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• BRIEF REPORTS •

Effects of frying oil and *Houttuynia cordata* thunb on xenobiotic-metabolizing enzyme system of rodents

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Abstract

AIM: To evaluate the effects of frying oil and *Houttuynia cordata* Thunb (*H. cordata)*, a vegetable traditionally consumed in Taiwan, on the xenobiotic-metabolizing enzyme system of rodents.

METHODS: Forty-eight Sprague-Dawley rats were fed with a diet containing 0%, 2% or 5% *H. cordata* powder and 15% fresh soybean oil or 24-h oxidized frying oil (OFO) for 28 d respectively. The level of microsomal protein, total cytochrome 450 content (CYP450) and enzyme activities including NADPH reductase, ethoxyresorufin O-deethylase (EROD), pentoxyresorufin O-dealkylase (PROD), aniline hydroxylase (ANH), aminopyrine demethylase (AMD), and quinone reductase (QR) were determined. QR represented phase II enzymes, the rest of the enzymes tested represented phase I enzymes.

RESULTS: The oxidized frying oil feeding produced a significant increase in phase I and II enzyme systems, including the content of CYP450 and microsomal protein, and the activities of NADPH reductase, EROD, PROD, ANH, AMD and QR in rats (P<0.05). In addition, the activities of EROD, ANH and AMD decreased and QR increased after feeding with *H. cordata* in OFO-fed group (P<0.05). The feeding with 2% *H. cordata* diet showed the most significant effect.

CONCLUSION: The OFO diet induces phases I and II enzyme activity, and the 2% *H. cordata* diet resulted in a better regulation of the xenobiotic-metabolizing enzyme system.

Key words: Frying oil; Houttuynia cordata thunb; Xenobiotic-metabolizing enzyme system

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INTRODUCTION

The liver is the primary site of drug metabolism and detoxification. Cytochrome P450 (CYP 450) enzymes are the predominant proteins in the hepatic endoplasmic reticulum representing about 5% to 25% of the total protein. They are responsible for the metabolism of most drugs, both endogenous and exogenous compounds, including steroid hormones and xenobiotics[1-3]. Cytochrome P450 enzymes, the major phase I components, have multiple roles in animals. They are important in bioactivation of chemical carcinogens, biotransformation of many endogenous vitamins and steroids, and detoxification of numerous xenobiotics[4,5]. Phase II components, which include transferases, dehydrogenases and glucuronidases, are involved in conjugation and detoxification reactions. Both phase I and phase II enzymes have important health implications for both animals and humans and have been reported to be influenced by various dietary factors[6] such as fried oil[7-9].

A high dietary intake of fat is prevalent in most developed countries, and fried foods are a principal fat source in the modern diet. According to a recent study[10], consumption of fried food is closely related to many chronic diseases, such as cancer and hyperlipidemia. One reason why the consumption of frying oil may influence the risk of chronic diseases is that it has a significant effect on CYP450. Polyphenols, which are plentiful in plants, also play an important role in CYP450 regulation[6]. *Houttuynia cordata* (*H. cordata*) Thunb, a traditional vegetable in Taiwan, which contains a high amount of polyphenol, has been showed to have both antioxidative and antimutagenic properties under OFO-feeding-induced oxidative stress[11]. This study was designed to assess the effect of frying oil and *H. cordata* thunb on CYP450 and xenobiotic-metabolizing enzyme system in rodents.

MATERIALS AND METHODS

Diets and animal care

H· cordata thunb was harvested and gathered at Taoyuan District's Agriculture Improvement Station, Taipei Branch, Northern Taiwan. The *H. cordata* was dried with a lyophilizer. One kilogram of fresh *H. cordata* produced about 150 g dry *H. cordata* powder after lyophilization. The OFO used in this experiment was prepared as that reported previously[11,12]. The OFO diets contained 15 g of OFO per 100 g of diet supplemented with 0%, 2% or 5% *H. cordata* powder as the O0, O20 and O50 groups, respectively. The control diets contained 15 g of fresh soybean oil per 100 g of diet, also supplemented with 0%, 2% or 5% *H. cordata* powder as the F0, F20 and F50 groups respectively (Table 1). Forty-eight male Sprague-Dawley (SD) rats were purchased from the National Science Council Animal Center, Taipei. The animals were treated in accordance with the *Guide for the Care and Use of Laboratory Animals* as reported previously[13].

Table 1 Composition of diets tested

Component	Diet					
	F0	F20	F50	O0	O20	O50
	g/kg diet					
Cornstarch	449.5	442.5	431.6	449.5	442.5	431.6
Casein	200	195.4	188.6	200	195.4	188.6
Sucrose	100	100	100	100	100	100
Fresh oil	150	150	150			
OFO[1]				150	150	150
Fiber	50	41.6	29.3	50	41.6	29.3
Minerals[2]	35	35	35	35	35	35
Vitamins[3]	10	10	10	10	10	10
L-Cysteine	3	3	3	3	3	3
Choline	2.5	2.5	2.5	2.5	2.5	2.5
Tert-butyl-hydroquinone	0.014	0.014	0.014	0.014	0.014	0.014
H. cordata powder	0	20	50	0	20	20

[1]OFO: oxidized frying oil; [2]AIN-93 mineral mixture; [3]AIN-93 vitamin mixture.

Tissue sampling and preparation

After 28 d of feeding, the rats were anesthetized with sodium pentobarbital. Livers were excised and weighed, and a portion of the liver sample was immediately homogenized in ice-cold 0.01 mol/L potassium phosphate buffer (pH 7.4) containing 11.5 g/L KCl. Aliquots of the crude homogenate 250 g/L were centrifuged at 12 000 *g* for 20 min to obtain the post-mitochondrial supernatant (PMS). Aliquots of the PMS were centrifuged at 105 000 *g* for 60 min to obtain the microsomal pellets (MPs). The MPs were suspended in 0.05 mol/L phosphate buffer (pH 7.7) containing 10 mmol/L EDTA and 30% glycerol. Both PMS and MPs were stored at -80 ℃ until being analyzed. The remaining liver portion was frozen in liquid nitrogen and stored at -80 ℃.

Xenobiotic-metabolizing enzyme system analysis

Microsomal protein was quantified using the Lowry method[14]. Total CYP450 content was determined using a dithionite CO-binding difference spectrum according to the procedure previously described[15]. NADPH reductase activity was determined using the method of Phillips and Langdon[16]. Ethoxyresorufin O-deethylase (EROD) and pentoxyresorufin O-dealkylase (PROD) activities were assayed according to the method of Lubet *et al*.[17]. Aniline hydroxylase (ANH) and aminopyrine demethylase (AMD) activities were assayed according to the methods of Kato *et al*. and Carpenter *et al*.[18,19]. Quinone reductase (QR) activity was assayed according to the method of Benson *et al*.[20]. The CYP450 content and the activities of NADPH reductase, EROD, PROD, ANH, and AMD represented the phase I enzymes, while QR represented the phase II enzymes.

Statistical analysis

Significant differences between the groups were analyzed using a two-way ANOVA and Duncan's multiple range test using the General Linear Model of the SAS package. $P<0.05$ was considered statistically significant.

RESULTS

Growth and nutritional status of animals

Following 28 d of feeding, the body weight gained in OFO-fed SD rats was significantly lower than that in the control animals (Table 2). The feeding efficiency of rats fed with the OFO diet (O0) was significantly lower than that of the controls (F0). However, supplementation with *H. cordata* (O20 and O50) improved the feeding efficiency to a value that was not different from that of the control groups.

Table 2 Body weight gain, food intake and feed efficiency of Sprague-Dawley rats fed with fresh/oxidized frying oil and 0%, 2% and 5% *H. cordata* thunb powder diets for 4 wk (mean±SD, $n = 8$)

Group	Body weight gain (g/wk)	Food intake (g/wk)	Feed efficiency (B.W gain/food intake)
F0	46.71±3.20	202.31±7.62	0.23±0.03
F20	44.85±2.82	206.22±7.82	0.22±0.04
F50	47.54±2.36	212.34±5.20	0.22±0.01
O0	35.62±2.10[1]	198.14±5.47	0.18±0.02
O20	37.91±4.75	185.25±4.66	0.20±0.03
O50	38.54±4.55	188.72±3.44	0.20±0.02
ANOVA[2]			
Dose	NS[3]	NS	NS
Oil	$P<0.05$	NS	NS
Dose×Oil	NS	NS	NS

[1]Mean values significantly differ between the two oil groups ($P<0.05$). [2]Analyzed by a two-way ANOVA. [3]NS: No significance.

Xenobiotic-metabolizing enzyme system analysis

The CYP450 content, microsomal protein, and NADPH reductase activities in OFO-fed rats were significantly higher than those in fresh oil-fed rats (Table 3). In the OFO-fed rats, the 2% *H. cordata* group had lower ANH and AMD activities than the 0% group (Table 4).

EROD activity was significantly higher in the OFO-fed rats than in the fresh oil-fed rats. In addition, EROD activity was lower in the 2% and 5% *H. cordata* groups. PROD activity expression showed a similar pattern to that of EROD (Table 5). In microsomal suspension, the QR activity of OFO-fed rats was significantly higher than that of the fresh oil-fed rats. In liver homogenate, OFO also induced QR activity. In the fresh oil-fed rats, the 2% and 5% *H. cordata* groups had higher QR activity than the 0% group (Table 6). We found that 2% *H. cordata* had the largest effect on the phase I and phase II enzymes, which suggested that *H. cordata* could regulate the xenobiotic-metabolizing enzyme system, possibly because there was a large amount of polyphenols in *H. cordata*.

Table 3 Liver microsomal protein content (CYP450) and the activity of liver NADPH reductase in Sprague-Dawley rats fed with the fresh/oxidized frying oil and 0%, 2% and 5% *H. cordata* thunb powder diets for 4 wk (mean±SD, $n = 8$)

Group	Microsomal protein (mg/g liver)	CYP450 (nmol/mg protein)	NADPH reductase (nmol/mg protein)
F0	13.36±4.71[1]	0.61±0.27[1]	202.31±28.1[1]
F20	15.66±0.96[1]	0.73±0.10[1]	228.42±30.4[1]
F50	15.79±1.35[1]	0.74±0.14[1]	225.64±25.3
O0	23.74±2.54	1.63±0.75	256.03±31.1[2]
O20	22.13±3.76	2.14±1.10	288.55±29.6[3]
O50	22.25±2.96	2.08±0.15	234.15±25.4[2]
ANOVA[4]			
Dose	NS[5]	NS	NS
Oil	$P<0.05$	$P<0.05$	$P<0.05$
Dose×Oil	$P<0.05$	$P<0.05$	NS

[1]Mean values significantly differ between the two oil groups ($P<0.05$). [2,3]Means not sharing common superscript letters significantly differ from one another by Duncan's multiple range test for the same oil group ($P<0.05$). [4]Analyzed by a two-way ANOVA. [5]NS: No significance.

Table 4 Activity of aniline hydroxylase (ANH) and aminopyrine demethylase (AMD) from the liver of Sprague-Dawley rats fed with the fresh / oxidized frying oil and 0%, 2% and 5% *H· cordata* thunb powder diets for 4 wk (mean±SD, n = 8)

Group	ANH (μmol / min / mg protein) ($\times 10^{-5}$)	AMD (nmol / min / mg protein)
F0	3.74±1.54[1]	6.76±0.42[1]
F20	3.05±1.06[1]	5.34±1.02
F50	4.21±1.25[1]	6.31±0.56[1]
O0	13.46±0.61[3]	8.91±0.23[3]
O20	8.18±0.43[2]	6.02±0.11[2]
O50	11.74±0.37[3]	9.03±0.54[3]
ANOVA[4]		
Dose	P<0.05	NS[5]
Oil	P<0.05	P<0.05
Dose×Oil	P<0.05	P<0.05

[1]Mean values significantly differ between the two oil groups (P<0.05). [2,3]Means not sharing common superscript letters significantly differ from one another by Duncan's multiple range test for the same oil group (P<0.05). [4]Analyzed by a two-way ANOVA. [5]NS: No significance.

Table 5 Activity of EROD and PROD from the liver of Sprague-Dawley rats fed with the fresh / oxidized frying oil and 0%, 2% and 5% *H· cordata* thunb powder diets for 4 wk (mean±SD, n = 8)

Group	EROD (pmol / min / mg protein)	PROD (pmol / min / mg protein)
F0	55.61±5.12[1]	8.32±4.11[1]
F20	52.14±3.20[1]	6.12±1.25[1]
F50	52.46±4.58[1]	5.98±1.54[1]
O0	88.62±5.26[3]	25.39±3.59
O20	78.23±4.41[2]	20.12±2.18
O50	75.26±5.89[2]	21.33±2.66
ANOVA[4]		
Dose	NS[5]	NS
Oil	P<0.05	P<0.05
Dose×Oil	NS	NS

[1]Mean values significantly differ between the two oil groups (P<0.05). [2,3]Means not sharing common superscript letters significantly differ from one another by Duncan's multiple range test for the same oil group (P<0.05). [4]Analyzed by a two-way ANOVA. [5]NS: No significance.

Table 6 The activity of quinone reductase from the liver of Sprague-Dawley rats fed with the fresh / oxidized frying oil and 0%, 2% and 5% *H· cordata* thunb powder diets for 4 wk (mean±SD, n = 8)

	Quinone reductase (nmol / min / mg protein)	
Group	Microsomal suspension	Liver homogenate
F0	106.31±25.42[1]	170.47±25.65[2]
F20	136.54±31.85	226.77±30.95[3]
F50	122.42±29.43[1]	216.37±29.85[3]
O0	145.17±30.62	475.92±22.10
O20	144.38±26.66	490.14±15.31
O50	149.74±24.85	484.31±15.12
ANOVA[4]		
Dose	NS[5]	P<0.05
Oil	P<0.05	P<0.05
Dose×Oil	NS	P<0.05

[1]Mean values significantly differ between the two oil groups (P<0.05). [2,3]Means not sharing common superscript letters significantly differ from one another by Duncan's multiple range test for the same oil group (P<0.05). [4]Analyzed by two-way ANOVA. [5]NS: No significance.

DISCUSSION

Chen *et al*.[21] reported that the CYP450 content in OFO-fed rats was 1.6 times that of fresh oil-fed rats. After fried, a linolenic acid-containing oil could induce CYP450 by 4 and 6.5 folds relative to fresh soybean oil and linolenic oil respectively.

ANH is an indicator of CYP2C11. During oil frying, some chemicals with hydroxyl or carbonyl groups are produced, and these substrates will induce CYP 2C11. Chen *et al*.[21] used soybean oil, red flower seed oil and shortening oil as study materials, and found that after fried, the amounts of carbonyl compounds in these three oils were 203 mEq/kg, 110 mEq/kg, and 3 mEq/kg, respectively. In addition, they also found that the ability of soybean oil to induce ANH activity was 2.5 times that of fresh soybean oil. Thus, it is suggested that these carbonyl compounds might induce the expression of CYP2C11.

We also compared the activities of ANH and AMD (an indicator of CYP2E1) and found that the increase in ANH was larger than that in AMD. This is because the aldehyde, acid and ketone that can induce AMD were easily vaporized and the accumulation of these in the OFO was not sufficient to strongly induce AMD[22].

PROD is an indicator of CYP2B1. Phenobarbital-type materials are the most common inducers of CYP 2B1[23]. The cyclic materials separated from the OFO resembled those of phenobarbital-type materials in structures[24]. Thus, the cyclic materials in OFO may be the major compounds to induce CYP 2B1. EROD is an indicator of CYP1A1. Polycyclic aromatic hydrocarbon (PAH) is an inducer. According to the Pon's study[24], there are cyclic compounds but not polycyclic compounds in OFO. In fact, during the oil oxidation process, polycyclic hydrocarbon compounds will not be produced unless the temperature is higher than 300 ℃ and pyrolysis occurs. This can explain why the degree of induction by OFO of PROD is larger than that of EROD. In addition, CYP1A1 is the major enzyme that activates procarcinogens; thus OFO is considered to have the potential to cause tumors, as reported in a number of previous studies, by induction of this enzyme.

The enzymes measured in our study were all clinically relevant. AMD was related to hepatotoxicity induced by alcohol. EROD and PROD were related to the activation of carcinogens, such as polycyclic aromatic hydrocarbons, alfatoxin and nitrosamines. QR could promote the metabolism and excretion of mutagens and carcinogens[20]. Thus, we assume that the inhibition of the phase I enzymes and the induction of the phase II enzymes by polyphenols in *H. cordata* is helpful to animal cells. The reason why polyphenols are meaningful in the regulation of the xenobiotic-metabolizing enzyme system is unclear at present. We know that polyphenols could influence the combined processing of fat-soluble materials, which are activated by the phase I enzymes and DNA or protein, thus decreasing the toxicity and damage to cells caused by xenobiotic fat-soluble materials[25], but further study is required.

In conclusion, the OFO diet induces phase I and phase II enzyme activities, and the 2% *H. cordata* diet results in a better regulation of the xenobiotic-metabolizing enzyme system. Our findings suggest that polyphenol in *H. cordata* can be an important and necessary factor in the defense against CYP450-mediated cancers and other chronic diseases.

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