toxicant is administered to the rat, the peroxide value of the liver showed about six times higher level than before the administration.

Some reports(20,37) indicate that rat's liver tissue suffered violent damage by some effective chemicals dosage, the remote cause of the damage is because of the lipids peroxides increase extravagantly.

And it is also quotable report that the nutrients such as protein and some kind of trace elements may be obsolated by certain factros, and then hepatic lipid peroxide increases rapidly and it leads to the damage of the liver(25,7). After all, according to these indications, certain toxicant's administration or impaired nutritional status that is, absorbance hindrance of essential nutrients particularly protein and trace elements caused hepatic tissue damage by increasing the lipid peroxides, and leading to the multiple disorders of organ.

It is certain that the mechanism of increment of hepatic homogenate thiobarbituric acid value of rat fed with concentrated extract of pteridium sp. for

Table 4. Effect on the Liver Homogenates TBA Values of rats Tube Feeded with Pteridium sp.'s Extracts

Group	3	7
Control	0.045±0.018	0.074±0.031
1	0.076±0.034	0.228±0.078
2	0.068±0.030	0.194±0.034

seven weekes is due to the exasperation of hepatic lipid hydroperoxides induced by the decrement of hepatic reduced glutathione level or oxidation of hepatic glutathion(24-32). And the increment of hepatic lipid hydroperoxides leads to weakened activation of hepatic microsome's cytochrome p-450, and the tissue damages are followed(33-38).

The present study data presumably suggests that the extract tube feeding induces the rat's liver lipid peroxides increment and then leads to the damage of certain organs and tissues. With the present results, I dare say the Pteridium sp. plant may not be disputed again to be loved as a greens or substitute foods.

But there is one advice for the consumers that is, when you boil and dry it, you might keep attention not to expose it to sunlight. If you do so, then you might suffer the bad effect of the lipid peroxides caused by(12) the Pteridium sp. plant.

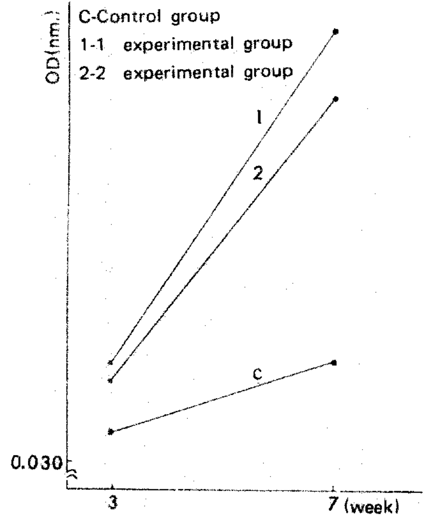

Fig. 4 Effect on the liver homogenate TBA Values of rat tube feeded with Pteridium sp.'s extracts. (Uchyiama method)

No significant differences was confirmed between both experimental group.

Pteridium sp's concentrated extracts(experimentals A and B) administration affected to the plasma lipids and it's peroxides level and to the hepatic lipid peroxides level in rats.

SUMMERY

Plasma lipids, lipid peroxides and hepatic lipid peroxides(2-thiobarbituric acid) level been demonstrated to vary with administration of Pteridium sp's concentrated extracts in rats.

Plasma triacylglyceride level increased remarkably with seven weeks of age on both experimental group.

Plasma phospholipid, free fatty acid and thiobarbituric acid value changed in unnoticeable on both experimental group with each age.

Hepatic homogenate thiobarbituric acid value showed significant increase on both experimental group with seven weeks of age.

要 約

Pteridium sp. 의 濃縮抽出液을 흰쥐에게 7週間 注入하여 血淸脂質과 脂質過酸物 및 肝組織脂質過酸化物의 變化를 分析하여 다음의 結果를 得했다.

7週의 血淸中性脂肪量은 比較群이나, 第3週의 1, 2群의 그것보다 크게 많이 增加했다.

血淸燒脂質 血淸遊離脂肪酸 및 血淸過酸化物 (TBA値)量은 各群別 및 3週, 7週 年令間에 別 變化差가 없었다.

肝組織의 脂質週酸化物(TBA)量은 7週年令이 3週에 比하여 크게 增加하였다. 但, 1, 2群間의 差는 크지 않았다.

여기서 이 抽出物 또는 그 食品의 攝取가 過量으로 長期間 계속되지 않는 限 生體에 미치는 영향은 거의 없는 것으로 思料됨

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