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The Effect of *Petasites japonicus* Extract on Hepatotoxicity in Rats

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Abstract: *Petasites japonicus* (Compositae) is a perennial herb which has been used as treatment of antitussive, expectorant, sedatives, paralysis, diurectics in folk remedies. The pharmacological studies of this natural drug have not yet estabilished. So, we examined anti-lipid peroxidative effects and liver protective effect on CCl_4 induced lipid peroxidation and hepatotoxicity in rats. In vivo liver lipid, MeOH Ex. revealed significant increase of anti-lipid peroxidative effects according to concentration dependently. In chemical parameters obtained from serum analysis, MeOH Ex. as a increase of medicine concentration (0.25 g/kg, 0.5 g/kg, 1.0 g/kg), GOT, GPT, A/P decreased. In 0.5 g/kg GPT administered group, there was relative in GOT, A/P. Cholesterol decreased in 1.0 g/kg and 1.0 g/kg administered group, BUN decreased relatively in 1.0 g/kg administered group.

Keywords: *Petasites japonicus*, anti-lipid peroxidation, hepatotoxicity

Introduction

Petasites japonicus Max. is a perennial herb which belongs to compositae and the grass called 'Bongdugcho', flower called 'Bongduhua' and root called 'Bongdugun' it is also distributed in middle and south part.[1)]

It blooms on May to June and it is a dioecism: female three has white flower, male tree has yellow flower. Leaves show after blooming, a petiole is long and lamina is the appearance of a kidney, 15 to 30 cm wide. Peduncle and petiole are edible, they have a special kind of smell and taste bitter. *Petasites saxatilis* Komarov and *Farfugium japonicum* Kitamura are cousin plants of *Petasites japonicus* Max.[2)]

This plant had been used for cough, phlegm and stomach. Leaves had attached on the skin for wound and insect, root had used in and outside for pain form injury and throat. In addition, it has been used for suppression, tumor, self-protection, diuresis and humid tetter. Nowadays it has bee reported as an effect of preventing paralytic.[3,4)]

According to research of butterbur, there were found β-sitosterol, angelic acid, caproic acid, caprylic acid, procatechuic acid in the root and segregated petasion in the leaves. Hemicelluose is segregated from the leaves and stem, Fukinolic acid which is polyphenol is segregated from the sclerophy and peduncle, Fukinanolide, fukinolide, petasin, isopetasin and S-isopetasin which are sesquiterpenoids are segregated from a flower bud.[5)] Also, from the root stem, they succeed to segregate eremopetasidione of sesquiterpenoid and petasiphenone of phenolic compound, and also found out new six eremophilenolide.[6)]

Moreover, two new eremophilenolide in ethylacetate fraction are segregated from the flower.[7)]

In a pharmacology research, it has been reported effect of growing in vitro and antioxidant effect by DPPH of MeOH Ex. for butterbur.[8)]

As is stated above, butterbur has used by people for self-protection, diuresis and humid tettert and it is easy to get though, as it is not proved by a research about its medical action, effect of geographicalperoxide and liver was measured by administering carbon tetrachloride which is experimental liver attacker to a white mouse MeOH Ex. from on the top of butterbur,[9)] so I would like to report my result.

Materials and Methods

Materials

Petasites japonicus Max. used in this experiment

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is purchased in Kyoung Dong maket and it was cut in small pieces after drying process to use as an experiment material.

Laboratory Animals

Sprague-dawley rats, weigh 200±20 g were raised in the same conditions for over a week so that they would get used to the lab environment. The temperature of the lab was 20±2°C, humidity rate was maintained at 50±10%. During the experiment process, the solid feed (from Sam Yang Ltd.) and water were sufficiently provided.

Preparation of Samples

Plants materials were extracted three times by MeOH for 4 hours and filtrated. The filtered liquid was concentrated to get MeOH Ex. This was suspended in 0.5% CMC solution.

Influence on the Inhibition of Lipid-peroxidation and the Liver Function

1. Medication

In the case of normal control group, saline was injected only, and in the case of positive, negative and 3 test control group 2 m*l*/kg solution of CCl_4-olive oil was injected respectively by a subcutaneous injection. After 1 hour, 0.5% Na-CMC solution was administered to normal and negative groups (by oral) and to the positive group silymarin 200 mg/kg was administered. On the other hand to test groups 0.25 g/kg, 0.5 g/kg, 1.0 g/kg of MeOH Ex. was administered for three days respectively. On the fourth day, only sample was administered and only water was provided for 24 hours (Table 1).

2. Preparation of Lipid

After the medication, the lab animals were lightly etherised and anatomized. The liver was extracted by perfusion using 0.15 M Ice cold KCl solution and removing the blood in liver. The liver was weighed and washed with 0.15 M Ice cold KCl solution and directly cut by 0.15 M Ice cold KCl of which the amount was 10 times the liver's weight. For about 5 minutes, the cut liver was homogenized in ice bath.

3. Thiobarbituric acid asssay (TBA assay)

In lipid peroxidation test, 0.02 M Phosphate buffer soln. 300 μ*l*, H_2O 100 μ*l*, 0.02 M $FeSO_2$ soln. 100 μ*l*, 0.02 M Ascorbic acid 100 μ*l* was taken to homogenate 300 μ*l* in a static test tube, and after vortex mixing, TBA-malondialdehyde complex was develped with the same method involved. And, upper part fluid that was central-separated for 15 minutes at 3000 rpm was measured by spectrophotometer in 535 nm.

concentration of homogenate (nM/m*l*) = (f/F) × 10 nM/m*l* tissue's homogenate

F: absorbance of standard sample
f: absorbance of sample (535 nm)

4. Weighing of the liver and the spleen

The weight percentage was accounted by measuring the weight of the liver and the spleen of normal control group, positive control group, negative control group, sampling group.

5. Measuring of GPT, GOT, A*l*P, Cholesterol, BUN contents

The content of GPT(glutamic pyruvic transaminase),

Table 1. Administration schedule

Group		1	2	3	4 (days)
Normal control		Saline + CMC soln			CMC soln.
Negative control		CCl_4-olive oil (1:1) + CMC soln.			CMC soln.
Positive control		CCl_4-olive oil (1:1) + silymarin silymarin			
MeOH Ex.	I	CCl_4-olive oil (1:1) + MeOH Ex.			MeOH Ex.
	II				
	III				

a)Saline and CCl_4-olive oil (1:1) were injected s.c (2 m*l*/kg).
b)CMC solution, silymarin (200 m*l*/kg) and each fraction were administered p.o 1 hr. after CCl_4 treatment.
I: 0.25 g/kg/day, II: 0.5 g/kg/day, III: 1.0 g/kg/day.

GOT(glutamic oxaloacetic transaminase), A/P (alkaline phosphatase), Cholesterol, BUN(blood urea nitrogen) in normal control group, positive control group, negative control group and in sampling group was measured by using blood autochemistry analyser.[10]

Results and Discussion

Inhibitory Effects of Lipid Peroxidation

Oxygen, which is a prerequisite for the survival of the body, is activated in the living body and used after turning to superoxide. The superoxide once again is said to be turned into H_2O_2, OH^- (hydroxy radical), O_2 and this oxygen free radicals deteriorate biological membranes by peroxide of the lipid of biological membranes thereby causing aging, coronary sclerosis, diabetes in addition to being relevant to the carcinogenesis and variation.[11]

A living body has enzyme system or materials that remove the remaining oxygen free radicals, and its main function is performed in the liver. However, if the liver is damaged by poisonous matters(CCl_4, Benzopyrene, Ethanol, etc.), it is reported that the internal enzyme system gets harmed by the peroxidation of lipid of liver's biological membranes, thereby increasing the amount of peroxidated lipid in blood and cells which cause diseases in other parts by the chain reaction of peroxidated lipid and oxygen free radicals.

Phospholipid can exist by the electric bond, hydrophobic bond, hydrogen bond of other lipids and hydrophobic proteins. Thus, it is difficult to extract only phospholipid from organic samples. Generally, the whole lipid is extracted and then the phospholipid is fractionated and refined. In cutting and extracting the hydrophobic bond of lipids, diethylether and chloroform, which are relatively polar solvents, are used. In cutting and extracting the electric bond, hydrophobic bond, hydrogen bond of other lipids and hydrophobic proteins, the polar solvent, methanol and ethanol which get rid of the related hydro molecules are used. And the separated limpid molecules are likely to be soluble in relatively non-polar solvents. Therefore, chloroform -methanol and ether-ethanol which are the mixed solvents of non-polar solvent and polar solvent are mainly used in extracting lipids.

In measuring the lipid and oxidization, there are oxygen uptake measuring method, hydroperoxide (ROOT) measuring method, hydroperoxidation outcome(especially aldehyde) measuring method, and conjugated diene measuring method.

It is hard to determine which is the best measuring method, but the simplest method, thiobarbituric acid(TBA) method which set the malondialdehyde (MDA) standard is generally used. The TBA method was first used by Kohn in 1944. The principle is that the decomposition of persoxide is promoted by ferrous solvent, and the decomposed malondialdehyde from the precipitated protein under the condition of below pH 3 by the trichloroacetic acid is used in fluorescent and colorimetric analysis of the red materials produced by the condensation with TBA of 2 molecule.

In order for the measurement of lipid and oxidation in serum, there is fluorescence method of Yagi et al. This method is assumed to catalyze the decomposition into phosphotungestic acid. There is a report on the importance of ratio of Fe^{+2} and Fe^{+3} in the formation of peroxidized lipid. The small amount of H_2O_2 and ascorbic acid promote the lipid and oxidation by maintaining the ratio of Fe^{+2} and Fe^{+3} with oxidation or reducing process.

In this experiment, the inhibitory effects of lipid peroxidation caused by the Fe^{+2}/ascorbic acid system in each fractionization were observed with the comparison with silymarin which is renowned

Table 2. Effect of *P. japonicus* methanol Ex. on lipid peroxidation

Group	Dose (g/kg/day)	MDA(nM/ml)
Normal		13.71±0.45
N.C		22.32±0.38
P.C		13.43±0.48***
Methanol Ex.	0.25	17.07±0.32**
	0.5	16.30±0.11**
	1.0	13.44±0.31***

a)N.C : Negative control, 2 ml/kg/day of 0.5% CMC soln, P.C: Positive control, 200 mg/kg/day of silymarin. The drugs were administered into p.o after s.c (CCl_4-olive oil, 2 ml/kg/day) for 3 days.

b)Each value represents the mean±S.E of 5 rats.

c)Significantly different from CCl_4 control:**$p<0.01$, ***$p<0.001$.

for its anti-oxidation activity.

CCl_4 and olive oil was injected in an equal amount respectively, so that the liver toxicity could be induced. Then, the MeOH Ex. of *Petasites japonicus* Max. was infected through the mouth with each concentration level to lad animals. And, the lipid peroxidation was promoted by adding Fe^{+2}/ ascorbic acid to homogenate which was extracted from the liver of the lad animals. As as result, the inhibitory effects increased by the increase of each sample's concentration level with valence involved (Table 2).

The Effects on a Serum Factor

In order to find the influence of samples on liver function, the liver toxicity was induced by injecting CCl_4-olive oil (1:1), and MeOH Ex. was injected by each dose, and the contained quantity of GOT, GPT, A/P, Cholesterol, BUN was measured from each serum. GOT, GPT, A/P show the increase of numbers in the case of infection and are enzymes which are used as index for the diagnosis of cardiovascular and biliary tract diseases. Cholesterol are chemical components in sera whose number increases in the case of liver cirrhosis. Although Bun is not a material for directing post in liver function, there is corelation

As for the influence on the liver function, the activities of GOT, GPT, A/P were restored to near the normal level. The contents of cholesterol and BUN showed inhibitory effects with valence. (Table 3).

Table 4. Effects of *P. japonicus* on Liver and Spleen weights

Group		Liver/Body Weight (%)	Spleen/Body Weight(%)
Normal		3.42±0.17	0.26±0.01
N.C		4.19±0.49	0.38±0.02
P.C		3.81±0.19	0.29±0.03
MeOH Ex.	I	3.63±0.29	0.37±0.02
	II	3.60±0.28	0.37±0.03
	III	3.77±0.19	0.34±0.01

a)N.C: Negative control, 2 ml/kg/day of 0.5% CMC soln, P.C: Positive control, 200 mg/kg/day of silymarin. I: 0.25 g/kg/day, II: 0.5 g/kg/day, III: 1.0 g/kg/day. The drugs were administered into p.o after s.c (CCl_4-oilve oil, 2 ml/kg/day) for 3 days.
b)Each value is mean ± S.E of 5 rats.
c)Significantly different from negative control: *p<0.05 **p<0.01 ***p<0.001

Effects of *P. japonicus* on Liver and Spleen weights

The weights of liver and spleen have reduced more than the negative control group but could not restore to the normal level (Table 4).

Conclusion

P. japonicus had been used for cough, phlegm and stomach. Leaves had attached on the skin for wound and insect, root had used in and outside for pain form injury and throat. In addition, it has been used for suppression, tumor, self-protection,

Table 3. Effects of *P. japonicus* on GOT, GPT, A/P, Cholsterol, and BUN contents activities

Group		GOT (U/L)	GPT (U/L)	A/P (IU/L)	Cholesterol (mg/dl)	BUN (mg/dl)
Normal		125.7±4.6	35.30±4.11	165.2±11.5	40.27±5.98	13.49±1.11
N.C		253.2±23.6	62.03±2.98	213.2±8.1	44.32±6.23	20.33±0.51
P.C		148.3±11.9***	33.50±0.60***	133.2±13.3***	31.59±1.6*	13.05±0.69***
MeOH Ex.	I	185.7±20.0*	50.59±3.98	173.9±16.4*	37.95±5.43	17.91±2.71
	II	162.1±27.9*	40.02±5.18*	163.2±7.9***	33.95±5.59*	17.82±3.21
	III	154.0±15.0*	40.22±5.34	153.9±13.7*	34.57±3.22*	16.83±1.46*

a)N.C: Negative control, 2 ml/kg/day of 0.5% CMC soln, P.C: Positive control, 200 mg/kg/day of silymarin. I: 0.25 g/kg/day II: 0.5 g/kg/day, III: 1.0 g/kg/day
The drugs were administered into p.o after s.c (CCl_4-oilve oil, 2 ml/kg/day) for 3 days.
b)Each value is mean ± S.E of 5 rats.
c)Significantly different from negative control: *p<0.05, **p<0.01, ***p<0.001.

diuresis and humid tetter. And it is widely used as food. In relation to this, the effects of *P. japonicus* on Liver and Blood-lipid metabolism to white rats were studied. For this purpose, carbon tetrachloride, which is used as a drug induced hepatitis, was injected to white rats in order to intentionally harm the liver system.

The results are as follows:

1. The inhibitory effects of lipid peroxidation were shown in accordance with the increase of samples' concentration level.

2. The weights of liver and spleen were not able to restore to the normal level. But on a general level, they were reduced more than the control group.

3. As for the influence on the parameter of serum, the activities of GOT, GPT, A/P were restored to near the normal level. MeOH Ex. as a increase of medicine concentration (0.25 g/kg, 0.5 g/kg, 1.0 g/kg), GOT, GPT, A/P decreased. In 0.5 g/kg GPT administered group, there was relative in GOT, A/P. Cholesterol decreased in 0.5 g/kg and 1.0 g/kg administered group, BUN decreased relatively in 1.0 g/kg administered group.

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