Kor. J. Env. Hlth., Vol. 32, No. 3, pp 235~239(2006)

The Effect of *Cichorium intybus* Extract on Hepatotoxicity in Rats

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(Received May 18, 2006/Accepted Jun. 5, 2006)

Abstract: *Cichorium intybus* (Compositae) has been used for fevers, dyspepsia, headache and jaundice, as a demulcent. Also, it has relaxation effects and relief effects against coffee and teas, and is widely used as food. We investigated anti-lipid peroxidative effects and liver protective activity on CCl_4 induced lipid peroxidation and hepatotoxicity in rats. MeOH Ex. enhanced the inhibition of anti-lipid peroxidative effects in liver lipid. In chemical parameters obtained from serum analysis, MeOH Ex. revealed significant decrease on hepatotoxicity. The results were as follows; 1. The inhibitory effects of lipid peroxidation were shown in accordance with the increase of samples' concentration level. 2. In chemical parameters obtained from serum analysis, the activities of GOT, GPT, AlP were restored to near the normal level. The contents of cholesterol and BUN showed inhibitory effects with valence. 3. 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.

Keywords: *Cichorium intybus*, anti-lipid peroxidation, hepatotoxicity

Introduction

Cichorium intybus L. is a polar alpine plant which belongs to the Compositae and an 1 or 2 years-old grass plant. It is called cichorium endivia. Its root shape is deep-seated cylinder figure and its branches are separated. The flowers bloom from May to the summer time. The colors of the flowers are deep sky-blue. It is a capitate flower and its diameter is about 3-4 cm, blooms in a wild chrysanthemum shape. Its leaves are used for a salad recipe in the springtime, and it has a bitter taste. The habitat is Europe, India, Egypt, and it is even distributed to the northern part of Lake Baikal in Central Asia.[1-4]

The studies on the composition of *Cichorium intybus* are performed by Waczinski *et al.*[5] about the lactucin and lactucopicrin which are bitterness-inducing ingredients; by Khali *et al.*[6] about the intybin which is a pigment material; and by Singh *et al.*[7] about chicoric acid of leaves. And, there are some more reports about other components and inulin(a kind of saccharide).[8-10]

In pharmacology, Beitter[11] reported that *Cichorium intybus* promote the activities of internal organs, digestive gland, stomach and intestines thereby improving the function of digestion, liver, gallbladder. In addition, Beitter reported that *Cichorium intybus* is good for diabetes due to the high level of insulin. Also, Chopra *et al.* reported that *Cichorium intybus* has tonic functions and is good for the cases when the spleen is expanded and in nausea caused by fever and dizziness, and that it could be used as an emmenagogue, too.

In a report of pharmacological activities it has on the influence of the stomach, intestines and heart also *Cichorium intybus* has sedation effect on dyspnoea and blood pressure in addition to the mitigation effect on coffee and teas.[12-14]

In this experiment, we report the effects of *Cichorium intybus* (which is commonly used as an antipyretic and food) on Liver and lipid metabolism in blood.

Materials and Methods

Materials

Cichorium intybus L. used in this experiment is purchased in the farm in Choon Chun, Kang Won in July, 2004, 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

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would get used to the lab environment. The temperature of the lab was 20 ± 20°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 our 3 test control groups 2 m*l*/kg solution of CCl_4-olive oil was injected respectively by a hypodermic[subcutaneous] injection. After 1 hour, 0.5% Na-CMC solution was administered to normal and negative groups (by oral) and to the positive group sylimarin 200 mg/kg was administered. On the other hand to our test groups 0.125 g/kg, 0.25 g/kg, 0.5 g/kg of methanol extractant was administered for three days respectively. On the forth 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 Phospahte 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, AlP, Total Cholesterol, TG, BUN Contents

The content of GPT(glutamic pyruvic transaminase), GOT(glutamic oxaloacetic transaminase), AlP (alkaline phosphatase), Total cholesterol, TG(triglyceride), 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.[16)]

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 II III		CCl_4 - olive oil (1:1) + MeOH Ex.		MeOH Ex.

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: 0.125 g/kg/day, II: 0.25 g/kg/day, III: 0.5 g/kg/day

Results and Discussion

The Effects on a Serum Factor

In order to find the influence of samples on liver function, the liver toxivity 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, AlP, Cholesterol, TG, BUN was measured from each serum. GOT, GPT, AlP 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, TG are chemical components in sera whose number increases in the case of liver cirrhosis. The BUN value is referred to the liver function indirectly.

The activities of GOT, GPT, AlP were recovered to the normal level. The contents of cholesterol and BUN were decreased effectively and the content of TG retained the valence in the group of 0.5 g/kg injection (Tables 2, 3).

Table 2. Effects of *Cichorium intybus* on GOT, GPT and AlP activities

Group		GOT (U/L)	GPT (U/L)	AlP (IU/L)
Normal		136.4 ± 3.70	48.50 ± 1.60	261.3 ± 4.10
N.C		253.8 ± 6.90	93.67 ± 3.70	358.7 ± 19.0
P.C		159.3 ± 3.00***	60.50 ± 2.20**	275.0 ± 4.90*
MeOH Ex	I	243.0 ± 7.60	75.00 ± 3.50*	254.0 ± 12.0*
	II	184.7 ± 5.30***	71.67 ± 1.80*	237.7 ± 6.50*
	III	148.8 ± 3.70***	51.33 ± 3.50**	214.3 ± 2.90*

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 : 0.125 g/kg/day, II : 0.25 g/kg/day, III : 0.5 g/kg/day
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<0.05, **p<0.01, ***p<0.001

Table 3. Effects of *Cichorium intybus* on Cholsterol TG, and BUN contents

Group		Cholesterol (mg/dl)	TG (mg/dl)	BUN (mg/dl)
Normal		51.50 ± 1.10	49.30 ± 2.61	24.50 ± 1.60
N.C		76.25 ± 2.80	69.20 ± 2.40	34.25 ± 1.80
P.C		52.01 ± 2.10**	68.00 ± 1.50	28.00 ± 1.10*
MeOH Ex	I	34.33 ± 1.90***	68.00 ± 1.50	28.00 ± 1.10*
	II	26.75 ± 1.31***	58.01 ± 6.30	5.75 ± 0.48*
	III	24.05 ± 2.11***	48.33 ± 4.40*	24.75 ± 0.63*

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 : 0.125 g/kg/day, II : 0.25 g/kg/day, III : 0.5 g/kg/day
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<0.05, **p<0.01, ***p<0.001

Table 4. Effects of *Cichorium intybus* on Liver and Spleen weights

Group		Liver/Body Weight (%)	Spleen/Body Weight (%)
Normal		3.81 ± 0.09	0.35 ± 0.05
N.C		4.96 ± 0.37	0.48 ± 0.04
P.C		3.82 ± 0.04	0.34 ± 0.05
MeOH Ex	I	4.43 ± 0.04	0.51 ± 0.06
	II	4.40 ± 0.35	0.43 ± 0.05
	III	4.15 ± 0.12	0.41 ± 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 : 0.125 g/kg/day, II : 0.25 g/kg/day, III : 0.5 g/kg/day
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

Effects of *Cichorium intybus* 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).

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.[18]

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 peroxide 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 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 *Cichorium intybus* 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 5).

Table 5. Effect of *C. intybus* methanol Ex. on lipid peroxidation

Group	Dose (g/kg/day)	MDA (nM/m*l*)
Normal		10.71 ± 0.35
N.C		20.32 ± 0.38
P.C		$11.43 \pm 0.38^{***}$
	0.125	$14.07 \pm 0.52^{**}$
Methanol Ex.	0.25	$12.90 \pm 0.11^{**}$
	0.5	$11.44 \pm 0.31^{***}$

a) N.C : Negative control, 2 m*l*/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 m*l*/kg/day) for 3 days.
b) Each value represents the mean ± S.E of 5 rats
c) Significantly different from CCl_4 control :
$^{**}p<0.01$, $^{***}p<0.001$

Conclusion

Cichorium intybus L. has relaxation effects and relief effects against coffee and teas, and is widely used as food. In relation to this, the effects of *Cichorium intybus* on Liver and lipid metabolism in blood to rats were studied. For this purpose, carbon tetrachloride, which is used as a drug induced hepatitis, was injected to 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) As for the influence on the parameter of serum, the activities of GOT, GPT, AlP were restored to near the normal level. The contents of cholesterol and BUN showed inhibitory effects with valence.

3) 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.

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