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Article

The Effects of *Oenanthe javanica* Extracts on Hepatic Fat Accumulation and Plasma Biochemical Profiles in a Nonalcoholic Fatty Liver Disease Model

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We tested the effects of *Oenanthe javanica* (OJ) on hepatic fat accumulation and plasma biochemical profiles in a nonalcoholic fatty liver disease model (NAFLD). Rats were divided 5 groups and fed the following diets for 6 weeks: normal rat chow diet (CHOW), high-fat high-cholesterol diet (HFCD), HFCD with the water extract of OJ (HFCD+W), HFCD with the *n*-butanol extract of OJ (HFCD+B), and HFCD with metadoxine (HFCD+M). Metadoxine (pyridoxine-pyrrolidone-carboxylate) is being used to treat alcoholic fatty liver and has been suggested that it can be effective for treating NAFLD. The HFCD gained significantly more body weight and visceral fat mass and had significantly higher levels of plasma glucose, triglyceride, cholesterol, and liver triglyceride content than the CHOW. The water- and *n*-butanol extracts of OJ improved the elevated plasma triglyceride and glucose levels induced by high-fat high-cholesterol diet and lowered triglyceride content in the liver. Similarly, the treatment with metadoxine reversed the increased plasma glucose and triglyceride and showed a tendency to decrease hepatic fat accumulation. However, these supplements did not change body weight or visceral fat mass. These results indicate that OJ extracts improve changes in hepatic fat accumulation, plasma glucose, and lipid profiles induced by high-fat high-cholesterol diet but did not affect visceral obesity. In conclusion, the water- and *n*-butanol extracts of OJ and metadoxine improved hepatic fat accumulation, hyperglycemia, and dyslipidemia induced by high-fat high-cholesterol diet although the effect of metadoxine on fatty liver was not significant, suggesting their potential use for the prevention and treatment of NAFLD.

Key words: *fatty liver, glucose, high-fat high-cholesterol diet, triglyceride*

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease, with increasing prevalence worldwide in association with increases in obesity, insulin resistance, dyslipidemia, and diabetes, characteristics of metabolic syndrome [McCullough, 2002; Choudhury and Sanyal, 2004; Lazo and Clark, 2008]. NAFLD represents a spectrum ranging from simple steatosis (fat accumulation) to nonalcoholic steatohepatitis (fat accumulation and inflammation) with further progression to fibrosis or cirrhosis [Angulo, 2002; Torres and Harrison, 2008; Vuppalanchi and Chalasani, 2009].

Fat accumulation in the liver results from the excess intake of dietary lipid and/or an imbalance between the synthesis and oxidation of fatty acids in the liver [Koruk *et al.*, 2003; Rector *et al.*, 2008]. Although fatty liver has been regarded as inconsequential, recent studies suggest that fatty liver increases the risk of progression of liver disease induced by various stresses and toxic stimuli such as endotoxin and alcohol [Yang *et al.*, 1997; Carmiel-Haggai *et al.*, 2003]. Therefore, the prevention or treatment of fat accumulation in the liver may provide a novel therapeutic strategy to control fatty liver disease.

Although the underlying mechanism of NAFLD is not completely understood, the histologic and pathologic changes of NAFLD resemble those of alcoholic fatty liver [Malhi and Gores, 2008]. It has been reported that oxidative stress and lipid peroxidation are implicated in

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; B, *n*-butanol-extract; FABP, fatty-acid binding protein; HFCD, high-fat high-cholesterol diet group; M, metadoxine; NAFLD, nonalcoholic fatty liver disease model; OJ, *Oenanthe javanica*; W, water-extract

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the pathogenesis of both alcoholic and non-alcoholic fatty liver. In fact, several experimental studies have shown that some antioxidants improve fatty liver disease [Marchi *et al,* 1990; Caballería *et al.,* 1998; Kwon *et al.,* 2009]. Metadoxine has been recently introduced for treatment of alcoholic fatty liver and its possible application for NAFLD has been suggested in several review articles due to the pathological similarities shared by alcoholic and non-alcoholic fatty liver [Kadayifci *et al.,* 2007; Fehér *et al.,* 2009]. To date, however, no medication has been approved for treating NAFLD [Lam and Younossi, 2009].

Oenanthe javanica (OJ), commonly called water dropwort, has been used as an herbal medicine to treat various diseases, including liver disease, in China [Li, 1978]. OJ extract has anti-hepatitis B virus activity and anti-diabetic effects by increasing insulin release from beta-cells [Yang *et al.,* 2000; Han *et al.,* 2008]. The OJ extract also has hepatoprotective effects against bromobenzene- and acetaminophen-induced liver damage by reducing levels of lipid peroxide, indicating an antioxidant effect [Park *et al.,* 1996; Park *et al.,* 2008]. In addition, OJ is widely consumed after binge drinking to overcome alcohol hangovers in Korea. Our previous study showed that the water extract and *n*-butanol fraction of OJ increased the rate of ethanol elimination as much as metadoxine [Kim *et al.,* 2009]. These studies indicated that OJ extract protects against liver damage induced by different insults. However, the effect of OJ extracts on NAFLD has not been determined.

Animal models to study NAFLD include nutritional models that provide free access to diets containing high fat or high carbohydrate content [Koteish and Diehl, 2001; Lee *et al.,* 2009]. Here, we examined the beneficial effects of water- and *n*-butanol extracts of OJ on hepatic fat accumulation and plasma biochemicals in the NAFLD model and the effects of OJ extracts were compared with that of metadoxine, a candidate for the treatment of NALFD.

Materials and Methods

Materials and chemicals. Water dropwort (*O. javanica* DC) was collected from agricultural farms at Cheongdo-Gun, Gyoungsangbuk-do, Korea. Metadoxine was kindly provided by Ilyang Pharmaceutical Co. Ltd (Yongin, Korea). All other reagents were obtained from the Sigma Chemical Company (St. Louis, MO, USA).

Preparation of water dropwort fractions. The dried and pulverized aerial part of water dropwort (3.6 kg) was extracted by refluxing with 36 L of boiling water for 1 h. After filtration, the extract was concentrated to 6 and 3 L of the extract was used as a water extract. The remaining 3 L was re-extracted three times with the same volume of *n*-butanol and then the extract was evaporated to produce a butanol extract (8.4 g). The butanol extract was dissolved in 3 L of saline.

Treatment of animals. Thirty male Sprague-Dawley rats (body weight: ~100 g) were obtained from Hyochang Sciences (Daegu, Korea). These rats were initially adapted for a week in the SPF animal facility, being fed rodent chow. The rats were then divided into five groups (6 rats per group) and fed on the following diets for 6 weeks: the normal rat chow diet group (CHOW), the high-fat high-cholesterol diet group (HFCD), and supplemented high-fat diet group with the water extract of OJ (HFCD+W), the *n*-butanol fraction of OJ (HFCD+B), or metadoxine (HFCD+M). Metadoxine was used as a reference in the present study. The high-fat high-cholesterol diet contained, in grams per 100 grams, 58 rat chow, 5 sucrose, 18 butter, 10 corn oil, 1 cholesterol, 5 casein, 0.2 methionine, 0.8 vitamins (AIN-76 vitamin mixture), and 2 minerals (AIN-76 mineral mixture). The rat chow, Purina Rodent Chow diet, was obtained from Purina Korea (Seoul, Korea) and contained, in percent of total calories, 58 carbohydrate, 14 fat, and 28 protein. The energy content of the high-fat high-cholesterol diet was 5.1 kcal/g, whereas that of the rat chow was 3.4 kcal/g. For the preparation of HFCD+W, HFCD+B and HFCD+M diets, 8% (v/w) water extract, 8% (v/w) *n*-butanol extract and 0.15 % metadoxine (w/w) were added to the high-fat high-cholesterol diet.

The supplementation of water- and *n*-butanol extracts of OJ was calculated to feed animals the same amount of fresh OJ (~5 g fresh OJ/kg body weight/day). HFCD+M was given about 150 mg/kg body weight/day of metadoxine. These doses were calculated based on measurements of animal food consumption. The rats were provided diets and water ad libitum. The body weight of each animal was recorded three times per week. We certify that all applicable institutional and governmental regulations concerning the ethical use of animals were followed, and this research was approved by Animal Care and Use Committee of Yeungnam University.

Visceral fat and biochemical analysis. All rats were fasted overnight before sacrifice after 6 weeks of feeding. The rats were anesthetized with ketamine (60 mg/kg). Blood was drawn from the abdominal aorta and centrifuged at 3,000×g for 20 min for the determination of plasma biochemicals. The epididymal and retroperitoneal fat pads were excised and weighed as visceral fat mass. Plasma levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using an assay kit (Asan Pharmaceutical Co., Seoul, Korea).

Plasma glucose, total cholesterol, and triglyceride concentrations were measured using Sigma enzyme kits. Triglyceride concentration in the liver was determined by extracting total lipids from clamp-frozen samples with chloroform-methanol (2:1, v/v) as described by Folch *et al.* [1957]. After separating the chloroform and methanol-water phases, phospholipids were removed and further processed by Frayn and Maycock's methods [1980]. Triglycerides were then quantified spectrophotometrically as glycerol using an enzymatic assay kit (Sigma).

Histological examination. At the time of sacrifice, the liver was fixed in 10% neutral buffered formalin. After fixation, 4-μm paraffin sections were stained with haematoxylin and eosin (H&E) for the evaluation of fat infiltration under light microscopy.

Statistical analysis Differences among groups were analyzed by one-way analysis of variance followed by the Tukey-Kramer test (PRISM version 4.0). All data are expressed as mean±SE. Differences were considered significant at $p<0.05$.

Results

Body weight and visceral fat mass. Body weights were monitored throughout the feeding period. After 6 weeks, the body weight of all HFCD groups was significantly higher than the CHOW, with a 24% greater weight gain for the HFCD (Table 1). No significant differences in body weight or weight gain occurred in the HFCD groups. Epididymal and retroperitoneal fat pads were weighed to measure total visceral fat mass. HFCD increased visceral fat mass about 2 fold compared with CHOW, as did the HFCD+W, HFCD+B and HFCD+M groups (Table 2). Thus, supplementing OJ extracts and metadoxine did not affect body weight or visceral fat mass in after a high-fat high-cholesterol diet.

Blood biochemistry. Next, we examined blood biochemistry from each group. Animals in the HFCD had significantly higher levels of plasma glucose, but HFCD+W, HFCD+B, and HFCD+M blocked this increase to the level of the CHOW (Table 3). Similarly, HFCD increased plasma triglycerides by 240% compared with CHOW. HFCD+W, HFCD+B and HFCD+M reduced this level by 84, 97, and 95%, respectively. HFCD increased total cholesterol by 124%, and this increase was significantly blocked by HFCD+B and HFCD+M (Table 4). HFCD+W also lowered plasma cholesterol, but not significantly. HFCD increased LDL cholesterol by 150% compared with CHOW, and HFCD+W, HFCD+B, and HFCD+M lowered this increase by 82, 71, and 78%.

Table 1. Effect of *Oenanthe javanica* extracts on body weight in rats fed a high-fat high-cholesterol diet for 6 weeks

	Body weight (g)		Gain in BW(g)
	Initial	After 6 weeks	
CHOW	138±2.7	374±5.7	236±4.4
HFCD	140±3.5	433±6.2*	293±5.7*
HFCD+W	138±2.3	424±12.9*	287±12.4*
HFCD+B	140±2.6	446±7.3*	306±7.7*
HFCD+M	136±3.4	416±21.4*	280±18.4*

CHOW, normal rat chow diet; HFCD, high-fat high-cholesterol diet; HFCD+W, HFCD with water-extract of OJ; HFCD+B, HFCD with *n*-butanol-extract of OJ; HFCD +M, HFCD with metadoxine.
Values are mean±SE for 6 animals per group. *$p<0.05$ vs. CHOW.

Table 2. Effects of *Oenanthe javanica* extracts on visceral fat in rats fed high-fat high-cholesterol diet for 6 weeks

Group	Retroperitoneal fat (g)	Epididymal fat (g)	Total (g)
CHOW	4.3±0.78	5.4±0.63	9.7±1.31
HFCD	10.5±1.76*	9.3±0.72*	19.8±2.17*
HFCD+W	8.3±1.09*	9.0±1.42*	17.3±2.47*
HFCD+B	10.4±0.92*	9.2±0.81*	19.6±1.62*
HFCD+M	8.9±2.24*	9.2±2.26*	18.1±4.48*

CHOW, normal rat chow diet; HFCD, high-fat high-cholesterol diet; HFCD+W, HFCD with water-extract of OJ; HFCD+B, HFCD with *n*-butanol-extract of OJ; HFCD +M, HFCD with metadoxine.
Values are mean±SE for 5 or 6 animals per group. *$p<0.05$ vs. CHOW.

Table 3. Effects of *Oenanthe javanica* extracts on plasma glucose and triglyceride concentrations in rats fed high-fat high-cholesterol diet for 6 weeks

Group	Glucose (mg/dl)	Triglyceride (mg/dl)
CHOW	129±7.2	19.9±2.24
HFCD	156±13.3*	47.8±7.91*
HFCD+W	131±3.1$^{\#}$	24.5±3.24$^{\#}$
HFCD+B	131±5.8$^{\#}$	20.7±2.32$^{\#}$
HFCD+M	129±4.3$^{\#}$	21.3±3.62$^{\#}$

CHOW, normal rat chow diet; HFCD, high-fat high-cholesterol diet; HFCD+W, HFCD with water-extract of OJ; HFCD+B, HFCD with *n*-butanol-extract of OJ; HFCD +M, HFCD with metadoxine.
Values are mean±SE for 6 animals per group. *$p<0.05$ vs. CHOW, $^{\#}p<0.05$ vs HFCD.

Table 4. Effects of *Oenanthe javanica* extracts on plasma cholesterol concentrations in rats fed high-fat high-cholesterol diet for 6 weeks

Group	Total-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)
CHOW	44.4±0.44	21.7±2.35	18.7±0.78
HFCD	55.2±1.80*	17.4±1.51	28.2±0.96*
HFCD+W	45.9±4.54	20.6±3.02	20.4±1.75$^{\#}$
HFCD+B	43.5±4.63$^{\#}$	17.9±3.10	21.5±1.22$^{\#}$
HFCD+M	43.5±2.94$^{\#}$	18.4±2.93	20.8±0.46$^{\#}$

CHOW, normal rat chow diet; HFCD, high-fat high-cholesterol diet; HFCD+W, HFCD with water-extract of OJ; HFCD+B, HFCD with *n*-butanol-extract of OJ; HFCD +M, HFCD with metadoxine.
Values are mean±SE for 6 animals per group. *p<0.05 vs CHOW, $^{\#}p$<0.05 vs HFCD.

Table 5. Effects of *Oenanthe javanica* extracts on plasma AST and ALT activities in rats fed high-fat high-cholesterol diet for 6 weeks

Group	AST (Karmen/mL)	ALT (Karmen/mL)
CHOW	32.0±0.79	25.0±0.67
HFCD	31.1±0.82	27.0±0.74
HFCD+W	31.0±0.48	26.1±0.91
HFCD+B	31.0±0.40	25.3±0.44
HFCD+M	31.3±0.12	25.3±0.19

CHOW, normal rat chow diet; HFCD, high-fat high-cholesterol diet; HFCD+W, HFCD with water-extract of OJ; HFCD+B, HFCD with *n*-butanol-extract of OJ; HFCD +M, HFCD with metadoxine.
Values are mean±SE for 6 animals per group.

Table 6. Effects of *Oenanthe javanica* extracts on hepatic triglyceride concentration in rats fed high-fat high-cholesterol diet for 6 weeks

Group	Triglyceride (mg/g tissue)
CHOW	9.9±0.37
HFCD	14.7±0.32*
HFCD+W	12.8±0.79*$^{\#}$
HFCD+B	12.6±0.35*$^{\#}$
HFCD+M	13.5±0.51*

CHOW, normal rat chow diet; HFCD, high-fat high-cholesterol diet; HFCD+W, HFCD with water-extract of OJ; HFCD+B, HFCD with *n*-butanol-extract of OJ; HFCD +M, HFCD with metadoxine.
Values are mean±SE for 6 animals per group. *p<0.05 vs CHOW, $^{\#}p$<0.05 vs HFCD.

HDL cholesterol and plasma ALT and AST did not change (Table 5). Therefore, these results show that the extracts of OJ and metadoxine improved the levels of plasma glucose, triglyceride and LDL-cholesterol with similar extent.

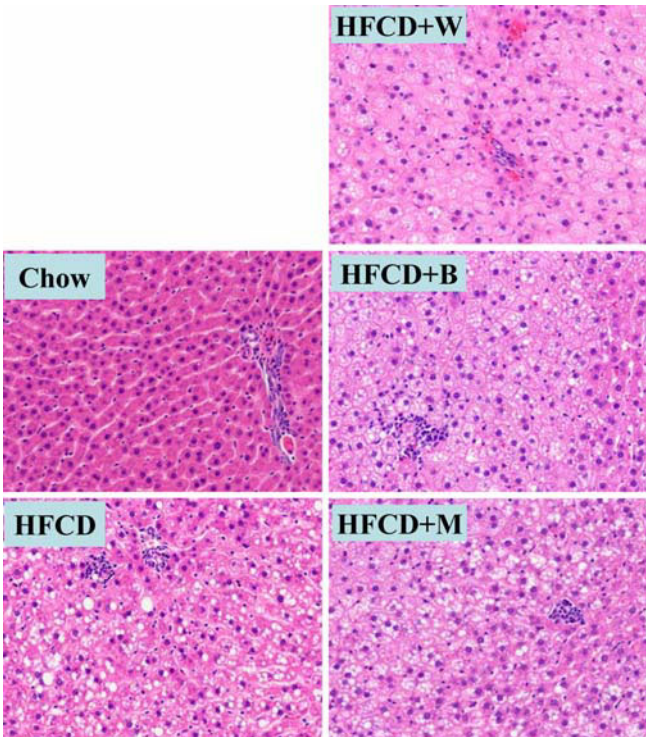

Fig. 1. Histological examination of liver tissue. Liver tissues were prepared as described in materials and methods. The data are representative H&E stained sections from each group (magnification×200). CHOW, normal rat chow diet; HFCD, high-fat high-cholesterol diet; HFCD+W, HFCD with water-extract of OJ; HFCD+B, HFCD with *n*-butanol-extract of OJ; HFCD+M, HFCD with metadoxine.

Triglyceride content in liver. HFCD increased liver triglyceride content (mg/g tissue) by 48% compared with CHOW (Table 6). Both HFCD+W and HFCD+B reduced this fat accumulation in the liver, but not to CHOW levels. HFCD+M also reduced liver triglyceride content, but not significantly. Histological examination of liver samples by H&E staining confirmed these results (Fig. 1). Liver from the CHOW showed normal histological features, whereas the HFCD exhibited mild to severe micro- and macrovesicular fat droplets. HFCD+W, HFCD+B, and HFCD+M reduced this fat accumulation and the extracts of OJ seemed to be more effective for controlling fatty liver than metadoxine.

Discussion

Here, we evaluated the effect of water- and *n*-butanol extracts of OJ on high-fat high-cholesterol diet-induced hepatic fat accumulation and plasma biochemical profiles

in a rat model of NAFLD.

NAFLD is the most common liver disease and has become a public health problem due to its growing prevalence worldwide. The pathogenesis of NAFLD is complex and is closely associated with obesity and insulin resistance. Here, we used high-fat high-cholesterol diet-fed rats as a model of NAFLD. High-fat high-cholesterol diet feeding for 6 weeks increased visceral fat mass, plasma triglyceride, and glucose levels, indicating the development of visceral obesity, dyslipidemia, and insulin resistance, respectively. These animals also showed fatty liver, as assessed by measurement of liver triglyceride content and histological examination. Therefore, our animal model reproduces the features of metabolic syndrome and liver histology seen in NAFLD.

Treatments for NAFLD include insulin-sensitizing agents, lipid-lowering agents and antioxidants [Fehér and Lengyel, 2003; Malaguarnera *et al.,* 2009]. Although these agents can improve NAFLD symptoms, they are not very effective. We tested the effects of OJ extracts and metadoxine as potential treatments for NAFLD. The water- and *n*-butanol extracts of OJ improved the elevated plasma triglyceride and glucose levels induced by high-fat high-cholesterol diet and lowered triglyceride content in the liver. Similarly, the treatment with metadoxine reversed the increased plasma glucose and triglyceride and showed a tendency to decrease hepatic fat accumulation. These results suggested that the OJ extracts and metadoxine ameliorate fatty liver and metabolic disturbances. Interestingly, the OJ extracts and metadoxine did not affect body weight or total visceral fat mass in high-fat high-cholesterol fed rats, indicating a lack of an anti-obesity effect or changes in visceral fat mass.

Visceral obesity is a critical determinant in the development of insulin resistance, dyslipidemia, and liver steatosis [Kral *et al.,* 1993; Nishina *et al.,* 1994; Shulman, 2000]. Circulating adipokines and cytokines play important roles in the network of adipose tissue and other tissues involved in systemic metabolism, such as the liver [Shulman, 2000; Shoelson *et al.,* 2007; Polyzos *et al.,* 2009]. Recently, attention has focused on the role of fatty-acid binding protein (FABP) in this network. FABP, also called aP2, is abundantly expressed in adipocytes, and inhibition of FABP improved dyslipidemia, insulin sensitivity and fatty liver in obese animal models induced by high-fat diet or genetic modification [Uysal *et al.,* 2000; Maeda *et al.,* 2005]. aP2 deficient ob/ob mice exhibited much lower plasma glucose, insulin, and triglyceride levels despite gaining more body weight and fat mass than ob/ob control mice [Uysal *et al.,* 2000]. Similarly, mice deficient in mal1, an FABP isoform, displayed increased insulin sensitivity without changes in total fat mass [Maeda *et al.,* 2003]. Therefore, OJ extracts might regulate FABP function without affecting adiposity, and OJ extracts and metadoxine might decrease fat accumulation in the liver by their antioxidant activity [Park *et al.,* 1996; Park *et al*., 2008; Fehér *et al.,* 2009]. Future studies are needed to define the action mechanisms of OJ extract and metadoxine in the pathogenesis of NAFLD with emphasis of their effects on lipid peroxidatoin and FABP activity.

In conclusion, the water- and *n*-butanol extracts of OJ and metadoxine improved hepatic fat accumulation, hyperglycemia, and dyslipidemia induced by high-fat high-cholesterol diet although the effect of metadoxine on fatty liver was not significant, suggesting their potential use for the prevention and treatment of NAFLD.

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