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■ Original Article ■

The Ameliorative Effects of Korean Bean-Leaves on Inflammation and Liver Injury in Obese Rat Model

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Obesity may cause metabolic syndrome and adult diseases. This study was undertaken to investigate the ameliorative or useful effects of beanleaves on inflammation and liver damage in obese rat models. Rats were divided into three groups: a control group (normal diet, n=6), a fat diet group (45%-fat diet, n=7), and a bean leaf group (45%-fat+Korean bean leaves diet, n=7). Body weights in the bean leaf group were lower than those of the fat group (P<0.05). Serum tumor necrosis factor-α (TNF-α) and prostaglandin E_2 (PGE_2) concentrations were lower in both the control and bean leaf groups than in the fat group (P<0.001). TNF-α concentrations in the bean leaf group were slightly higher than in the control group but statistically significant (P<0.05). The bean leaf group histologically exhibited lower fatty degeneration, spotty necrosis, and leukocyte infiltrations in hepatic tissues than those of the fat group. In the homogenized liver tissues, the cyclooxygenase-2 (COX-2) gene was only expressed in the fat group. The gene expression levels of hepatic TNF-α, inducible nitric-oxide synthase, peroxiome proliferator-activated receptor-α (PPAR-α), poly (ADP-ribose) polymerase (PARP), and transforming growth factor-β1 (TGF-β1) were weaker in the bean leaf group than in the fat group. These results suggest that adding bean-leaves to the diet may ameliorate obesity-induced systemic inflammation and liver damage and that bean leaves may be a useful food for preventing obesity and thereby metabolic syndrome and adult diseases.

Key Words: Obesity, Bean-leaves, Liver, Inflammation, Fatty liver, COX-2, iNOS, PPAR-α, PARP, TGF-β1

INTRODUCTION

Obesity has been announced as a global pandemic disorder by the World Health Organization (WHO). At least 2.6 million people are dying each year due to being overweight and/or obese. The incidence of obesity is dramatically increasing, and WHO predicts that 2.3 billion people will be overweight and more than 700 million will be obese by the year 2015 (Torres et al., 2012). As everybody knows, overweight and/or obesity may contribute to metabolic syndrome and adult disease. Obesity, especially, is a potential causation of metabolic syndrome, which is characterized by insulin resistance, hyperglycemia, central (abdominal) obesity, dyslipidemia, and/or hypertension (Grundy et al., 2004; Kahn et al., 2005). Metabolic syndrome may be associated with an increased risk factor for developing type II diabetes, cardiovascular and cerebrovascular diseases, and some

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cancers (Grundy et al., 2004; Goldstein et al., 2006; Aschner et al., 2010). Pediatric and child diabetes with obesity have recently become social problems. The causes of obesity include overeating, lack of exercise, prolonged television-watching, family history, etc. To date, many investigators have tried both prevention and treatment on obesity. Nevertheless, both interventions are very difficult. Particularly, the use of western anti-obesity drugs has been limited by side effects including mood changes, suicidal thoughts, and gastrointestinal or cardiovascular complications (Sui et al., 2012). Recently, some studies have been interested in herbal or food ingredient interventions for preventing and curing obesity and diabetes. Zhao et al. (2012) have demonstrated that a berberin-containing Chinese herbal medicine has anti-diabetic effects, and Sui et al. (2012) have reviewed the effectiveness and safety of traditional Chinese medicine including herbal medicine, for the treatment of obesity. In addition, green tea, Korean red ginseng, and *Artemisia princeps* were reported to exert therapeutic effects in terms of nonalcoholic fatty livers, improvement of insulin sensitivity, anti-obesity and anti-diabetes (Masterjohn and Bruno, 2012).

On the other hand, soybean can provide an important protein source and an important part of the diet worldwide. The physical functions of soybean have been studied, but no interest has been paid to the utilization of soybean leaves (*Glycine mas.MERR*). The leaves of some kinds of soybeans contain flavonoids which are not found in soybeans themselves, as well as isoflavones. Flavonoids are polyphenolic compounds widely distributed throughout the plant kingdom. They are important to protect plant leaves from ultra violet (UV) rays during their growth Most flavonoids are natural antioxidants which reduce the risk of cancer, aging and cardiovascular diseases. Their important roles include free radical scavenging, reduction, protection against lipid peroxidation and quenching of the reactive oxygen species (Zang et al., 2011). The bean leaves (*Glycine mas.MERR*) have been traditionally eaten, especially in the southern region of Korea, because they are believed to have an anti-obesity effect. We have designed this study to scientifically determine whether Korean bean-leaves (*Glycine mas.MERR*) have anti-inflammatory, anti-obesity and protection effects of nonalchoholic induced-liver damage.

MATERIALS AND METHODS

Experimental animal and grouping

The Male Sprague-Dawley rats purchased from Joong-Ang Animal company (Seoul, Korea) were six weeks of age. The rats were housed under pathogen-free conditions in enclosed filter-topped cages. 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℃ and humidity of 60%. After an adaptation period of one week, the rats were divided into two groups: the control group (n=6) which was fed a normal diet, the fat diet group (n=7) which was fed a fat diet (containing 45% Kcal pellet)(Central Lab. Animal Inc., Seoul, Korea), and the bean-group (n=7) which was fed a 45% -fat + Korean bean-leaves diet. In the bean-group, Korean bean-leaves (*Glycine mas.MERR*) were lyophilized and powdered. The powder was mixed with 0.4 mL of normal saline and fed as 10% of the total feed per day (about 2.50 g). Korean bean-leaves were purchased from a farmhouse of organic culture. The each diet was feed to each group for eight weeks. This study was approved by the Animal Ethics Committee of the Catholic University of Pusan.

Dissection and sample collection

After eight weeks, all rats were fasted for 24 hours and were then anesthetized by ether and fixed on the Rat Operating Table (Dong Sew Science, Seoul, Korea). The abdominal cava was exposed by lower abdominal incision, and 8 mL of blood was collected directly from the abdominal cava. Systemic irrigation was performed through the left ventricles with a phosphate buffer solution and the livers were harvested from all rats. Separated sera and pieces of harvested livers were kept at -70℃ until analyses of tumor necrosis factor-α (TNF-α), prostaglandin E_2 (PGE_2), cyclooxygenase-2 (COX-2) gene, inducible nitric-oxide synthase, peroxiome

proliferator-activated receptor-α (PPAR-α), poly (ADP-ribose) polymerase (PARP) and transforming growth factor-β1 (TGF-β1) could be performed. Sections of the rest of the liver tissues were fixed in 10% neutral buffered formalin for histological findings.

Analysis

Body weights: The body weights were measured and compared among three groups.

Serum tumor necrosis factor-α (TNF-α): ELISA method was applied for measuring TNF-α concentrations in the serum. Biotrak Ⅱ microplate reader (Biochrom Ltd., Vienna, Austria) with Thermo Scientific rat TNF-α ELISA kit (Pierce Biotechnology, LA, USA) were used for it.

Serum prostaglandin E_2 (PGE_2): The serum PGE_2 concentration was measured using an ELISA kit according to the manufacturer's instructions. The PGE_2 ELISA kit was purchased from R&D System (Minneapolis, MN, USA).

Preparation for paraffin-embedded tissues: The harvested liver tissues were fixed in 10% neutral buffered formalin for 12 hours before being processed to paraffin wax. After the tissues were fixed, they were processed automatically for dehydrating, clearing, filtrating and embedding in paraffin. For histological examination, the paraffin-embedded tissues were cut into sections of 4 μm thickness by a rotary microtome (Leicca, Nussloch, Germany) and placed on glass slides.

Hematoxylin and eosin (H&E) stain: The paraffin wax of the slides was removed with xylene, and the sections were subsequently rehydrated with ethanol (100% → 95% → 90% → 70%). After washing in running tap water, the sections were stained with hematoxylin for 6 minutes and rinsed, then stained with eosin for 2 minutes. The stained sections were dehydrated with graded strengths of ethanol (70% → 90% → 95% → 100%). The sections were cleared in two changes of xylene and finally cover slipped using a permanent mounting with malinol. The slides were examined based on the World Health Organization (WHO) criteria (Travis et al., 2006).

Immunoblot analysis: Liver tissues were homogenized in protein extraction solution (Intron Biotechnology, Gyeonggi-do, Korea) at 4℃. The homogenate was centrifuged at 13,000 rpm for 10 minutes to discard cell debris, and then the protein concentration of the supernatant was measured. Protein concentration was determined with a commercial protein assay kit (Bio-Rad, Hercules, CA, USA) using bovine serum albumin as a standard. Equal protein concentrations were subjected to 7.5-12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene fluoride membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). After blocking with TBS containing 5% non-fat dry milk for 1 hour at room temperature, each membrane was incubated with specific primary antibody overnight at 4℃. β-actin monoclonal antibody (mAb), iNOS polyclonal antibody and COX-2 polyclonal antibody was purchased from Cell Signaling Technology (Beverly, MA). TNFα mAb, TGFβ polyclonal antibody, PARPα polyclonal antibody, PPARγ polyclonal antibody and NOX4 mAb were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). After washing twice with TBS containing 0.1% Tween-20 (TBST), each membrane was immunoblotted with horseradish peroxidase-conjugated anti-mouse, anti-rabbit or anti-goat IgG antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 hour at room temperature, followed by washing three times in TBST and visualization by enhanced ECL (Amersham Pharmacia Biotech, Buckinghamshire, UK). Vision Works Image Software (UVP, Cambridge, UK) was carried out to measure band intensities.

RESULTS

Body weights

The body weights in the fat group were significantly

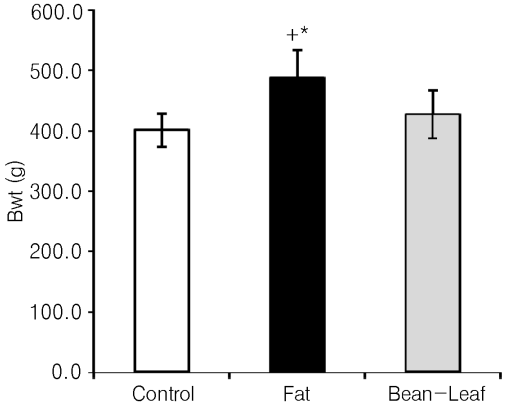

Fig. 1. Comparison of body weights among three groups (the control, fat and bean-leaf groups). The body weights in the fat group were higher than those of the Control and the bean-leaf groups, but there was no significance between the control and the bean-leaf groups. (+, P<0.01 compared with the Control group; *, P<0.04 compared with the bean-leaf group).

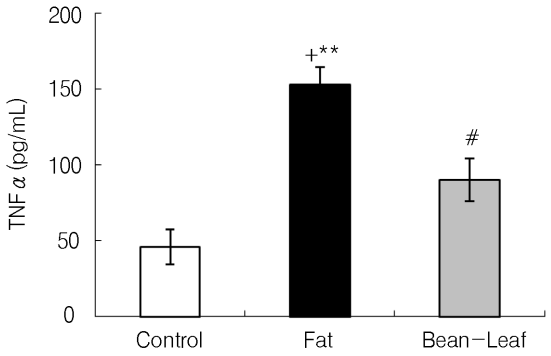

Fig. 2. Comparison of serum tumor necrosis-α (TNF-α) concentration among three groups (the control, fat, and bean -leaf groups). The serum TNF-α concentrations in the fat group were higher than those of the Control and the bean-leaf groups, while those of the bean-leaf group were greater than those of the control group (+, P=0.001 compared with the control group; **, P=0.003 compared with the bean-leaf group #, P=0.03 compared with the control group). BF, the bean-leaf group.

increased than those of the control and the bean leaf groups (P<0.01 and P<0.05, respectively) (Fig. 1).

Serum tumor necrosis factor-α (TNF-α)

Serum TNF-α concentrations were significantly higher in the fat group than in the control and the bean leaf groups (P<0.001). However, serum TNF-α concentrations in the bean-leaf group were greater than those of the control group (P<0.05) (Fig. 2).

Serum prostaglandin E_2 (PGE_2)

Serum PGE_2 concentrations were pronouncedly higher in the fat group than in the control and the bean leaf groups (P<0.001). There was no difference between the control and bean leaf groups (P>0.05) (Fig. 3).

Hematoxylin and eosin stain

The bean leaf group (Fig. 4-C) showed significantly lower fatty liver degeneration (steatosis) than the fat group (Fig. 4-B). The degrees of spotty necrosis in the lobule and inflammatory cell infiltration in the portal tract were markably low in the bean leaf group (Fig. 5-C) compared with the fat group (Fig. 5-B).

Immunoblot analysis

We studied immunoblot analysis to investigate obesity-

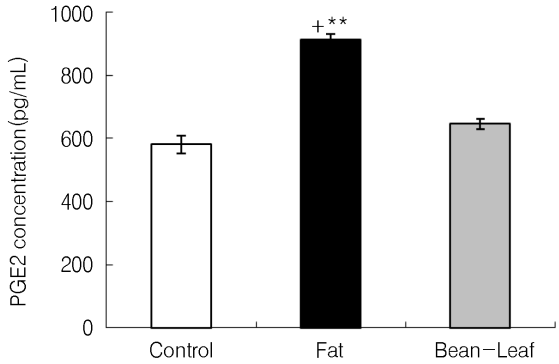

Fig. 3. Comparison of Prostaglandin E_2 (PGE_2) concentration among three groups (the control, fat, and bean -leaf groups). The prostaglandin concentrations in the fat group were higher than those of the control and the bean-leaf groups (+, P=0.001 compared with the control group; **, P=0.003 compared with the bean-leaf group). BF, the bean-leaf group.

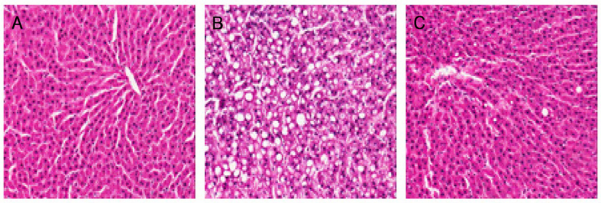

Fig. 4. Light microphotographs of liver tissues by H&E stain (x200 A, the control group; B, the fat group; C, the bean-leaf group). The bean-leaf group (C) showed significantly lower liver fatty degeneration (steatosis) than the fat group (B), suggesting that bean leaves may have a protective effect for the fatty liver injury.

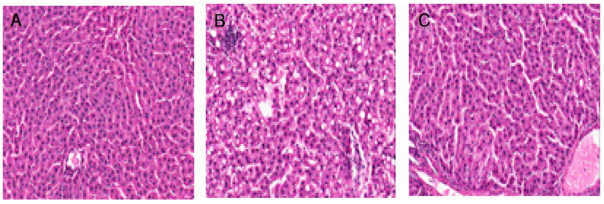

Fig. 5. Light microphotographs of liver tissues by H&E stain (x200; A, the control group; B, the fat group; C, the bean-leaf group). The degrees of spotty necrosis in lobule and inflammatory cell infiltration in portal tract were markedly low in the bean-leaf group (C), compared with the fat group (B), indicating that bean leaves have useful effect for liver protection.

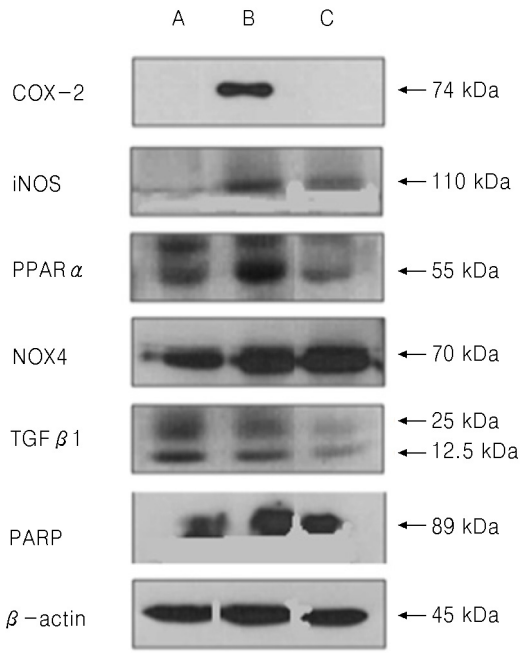

Fig. 6. Comparison of each gene expression in the liver tissues among three groups by Western blotting. (A), the control (B), the fat group; (C), the bean-leaf group.

induced liver damage and the protection effects of Korean bean leaves.

The fat group obviously showed strong and over expression of COX-2, whereas the control and bean leaf groups did not show Western blotting (Fig. 6). iNOS expression in the fat group was stronger than that of the control and bean leaf groups (Fig. 6). PPARα expression in the bean leaf group was weaker than that of the other groups, and it was strongest in the fat group (Fig. 6). There was no difference in NOX4 expression in the fat and bean leaf groups (Fig. 6). TGFβ1 expression in the bean leaf group was weaker than that of the fat group (Fig. 6). PARP expression in the fat group was stronger than that of the control and bean leaf groups (Fig. 6).

DISCUSSION

Nonalcoholic fatty liver disease (NAFLD), which is identified by excess lipid accumulation in the liver, is strongly associated with obesity, insulin resistance, hypertension, dyslipidemia and metabolic syndrome (Farrell et al., 2006; Torres et al., 2008). NAFLD starts with hepatic steatosis, which can progress with inflammation to nonalcoholic steatohepatitis, and a subset of patients develop progressive fibrosis and ultimately cirrhosis. In the majority of cases, NAFLD is associated with (components of) the metabolic syndrome. Obesity, diabetes and hepatic steatosis are also independent risk factors for hepatic fibrosis in different chronic liver diseases.

In the fat group, we found increased body weights and fatty livers and subsequent inflammation and liver injury. An elevated body weight may lead to obesity, which can be implicated in the pathogenesis of insulin resistance, the excess of visceral adipose tissue and increased production of adipokines (Gnacińska et al., 2009). In addition, obesity may contribute to metabolic syndrome, as it increases the risk of developing hypertension, diabetes, dyslipidemia, hyperglycemia, hyperuricemia, and proinflammatory status (Gnacińska et al., 2009). Serum TNF-α and PGE_2 concentrations were increased, and in the light microscopic observations, fatty liver degeneration (steatosis), and spotty necrosis in the lobule and inflammatory cell infiltration in the portal tract appeared in the fat group. Previous studies have demonstrated that the proinflammatory cytokine, TNF-α was overexpressed in obesity (Hotamisligil et al., 1993; Xu et al., 2002; Choi et al., 2012). The role of TNF-α in the development of insulin resistance has been demonstrated in various animal obesity models. The expression of TNF-αmRNA in adipose tissue increases in obesity and hyperinsulemia (Hotamisligil et al., 1995).

PGE_2, a product of prostanoids, is formed from

arachidonic acid by the prostaglandin synthesizing with cyclooxygenase (COX) enzymes and prostaglandin synthase (Simmons et al., 2004), which is involved in many diseases. During an inflammatory response, the concentrations of PGE_2 production can change dramatically. While PGE_2 concentrations are generally very low in uninflamed tissues, they increase immediately with acute inflammation prior to the leukocyte recruitments (Tilley et al., 2001). PGE_2 exhibits biphasic effects on bone formation, stimulating bone formation at low concentrations, but inhibiting it at high concentrations (Raisz et al., 1999). PGE_2 mediates inflammation, tissue destruction, and pain in osteoarthritis (Simmons et al., 2004). PGE_2 has been involved in human obesity, in which increased circulating levels of PGE_2 have been observed (Fain et al., 2001). PGE_2 is a lipid mediator with effects in the central nervous system, including activation of the hypothalamic-pituitary-adrenal (HPA) axis and febrile (Derijk et al., 1991). PGE_2 has also been shown to inhibit lipolysis in white adipose tissue and stimulate the secretion of leptin, suggesting that PGE_2 signaling is important for body weight homeostasis (Fain et al., 2001).

Finally, an 8-week fat diet in this study induces obesity, thus fat diet can cause increase of TNF-α and PGE_2 levels as well as, fattiness and injury of liver tissues. Fat diet-induced obesity is considered to be the cause of a joint elevation in TNF-α and PGE_2 levels that may contribute to the potential of the development of several diseases.

Nevertheless, in the bean leaf group, the above mentioned findings were relieved with soybean leaves, suggesting anti-obesity, anti-inflammatory and anti-liver damage effects of Korean bean-leaves.

On the Western blotting, the degrees of COX-2, iNOS, PPARα, TGFβ1 and PARP expression in the bean leaf group were weaker than those of in the Fat group, indicating that Korean bean leaves have ameliorative or protective effects on liver tissues.

Cyclooxygenase (COX) is a crucial enzyme in the biosynthesis of prostaglandins. There are two COX isoforms: COX-1 is constitutively expressed in a number of cell types and is involved in the homeostatic functions of prostaglandins, whereas COX-2 is inducible by a variety of proinflammatory stimuli, such as cytokines and lipopolysaccharide. In the liver, productions of COX-2 and prostaglandins have been implicated in hepatic regeneration, liver matrix remodeling and portal hypertension. In animal models of alcohol-induced liver disease COX-2 has been demonstrated to be related to necro-inflammatory activity. In viral hepatitis, hepatocellular COX-2 expression was observed and associated with fibrosis progression. More interesting has been the demonstration of the role of COX-2 in the development of hepatocellular carcinoma and cholangiocarcinoma, in experimental models that included human samples. It has also been demonstrated that COX-2 was implicated in carcinogenesis through apoptosis inhibition and increased proliferation of human tumor cells (Núñez Martínez et al., 2003). COX-2 expressed in the liver may be a marker to reflect the degree of inflammation and injury of liver tissue (Niu et al., 2011). COX-2 has been implicated in the carcinogenesis of various human cancers, including colorectal cancer and also cholangiocarcinoma (Niu et al., 2005). In our study, the fat group had higher TNF-α and COX expression compared with the bean leaf group, suggesting that fat diet can induce inflammation and steatohepatitis, and that Korean bean-leaves have anti-inflammatory and protective effects on liver injury. In the inflamed liver, proinflammatory cytokines including TNF-α, IL-1beta, and IFN-gamma stimulate the induction of iNOS gene expression, leading to excess production of NO and resulting in liver injury (Ozaki et al., 2010). Homologues of NADH oxidases (NOXs) are major sources of reactive oxygen species (ROS) which play a key role in chronic liver injury and fibrosis. Of NOXs, NOX-4 was shown to be critical in lung and kidney fibrosis by mediating activation of myofibroblasts (Hecker et al., 2009; Barnesety al., 2011). In the liver, NOX-4 is primarily expressed in hepatocytes, stellate cells, and endothelial cells (Reinehr et al., 2005). NOX-4 has been overexpressed in hepatitis C, and causes the formation of ROS, most via TGF-β induction (de Mochel et al., 2010). NOX-4 mediates TGF-β-induced hepatocyte apoptosis (Carmona-Cuenca et al., 2008). These studies have demonstrated that NOX-4

is an important proapoptotic and fibrogenic factor in the liver. More recently, Jiang et al. (2012) showed that NOX-4 is a key element in HSC activation, and liver fibrosis *in vivo*. However, in this study, we observed nearly no difference in the degree of NOX-4 expression between the fat and the bean leaf groups, indicating a need for further studies. PARPs [poly (ADP-ribose) polymerase genes] have been demonstrated to be important in many cellular processes, such as DNA replication, repair, and recombination, cell proliferation and death, gene transcription, telomere maintenance, inflammation, as well as in carcinogenesis (Piskunova et al., 2008). PARP-1 has been involved in the pathogenesis of inflammatory and neurodegenerative diseases and cancers (Piskunova et al., 2007; Hassa et al., 2008). An inactivation or inhibition of PARP-1 not only leads to acceleration of aging, shortened life span, and increased spontaneous carcinogenesis but also increases anticancer activation (Curtin et al., 2005). However, excessive activation of PARP depletes cellular NAD^+, which subsequently triggers ATP depletion and necrotic cell death (Ha et al., 1999). Furthermore, high PARP activity may promote DNA repair and genomic stability in normal cells as well as cancer cells, thus it can lead to resistance to DNA-damaging anticancer therapy. However, low PARP activity may lead to reduced proinflammatory mediators, tissue injury, necrosis and reperfusion injury (Zaremba et al., 2011). Therefore, our findings that the bean leaf group had a weaker PARP expression compared with the fat group may represent the useful effect of Korean bean leaves on the liver. Transforming growth factor beta (TGF-β) is an important suppressor factor in the adult liver, inhibiting hepatocyte DNA synthesis and inducing active cell death (Rossmanith et al., 2001). However, TGF-β overexpression is frequently observed in human hepatocellular carcinomas, suggesting that liver tumor cells, as with many other tumor cells, can overcome the suppressive effects of TGF-β (Breuhahn et al., 2006; Massagué J, 2008). Although TGF-β suppresses early stages of tumor development, it later contributes to tumor progression when cells become resistant to its suppressive effects. In addition to circumventing TGF-β-induced growth arrest and apoptosis, malignant tumor cells become capable of undergoing epithelial-to-mesenchymal transition (EMT), favoring invasion and metastasis (Franco et al., 2010). Moreover, once injured, hepatocytes undergo apoptosis. TGF-β, whose levels increase during the development of liver fibrosis, could be involved in hepatic stellate cell activation and collagen deposition (Matsuzaki et al., 2009). Thus, TGF-β inhibits growth and induces apoptosis of hepatocytes and also contributes to the activation of hepatic stellate cells (Sanchez et al., 1996; Proell et al., 2007). Therefore, in this study, it appears that a high fat diet may induce potentially degenerative and/or inflammatory damages on the liver, resulting in stronger TGF-β1 expression. Peroxisome proliferation-activated receptors (PPARs) are ligand-activated transcription factors and members of the nuclear hormone receptor super family, which regulate energy homeostasis (glucose and lipid metabolisms), inflammation, proliferation, and differentiation (Desvergne et al., 1999; Escher et al., 2000). Especially, PPARα acts as a master regulator of fatty acid oxidation by controlling the transcription of its target genes (Roepstorff et al., 2005). PPARα is mainly expressed in tissues with high lipid catabolic capacities, such as liver, skeletal muscle, and brown adipose tissues (Mandard et al., 2004). Previous studies have suggested that the activation of PPARα promotes fatty acid oxidation in the liver and decreases the levels of circulating and cellular lipids in obese diabetic patients (Goldenberg et al., 2008). In addition, PPARα seems to reduce inflammation, mainly through direct interaction with NF-κB, causing inhibition of its signaling pathway or decreasing the activated levels of NF-κB and subsequent inflammation (Vanden et al., 2003; Poynter et al., 1998). Unexpectedly, our results were discordant with previous studies, because PPARα expression was the strongest compared with the control and the bean leaf groups. However, other studies have demonstrated adverse effects of PPARα. Tordjmanet al. (2002) reported that PPARα-stimulated fatty acid oxidation can impair pancreas-β cell function, indicating that PPARα suppresses insulin secretion, leading to diabetes. Fink et al. (2002) demonstrated that "(a) PPARα is a critical regulator of myocardial fatty acid uptake and utilization, (b) activation (overexpression) of cardiac PPARα regulatory

pathways results in a reciprocal repression of glucose uptake and utilization pathways, and (c) derangements in myocardial energy metabolism typical of the diabetic heart can become maladaptive, leading to cardiomyopathy."

In addition, hepatic activation of the PPARα pathway provides a mechanism underlying aryl hydrocarbon receptor-mediated insulin resistance (Wang et al., 2011). These recent studies imply that PPARα overexpression results from the fat diet-induced fatty liver. Such literature supports our findings that PPARα overexpression may be harmful and may be ameliorated by a diet that includes bean-leaves diet. Soybean have been an important food for humans for over five thousand years. Since ancient times, soybean foods have been classified into two major types, the fermented (e.g. soy sauce, bean-paste, miso, and fermented tofu) and nonfermented (e.g. tofu, soymilk, soybean sprouts, soy flour, and various forms of soybean proteins). Intake of soybean has been increasing because of their useful effects on preventing certain cancers (Pollard et al., 2000), reducing the risk of osteoporosis in women (Arjmandi et al., 1998), lowering serum cholesterol levels (Ho et al., 2003), exerting antiatherosclerosis (Wilson et al., 1998), and decreasing the risk of coronary heart disease (Wilcox et al., 1995). However, we have littler data on the soybean leaf worldwide because it has only been consumed in the southern regions (e.g. Kyeong-Nam and Je-Ju Do) of Korea. Ho et al. (2003) reported that soy leaf powder in hamster experiments decreased but didn't affect on the triglyceride levels.

In summary, Korean bean-leaves may have useful effects in the areas of, anti-obesity, anti-inflammation, and liver protection. However, further studies should be performed to determine which useful elements come directly from the bean leaves.

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REFERENCES

Arjmandi BH, Getlinger MJ, Goyal NV, Alekel L, Hasler CM, Juma S, Drum ML, Hollis BW, Kukreja SC. Role of soy protein with normal or reducedisoflavone content in reversing bone loss induced by ovarian hormone deficiency in rats. Am J Clin Nutr. 1998. 68: 1358S-1363S.

Aschner PJ. The role for saxagliptin within the management of type 2 diabetes mellitus: an update from the 2010 European Association for the Study of Diabetes (EASD) 46th annual meeting and the American Diabetes Association (ADA) 70th scientific session. Diabetol Metab Syndr. 2010. 15: 2-69.

Barnes JL, Gorin Y. Myofibroblast differentiation during fibrosis: role of NAD(P)H oxidases. Kidney Int. 2011. 79: 944-956.

Breuhahn K, Longerche T, Schirmacher P. Dysregulation of growth factor signaling in human hepatocellular carcinoma. Oncogene. 2006. 25: 3787-3800.

Carmona-Cuenca I, Roncero C, Sancho P, Caja L, Fausto N, Fernández M, Fabregat I. Upregulation of the NADPH oxidase NOX4 by TGF-beta in hepatocytes is required for its pro-apoptotic activity. J Hepatol. 2008. 49: 965-976.

Choi SC, Seok SJ. The effect of fat diet on inflammatory markers and blood coagulation system in rats. J Exp Biomed Sci. 2012. 18: 237-243.

de Mochel NS, Seronello S, Wang SH, Ito C, Zheng JX, Liang TJ, Lambeth JD, Choi J. Hepatocyte NAD(P)H oxidases as an endogenous source of reactive oxygen species during hepatitis C virus infection. Hepatology. 2010. 52: 47-59.

Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev. 1999. 20: 649-688.

Escher P, Wahli W. Peroxisome proliferator-activated receptors: insight into multiple cellular functions. Mutat Res. 2000. 448: 121-138.

Franco DL, Mainez J, Vega S, Sancho P, Murillo MM, de Frutos CA, Del Castillo G, López-Blau C, Fabregat I, Nieto MA. Snail1 suppresses TGF-beta-induced apoptosis and is sufficient to trigger EMT in hepatocytes. J Cell Sci. 2010. 123: 3467-3477.

Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology. 2006. 43: S99–S112.

Goldstein LB, Adams R, Alberts MJ, Appel LJ, Brass LM, Bushnell CD, Culebras A, DeGraba TJ, Gorelick PB, Guyton JR, Hart RG, Howard G, Kelly-Hayes M, Nixon JV, Sacco RL. American Heart Association; American Stroke Association Stroke Council. Primary prevention of ischemic stroke: a guideline from the American Heart

Association/American Stroke Association Stroke Council: cosponsored by the Atherosclerotic Peripheral Vascular Disease Interdisciplinary Working Group; Cardiovascular Nursing Council; Clinical Cardiology Council; Nutrition, Physical Activity, and Metabolism Council; and the Quality of Care and Outcomes Research Interdisciplinary Working Group. Circulation. 2006. 24: e873-e923.

Fain JN, Kanu A, Bahouth SW, Cowan GS Jr, Hiler ML, Leffler CW. Comparison of PGE2, prostacyclin and leptin release by human adipocytes versus explants of adipose tissue in primary culture. Prostaglandins Leukot Essent Fatty Acids. 2002. 6: 467-473.

Finck BN, Lehman JJ, Leone TC, Welch MJ, Bennett MJ, Kovacs A, Han X, Gross RW, Kozak R, Lopaschuk GD, Kelly DP. The cardiac phenotype induced by PPARalpha overexpression mimics that caused by diabetes mellitus. J Clin Invest. 2002. 109: 121-130.

Gnacińska M, Małgorzewicz S, Stojek M, Lysiak-Szydlowska W, Sworczak K. Role of adipokines in complicationsrelated to obesity. Adv Med Sci. 2009. 2: 150-157.

Goldenberg I, Benderly M, Goldbourt U. Update on the use of fibrates: focus on bezafibrate. Vasc Health Risk Manag. 2008. 4: 131-141.

Grundy SM, Cleeman JI, Merz CN, Brewer HB Jr, Clark LT, Hunninghake DB, Pasternak RC, Smith SC Jr, Stone NJ. Coordinating Committee of the National Cholesterol Education Program. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. J Am Coll Cardiol. 2004. 3: 720-732.

Hassa PO, Hottiger MO. The diverse biological roles of mammalian PARPS, a small but powerful family of poly-ADP-ribose polymerases. Front Biosci. 2008. 13: 3046-3082.

Curtin NJ. PARP inhibitors for cancer therapy. Expert Rev Mol Med. 2005. 7: 1-20.

Ha HC, Snyder SH. Poly (ADP-ribose) polymerase is a mediator of necrotic cell death by ATP depletion. Proc Natl Acad Sci. 1999. 96: 13978-13982.

Hecker L, Vittal R, Jones T, Jagirdar R, Luckhardt TR, Horowitz JC, Pennathur S, Artinez FJ, Thannickal VJ. NADPH oxidase-4 mediates myofibroblast activation and fibrogenic responses to lung injury. Nat Med. 2009. 5: 1077-1081.

Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science. 1993. 259: 87-91.

Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest. 1995. 5: 2409-2415.

Ho HM, Leung LK, Chan FL, Huang Y, Chen ZY. Soy leaf lowers the ratio of non-HDL to HDL cholesterol in hamsters. J Agric Food Chem. 2003. 51: 4554-4558.

Jiang JX, Chen X, Serizawa N, Szyndralewiez C, Page P, Schröder K, Brandes RP, Devaraj S, Török NJ. Liver fibrosis and hepatocyte apoptosis are attenuated by GKT137831, a novel NOX4/NOX1 inhibitor in vivo. Free Radic Biol Med. 2012. 53: 289-296.

Kahn HS. The "lipid accumulation product" performs better than the body mass index for recognizing cardiovascular risk: a population-based comparison. BMC Cardiovasc Disord. 2005. 8: 5-26.

Masterjohn C, Bruno RS. Therapeutic potential of green tea in nonalcoholic fatty liver disease. Nutr Rev. 2012. 1: 41-56.

Massagué J. TGF-beta in cancer. Cell. 2008. 134: 215-230.

Matsuzaki K. Modulation of TGF-beta signaling during progression of chronic liver diseases. Front Biosci. 2009. 14: 2923–2934.

Mandard S, Müller M, Kersten S. Peroxisome proliferator-activated receptor alpha target genes. Cell Mol Life Sci. 2004. 61: 393-416.

Niu YH, Yi RT, Liu HL, Chen TY, Zhang SL, Zhao YR. Expression of COX-2 and PPARg in the livers of patients with acute on chronic HBV-related liver failure and their relationship with clinic parameters. Zhonghua Gan Zang Bing ZaZhi. 2011. 7: 511-516.

Núñez Martínez O, Clemente Ricote G, García Monzón C. Role of cyclooxygenase-2 in the pathogenesis of chronic liver diseases. Med Clin (Barc) 2003. 121: 743-748.

Ozaki T, Habara K, Matsui K, Kaibori M, Kwon AH, Ito S, Nishizawa M, Okumura T. Dexamethasone inhibits the induction of iNOS gene expression through destabilization of its mRNA in proinflammatory cytokine-stimulated hepatocytes. Shock. 2010. 33: 64-69.

Piskunova TS, Yurova MN, Ovsyannikov AI, Semenchenko AV, Zabezhinski MA, Popovich IG, Wang ZQ, Anisimov VN. Deficiency in Poly (ADP-ribose) Polymerase-1 (PARP-1) Accelerates Aging and Spontaneous Carcinogenesis in Mice. Curr Gerontol Geriatr Res. 2008. 7541-7590.

Piskunova TS, Iurova MN, Zabezhinskiĭ MA, Anisimov VN. Poly (ADP-ribosa) polymerase-the relationships with life span and carcinogenesis. Adv Gerontol. 2007. 20: 82-90.

Pollard M, Wolter W, Sun L. Prevention of induced prostate-related cancer by soy protein isolate/isoflavone-supplemented diet in Lobund-Wistar rats. In Vivo. 2000. 14: 389-392.

Poynter ME, Daynes RA. Peroxisome proliferator-activated receptor alpha activation modulates cellular redox status, represses nuclear factor-kappaB signaling, and reduces inflammatory cytokine production in aging. J Biol Chem. 1998. 273: 32833-32841.

Proell V, Carmona-Cuenca I, Murillo MM, Huber H, Fabregat I. TGF-beta dependentregulation of oxygen radicals during transdifferentiation of activated hepaticstellate cells to myofibroblastoid cells. Comp Hepatol. 2007. 6: 1-10.

Raisz LG. Physiology and pathophysiology of bone remodeling. Clin Chem. 1999. 8: 1353-1368.

Reinehr R, Becker S, Eberle A, Grether-Beck S, Häussinger D. Involvement of NADPH oxidase isoforms and Src family kinases in CD95-dependent hepatocyte apoptosis. J Biol Chem. 2005. 29: 179-194.

Rossmanith W, Schulte-Hermann R. Biology of transforming growth factor beta in hepatocarcinogenesis. Microsc Res Tech. 2001. 52: 430-436.

Roepstorff G, Halberg N, Hillig T, Saha AK, Ruderman NB. Malonyl-CoA and carnitine in regulation of fat oxidation in human skeletal muscle during exercise. Am J Physiol Endoclinol Metab. 2005. 288: E133-E142.

Sanchez A, Alvarez AM, Benito M, Fabregat I. Apoptosis induced by transforming growth factor-beta in fetal hepatocyte primary cultures: involvement of reactive oxygen intermediates. J Biol Chem. 1996. 271: 7416-7422.

Simmons DL, Botting RM, Hla T. Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. Pharmacol Rev. 2004. 3: 387-437.

Sui Y, Zhao HL, Wong VC, Brown N, Li XL, Kwan AK, Hui HL, Ziea ET, Chan JC. A systematic review on use of Chinesemedicine and acupuncture for treatment of obesity. Obes Rev. 2012. 5: 409-430.

Tilley SL, Coffman TM, Koller BH. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. J Clin Invest. 2001. 1: 15-23.

Torres-Rovira L, Astiz S, Caro A, Lopez-Bote C, Ovilo C, Pallares P, Perez-Solana ML, Sanchez-Sanchez R, Gonzalez -Bulnes A. Diet-induced swine model with obesity/leptin resistance for the study of metabolic syndrome and type 2 diabetes. Scientific World Journal. 2012. 21: 101-149.

Torres DM, Harrison SA. Diagnosis and therapy of nonalcoholicsteatohepatitis. Gastroenterology. 2008. 134: 1682-1698.

Tordjman K, Standley KN, Bernal-Mizrachi C, Leone TC, Coleman T, Kelly DP, Semenkovich CF. PPARalpha suppresses insulin secretion and induces UCP2 in insulinoma cells. Environ Health Perspect. J Lipid Res. 2002. 6: 936-944.

Travis WD, Garg K, Franklin WA, Wistuba II, Sabloff B, Noguchi M, Kakinuma R, Zakowski M, Ginsberg M, Padera R, Jacobson F, Johnson BE, Hirsch F, Brambilla E, Flieder DB, Geisinger KR, Thunnissen F, Kerr K, Yankelevitz D, Franks TJ, Galvin JR, Henderson DW, Nicholson AG, Hasleton PS, Roggli V, Tsao MS, Cappuzzo F, Vazquez MJ. VandenBerghe W, Vermeulen L, Delerive P, De Bosscher K, Staels B, Haegeman G. A paradigm for gene regulation: inflammation, NF-kappaB and PPAR. Adv Exp Med Biol. 2003. 544: 181-196.

Wang C, Xu CX, Krager SL, Bottum KM, Liao DF, Tischkau SA. Aryl hydrocarbon receptor deficiency enhances insulin sensitivity and reduces PPAR-α pathway activity in mice. Environ Health Perspect. 2011. 12: 1739-1744.

Wilcox JN, Blumenthal BF. Thrombotic mechanisms in atherosclerosis: potential impact of soy proteins. J Nutr. 1995. 125: 631S-638S.

Wilson TA, Meservey CM, Nicolosi RJ. Soy lecithin reduces plasma lipoprotein cholesterol and early atherogenesis in hypercholesterolemic monkeys and hamsters: beyond linoleate. Atherosclerosis. 1998. 140: 147-153.

Wu T. Cyclooxygenase-2 and prostaglandin signaling in cholangiocarcinoma. Biophys Acta. 2005. 1755: 135-150.

Xu H, Uysal KT, Becherer JD, Arner P, Hotamisligil GS. Altered tumor necrosis factor-alpha (TNF-alpha) processing in adipocytes and increased expression of transmembrane TNF-alpha in obesity. Diabetes. 2002. 6: 1876-1883.

Zang Y, Sato H, Igarashi K. Anti-diabetic effects of a kaempferol glycoside-rich fraction from unripe soybean (Edamame, Glycine max L. Merrill. 'Jindai') leaves on KK-A(y) mice. Biosci Biotechnol Biochem. 2011. 75: 1677-1684.

Zhao HL, Sui Y, Qiao CF, Yip KY, Leung RK, Tsui SK, Lee

HM, Wong HK, Zhu X, Siu JJ, He L, Guan J, Liu LZ, Xu HX, Tong PC, Chan JC. Sustained antidiabetic effects of a berberine-containing Chinese herbal medicine through regulation of hepatic gene expression. Diabetes. 2012. 4: 933-943.

Zaremba T, Thomas HD, Cole M, Coulthard SA, Plummer ER, Curtin NJ. Poly (ADP-ribose) polymerase-1 (PARP-1) pharmacogenetics, activity and expression analysis in cancer patients and healthy volunteers. Biochem. 2011. 436: 671-679.