

## Extracts of Korean Natural Herb Alleviate Pathophysiology of Nonalcoholic Fatty Liver

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### Abstract

*This study was designed to examine effects of Korean Acer tegmentosum Maximowocz extract, which has traditionally been used for the treatment of liver damages in fat diet-induced obese rats. 40 male SD rats were divided into three groups: normal diet (Control group, n=10), 40% fat diet-induced obese rats (Obesity group, n=15), and Acer tegmentosum Maximowoczextract diet with 40% fat diet-induced obese rats (ATM group, n=15). Hematological and biochemical variables were measured. Histological examinations of liver and adipose tissues were performed with hematoxylin & eosin stain. Total leukocyte, neutrophil, neutrophil/lymphocyte ratio, erythrocyte, platelet, hemoglobin, and red distribution width levels were significantly higher, whereas monocyte levels were lower in obesity group than in control and ATM group. Glucose, total cholesterol, triglyceride, LDL-cholesterol, AST, ALT, and BUN levels in obesity group were significantly greater than those of ATM. ATM group showed significantly lower liver fatty degeneration (steatosis) than obesity group. ATM group exhibited regular morphology of adipocytes and adipocyte sizes are smaller than obesity group, whereas obesity group showed larger and irregular shapes of adipocytes than ATM and control groups. These results suggest that Korean Acer tegmentosum Maximowocz exerts anti-inflammatory and anti-obesity effects.*

**Key words:** *Korean Acer tegmentosum Maximowocz, Nonalcoholic fatty liver, Rat, Hematological variable, Biochemical variable, Histological examination*

### 1. Introduction

Morbidity attributable to nonalcoholic fatty liver disease (NAFLD), in which excessive fat accumulates in the liver of a patient without a history of alcohol abuse is increasing in Korea. Recent investigations have reported that 10-20% of people subjected to a medical examination had NAFLD with abnormal liver functions, insulin resistance with genetic or environmental factor induces subsequent NAFLD. NAFLD combined with oxidative stress or hepatic fibrosis may be progressed to steatohepatitis and/or liver cirrhosis (Abdelmalek and Diehl, 2007). Although simple fatty liver is first clinical manifestation, 30% of them may progress towards liver cirrhosis and/or hepatic cancer (Cohen *et al.*,

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2011). In addition to diabetes, there is a strong relationship between NAFLD and subclinical manifestation of atherosclerosis, which may lead to increased intima-media thickness, endothelial dysfunction, arterial stiffness, impaired left ventricular function and coronary calcification (Farion *et al.*, 2014). The therapy on NAFLD includes diet, regular exercise, and/or application of antioxidants. However, diet or regular exercise has potential of failure. The applications of antioxidants, vitamin E or C, for treatment of NAFLD also have arguments (Harrison *et al.*, 2003; Kugelmas *et al.*, 2003; Pacana and Sanyal, 2012). This study was performed to examine effects of Korean *Acer tegmentosum Maximowocz* extract, which has traditionally been used for the treatment of liver damages in fat diet-induced obese rats.

## 2. Materials and Methods

Sprague-Dawley strain male albino rats (aged-6 weeks, weighing 161-179 g) had free access to standard laboratory L feed and water *ad libitum*. Clean food and water were provided *ad libitum*. All of the rats were kept on a 12:12-hrs light/dark cycle at a temperature of 25°C and humidity of 60%. After an adaptation period of one week, the rats were divided into three groups: normal diet (Control group, n=10), 40% fat diet-induced obese rats (Obesity group, n=15), and *Acer tegmentosum Maximowocz* extract diet with 40% fat diet-induced obese rats (ATM group, n=15). After the eight weeks of experiments, all of rats were fasted for 24 hours, were anesthetized by ether and 7 mL of blood was collected directly from the abdominal cava. Liver and adipose tissues were harvested. The blood and serum was used for measuring hematological and biochemical variables. Histological examinations of liver and adipose tissues were performed with hematoxylin & eosin stain. The study was approved by the Animal Ethics Committee of Catholic University of Pusan.

## 3. Results

The weights after eight weeks were higher in the obesity group than in the control and ATM groups ( $P<0.05$ ) (Table 1). Total leukocyte, neutrophil, neutrophil/lymphocyte ratio, erythrocyte, platelet count, hemoglobin and red blood cell distribution width levels in the obesity group were higher, whereas monocyte counts were lower than those of the control and ATM groups ( $P<0.05$ ) (Table 2). Glucose, total cholesterol, triglyceride, low density lipoprotein, AST, ALT, ALP and BUN levels in the obesity group were higher than in ATM group ( $P<0.05$ ) (Table 3). Histological examination on the liver in the obesity group had hepatic steatosis and Mallory body, whereas those of ATM group were similar to the control group (Figure 1). ATM group exhibited regular morphology of adipocytes and adipocyte sizes are smaller than obesity group, whereas obesity group showed larger and irregular shapes of adipocytes than ATM and control groups (Figure 2).

## 4. Discussion

40% fat-diet for eight weeks led to higher body weights in obesity group, suggesting fat-diet can contribute to obesity. Also, obesity group has relatively much fat mass in lower abdomen with compared with control and ATM groups. Obesity may cause cancers, metabolic syndrome, sterility, renal disorders, hypertension, cardiovascular disease, diabetes, and/or asthma (Walzem and Chen, 2014; Hauner and Hauner, 2014; Vaněčková *et al.*, 2014; Prasad, 2014; Bruder-Nascimento *et al.*, 2014; Golia *et al.*, 2014; Permaul *et al.*, 2014). This finding indicates that Korean *Acer tegmentosum Maximowocz* extract exert anti-obesity effects. In the present ATM group had significantly lower total leukocyte, neutrophil and neutrophil/lymphocyte ratio levels than obesity group. These results may reflect that obesity can contribute increased systemic inflammation and that

Korean *Acer tegmentosum Maximowocz* possesses antiinflammation. Many studies provide evidence of strong association between total leukocyte and neutrophil counts and increased morbidity and mortality of cardiovascular disease in adults with middle grade and chronic renal disease (Dregu *et al.*, 2008; Sekitani *et al.*, 2010; Straburzynska-Migaj *et al.*, 2010). Moreover, elevated total leukocyte and neutrophil counts were closely related with increased morbidity and mortality of cardiovascular disease in even healthy adults (Yen *et al.*, 2006; Nordestgaard *et al.*, 2010; Oda and Kawai, 2010). Obesity group also had higher platelet counts than ATM group. Thaulow showed that in even health adults, elevated platelet counts may lead to faster aggregation, the development of coronary artery disease and sudden death (Thaulow *et al.*, 1991). Although further studies should be performed, it is suggested that Korean *Acer tegmentosum Maximowocz* may have anticoagulant function. The findings of higher RBC, hemoglobin and RDW levels in obesity group may cause disturbance of blood flow due to increased blood viscosity and activation of coagulation system, leading to thrombosis. Especially, RDW is an independent predictor of mortality in patients with stable coronary artery disease (Wang *et al.*, 2011; Osadnik *et al.*, 2013). RDW has been reported to be a risk marker of morbidity and mortality for cardiovascular diseases in various study populations (Wang *et al.*, 2011; Osadnik *et al.*, 2013). Patients with NAFLD were more likely to have high levels of RDW. Moreover, NAFLD was associated with body weights, RDW, fasting plasma glucose, and triglyceride (Masoudkabar *et al.*, 2011; Yang *et al.*, 2014). The present study demonstrated that obesity group had NAFLD, whereas histological findings on the liver tissue in ATM group had similar to control group (Figure 1), suggesting useful effects of Korean *Acer tegmentosum Maximowocz* from the viewpoint of obesity, hematology, and NAFLD. On the other hand, in the biochemical variables, ATM group had lower glucose, total cholesterol, triglyceride, and LDL-cholesterol, AST, ALT, ALP, and BUN concentrations compared with obesity group. On the histological observations by H&E stain, ATM group had similar to control group, but obesity group exhibited the fatty droplets, Mallory bodies, fatty degenerative findings, larger and irregular shapes of adipocytes.

In conclusions, Korean *Acer tegmentosum Maximowocz* is considered to have antiobesity, anti-inflammatory and liver protective effects as well as protection of metabolic syndrome. However, further study should be performed in human.

## Appendix

**Table 1. Demographic Characteristics of Three Groups**

Variable	Group		
	Control	Obesity	ATM
Total number (n)	10	15	15
Gender	Male	Male	Male
Age (week)	6	6	6
BW before experiment (g)	162.30±8.77	160.10±7.86	162.06±8.56
BW after experiment (g)	319.03±10.25	385.17±11.54*	360.10±9.34
Experimental period (week)	for 8	for 8	for 8

Data are expressed as mean±standard deviation (SD). \*,  $P < 0.05$  (compared with control and ATM groups). Abbreviation: Control, normal diet group; Obesity, fat diet group; ATM, extract of *Acer tegmentosum Maximowocz*-diet group with fat diet rat.; BW, body weight.

**Table 2. Hematological Variables in Three Groups**

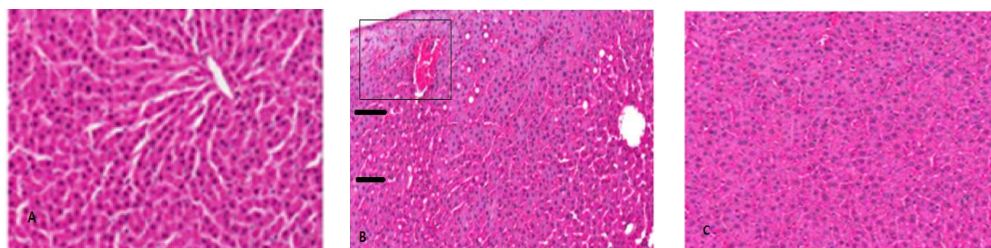
Variable	Group		
	Control	Obesity	ATM
T-leukocyte ( $10^3/\mu\text{L}$ )	3.99±3.12	5.76±2.48*	39.32±5.40
Neutrophil (%)	24.12±2.61	32.97±3.11*	23.40±4.02
Lymphocyte (%)	72.20±1.82	72.40±3.02	70.3±3.73
Monocyte (%)	3.71±0.60	2.60±0.51*	3.17±0.62
N/L ratio	0.33±0.02	0.46±0.16*	0.33±0.03
RBC ( $10^6/\mu\text{L}$ )	731.42±12.88	859.50±24.66*	704.13±79.90
Platelet ( $\times 10^3/\mu\text{L}$ )	475.53±77.68	641.27±34.65*	499.15±41.50
Hemoglobin (g/dL)	14.20±2.56	15.16±3.42*	14.04±2.40
MCV (fL)	57.86±4.67	58.83±5.08	57.82±4.08
MPV (fL)	6.56±1.74	6.88±1.82	6.60±1.05
RDW (%)	12.10±1.02	13.85±1.03*	12.00±1.11

Data are expressed as mean±SD. \*,  $P < 0.05$  (compared with control and ATM group). Abbreviation: T-, total; RBC, red blood cells; N/L ratio, neutrophils/lymphocytes ratio; MCV, mean corpuscular volume; MPV, mean platelet volume; RDW, red blood cell distribution width; PDW, platelet distribution width.

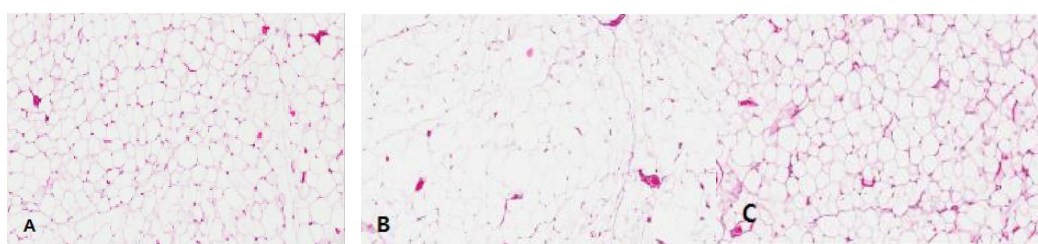
**Table 3. Biochemical Markers of the Study Population**

Variable	Group		
	Control	Obesity	ATM
Glucose (mg/dL)	123.250±9.41	205.53±9.42*	120.50±8.47
T-cholesterol (mg/dL)	45.00±13.89	65.33±14.50*	39.37±11.24
Triglyceride (mg/dL)	29.30±8.83	63.71±11.68*	23.60±9.96
HDL-cholesterol (mg/dL)	15.66±9.82	9.10±7.08*	9.74±8.00 <sup>+</sup>
LDL-cholesterol (mg/dL)	10.00±3.37	11.00±3.87*	5.55±2.33 <sup>+</sup>
Apolipoprotein-A1 (mg/dL)	<20	<20	<20
Apolipoprotein-B (mg/dL)	<20	<20	<20
AST (IU/L)	69.00±13.63	95.16±15.32*	57.15±9.96 <sup>+</sup>
ALT (IU/L)	39.3±9.89	52.00±10.39*	36.40±8.81
ALP (IU/L)	89.66±17.12	155.89±20.02*	134.14±18.14
GGT (IU/L)	6.00±1.05	6.51±1.70	5.81±1.20
T-bilirubin (mg/dL)	<0.1	<0.1	<0.1
BUN (mg/dL)	15.51±2.31	16.23±2.06*	13.77±1.85 <sup>+</sup>
Creatinine (mg/dL)	0.61±0.10	0.67±0.16	0.62±0.10
Uric acid (mg/dL)	1.10±0.44	1.76±0.25	1.20±0.10

Data are expressed as mean±SD. \*,  $P < 0.05$  (compared with control and ATM group); +,  $P < 0.05$  (compared control and obesity group). Abbreviation: T, total; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; BUN, blood urea nitrogen.



**Figure 1. Histological Findings of Livers in Three Groups (by HE staining, 100X). ATM Group (C) showed Significantly Lower Liver Fatty Degeneration (steatosis) than Obesity Group (B). In Obesity Group (B) the Fatty Droplets are Spread, Black Arrows are Mallory Bodies, and a Square Shows Fatty Degenerative Findings, Suggesting that *Acer tegmentosum Maximowocz* may have a Protective Effect in Nonalcoholic Fatty Liver Injury**



**Figure 2. Histological Findings of Adipose Tissue in Three Groups (by HE staining, 100X). Obesity Group (B) Showed Larger and Irregular Shapes of Adipocytes, whereas ATM Group (C) Exhibited Regular Morphology of Adipocytes and Adipocyte Sizes are Smaller than Obesity Group (B), Indicating that *Acer tegmentosum Maximowocz* may Exert Anti-Obesity Effects**

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