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Effects of green tea catechins on nonalcoholic steatohepatitis (NASH) patients

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ABSTRACT

Nonalcoholic steatohepatitis (NASH) is a form of chronic progressive liver disease wherein patients appear to have a higher likelihood of progression to cirrhosis. The present study evaluates the effects of green tea catechin treatment on NASH patients. Thirty-eight NASH patients (22 male, 16 female, mean age: 50 ± 16 years, mean body mass index [BMI]; 29.9 ± 5.0 kg/m^2, ±s.d.) with governed dietary and physical exercise participated in this case study. Subjects were treated using an alimentotherapy and an exercise therapy. Twenty six patients (13 male; 13 female) took 600 mg of green tea catechins per day for 6 months, while 12 patients were assigned to the control (placebo) group. Blood aliquots were checked at baseline and during 6 month trial for various physiochemical parameters estimation. An accumulation of visceral fat and subcutaneous fat were also measured along with the liver-to-spleen attenuation ratio using computed tomography (CT) at baseline and after 6 month trial. Green tea catechins consumption showed a significant decrease in BMI with lower serum lipid profiles and blood sugar levels. The alanine transaminase (ALT) and aspartate transaminase (AST) levels remained under the limits for most of the NASH patients of catechin treatment group, whereas an increase was systematically registered in for NASH patients in placebo group. Further, lowered high sensitive C-reactive protein (hs-CRP) level as well as improved visceral fat to subcutaneous fat (V/S) area ratio and liver to spleen (L/S) ratios suggest that green tea catechins along with controlled diets and exercise therapy can improve anthropometric parameters and therefore help to prevent the progression of NASH through their antioxidant and anti-inflammatory properties by reducing oxidative stress in the NASH patients.

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1. Introduction

Ludwig, Viggiano, McGill, and Oh (1980) introduced the term nonalcoholic steatohepatitis (NASH) to address a form of liver disease having similar symptoms of alcoholic steatohepatitis (ASH) even in individuals without any history of alcohol consumption. In 2003, the American Association for the Study of Liver Diseases (AASLD) outlined the guidelines for NASH and related histopathologic abnormalities (Neuschwander-Tetri & Caldwell, 2003). NASH was defined as the presence of concomitant necroinflammatory changes that may progress to cirrhosis and fibrosis, wherein fat infiltration of the liver dysfunction accompanies macrovesicular steatosis, hepatocellular carcinoma, lobular inflammation, balloon degeneration of hepatocytes, and zone 3 pericellular fibrosis (Brunt, 2005; Chitturi & Farrell, 2001). The prevalence of NASH in the American general population is estimated to be in the range of 6–17%. In the Asia-Oceania region (Hashimoto et al., 2009; Lirussi, Azzalini, Orando, Orlando, & Angelico, 2007) the prevalence of nonalcoholic fatty liver disease (NAFLD) is increasing and is estimated to be between 10% and 30%, however, the estimated prevalence of biopsy proven NASH among healthy Japanese adults ranges only from 1% to 3%, therefore liver biopsy is required to distinguish NASH from NAFLD because both share common clinical and pathological features (Matteoni et al., 1999). A role of lipid peroxidation in NASH has been widely suggested in both animal models and humans with steatosis of different etiologies (Cortez-Pinto et al., 1996; Day & James, 1998). Also, a growing body of evidence supports a logical role of lipid peroxidation in the pathogenesis of alcohol induced hepatitis as well as fibrosis (Day, 1996). Thus, oxidative stress could play an important role in the pathogenesis of NASH. Reactive oxygen species (ROS) resulting from alcohol metabolism are thought to be involved in oxidative stress generation that can initiate primary hepatocellular injury and activate inflammatory cytokines that progresses hepatitis and NASH (Baskol, Baskol, & Kocer, 2007; Day & James, 1998; Machado et al., 2008). Since, most NASH patients have insulin resistance (Pagano et al., 2002; Seppala-lindroos et al., 2002), insulin resistance may be an important parameter in the accumulation of hepatocellular fat, whereas adenosine triphosphate (ATP) depletion, excess intracellular fatty acid, mitochondrial dysfunction along with aforementioned oxidant stress could be another important cause of hepatocellular injury in the steatotic liver. NASH has also been suggested to be associated with diabetes, obesity and hyperlipidemia since insulin resistance is a key feature of obesity and type 2 diabetes. Thus, the onsets of NASH are closely related to lifestyle, particularly eating habits and lack of exercise, and it would be expected that weight loss should be therapeutic as the first line management. In addition to the earlier associated medical disorders, genetic factors, age and gender, iron storage disorders, and oxidative stress cannot be ignored for the development of NASH (Angulo, Keach, Batts, & Lindor, 1999; George et al., 1998; Sturben, Hespenheide, & Caldwell, 2000).

There is no worldwide consensus on the treatment of NASH as natural history and progression of NASH is not well characterized (Vuppalanchi & Chalasani, 2009; Vuppalanchi et al., 2012). Suzuki et al. (2005) evaluated hyaluronic acid as a biomarker predictor of fibrosis in 79 patients, while Degertekin, Ozenirler, Elbeg, and Akyol (2007) evaluated 40 patients biopsy proven NASH using endothelin-1 as predictive biomarker. Pagano et al. (2002) reported the presence of metabolic syndrome in primary NASH and assessed the role of liver diseases in the genesis of peripheral hyperinsulinemia. Marchesini et al. (2001) revealed that fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum fatty acids independent of obesity in normal weight and moderately overweight subjects. Baskol et al. (2007) studied the oxidative stress and antioxidant defense in serum of NASH patients and revealed that effective antioxidant therapy is highly necessary to inhibit oxidative stress. Cortez-Pinto and Machado (2008) employed blood oxidative stress markers in NASH patients and evaluated their redox states and correlated to dietary intake of fiber, carbohydrates, vitamins and fatty acids. Knobler et al. (1999) studied 48 patients who underwent dietary restriction.

Although several drugs (antiviral agents, non-steroidal anti-inflammatory drug such as aspirin, antiepileptic sodium valproate and antibiotics) are associated with the development of steatosis, only one class of drugs (cationic amphiphilic amines) is associated with classical steatohepatitis. Concerning pharmaceutical interventions, various therapeutic agents widely studied in the setting of NASH are metformin, ursodeoxycholic acid, gemfibrozil, thiazolidinediones, and lofibrate wherein metaformin was shown to be a relatively safe pharmaceutical intervention to improve liver histology (Ratziu et al., 2006). Furthermore, much focus has also been directed to the role of antioxidants such as vitamin E (Sanyal et al., 2010) in NASH therapy, however, at present there is insufficient information to either support or refute the use of antioxidant supplements for NASH patients. Therefore, it is necessary to perform randomized/controlled trials to verify the effects of antioxidants on NASH treatments.

Lifestyle intervention including improvement of eating habits are commonly recommended for NASH. Since, NASH is mainly a manifestation of metabolic syndrome and obesity, and is characterized by excess calorie consumption and lack of optimal health related physical activity (Krasnoff, Painter, Wallace, Bass, & Merriman, 2008), green tea supplementation could be an ideal therapeutic agent for the treatment of NASH as it has anti-mutagenic, hypolipidemic, antibacterial and antioxidative features (Kanematsu, Hara, & Kada, 1980; Okubo, Ishihara, Oura, Serit, & Kim, 1992). Nakagawa et al. (1999) reported the effect of green tea catechin supplementation on antioxidant capacity of plasma in humans by estimating plasma phosphatidylcholine hydroperoxide level as a marker of oxidized lipoproteins. Basu et al. (2010) suggested the role of green tea catechins in improving body weight, lipids and lipid peroxidation in obese subjects with metabolic syndrome.

Zhang et al. (2012) reported the effects of catechin-enriched green tea beverage on visceral fat loss in adults with a high proportion of visceral fat. Furthermore, green tea catechins *in vivo* effects on antioxidation, intestinal bacterial flora, cancer prevention, cardiovascular health renal disease and diabetes are well documented in a number of clinical studies (Weisburger, 2003; Yamane, 1996). Thus, it is highly likely that the risk factors of hepatitis could be reduced by using green tea catechins in human therapy.

In the present study, the control of NASH parameters through a restricted diet along with exercise therapy is recommended. Based on the epidemiological evidences that consumption of green tea catechins is associated with major parameters associated with NASH (lower circulating level of aminotransferases, triacylglycerols, and atherogenic lipoproteins), we have suggested to test the hepatoprotective effect of green tea catechins in human NASH. Also, it is generally believed that weight loss is beneficial for patients with NASH, even though the precise hepatic and extrahepatic benefits of weight loss are not well understood. Since, there exists no report on randomized, controlled clinical trials of the effect of green tea catechins on NASH patient treatments to date, we performed a controlled trial to examine the combined therapeutic effects of a restricted diet and exercise therapy on NASH patients. The study is especially designed to determine if long term ingestion of green tea catechins could provide any useful therapeutic effect to NASH patients prior to recommending green tea catechins as a useful prophylactic against NASH in humans. The trial also compared to NASH patients without green tea catechins treatment. Experimental evidences support a role of green tea catechins in protecting against NASH by regulating energy homeostasis and decreasing oxidative stress and inflammatory responses. The results of improved blood chemical data are reported with significant levels of the proposed therapy. Further, the effects of calorie restriction combined with exercise to treat NASH with green tea catechins are evaluated and discussed.

Table 1 – Baseline characteristics comparison of green tea catechins treatment group and control (placebo) group of NASH patients.

	Catechins treatment group (*n* = 26)	Control (placebo) group (*n* = 12)
Gender ratio (male/female)	13/13	10/2
Age (years)	53.9 ± 15.2	48.4 ± 15.8
ChE (IU/L)	377.8 ± 75.0	331.2 ± 122.5
Hyaluronic acid (mg/dL)	45.3 ± 59.7	58.2 ± 78.9
Ferritin (ng/mL)	239.2 ± 193.5	398.8 ± 545.9
HbA1c (%)	5.9 ± 0.7	7.0 ± 2.2
BW (kg)	78.1 ± 13.2	84.6 ± 9.4
BMI	30.3 ± 4.7	30.2 ± 2.9
W (cm)	93.6 ± 12.7	88.3 ± 6.1
TG (mg/dL)	167 ± 70	182 ± 62
TC (mg/dL)	206 ± 32	201 ± 27
HDL-C (mg/dL)	52 ± 17	50 ± 15
LDL-C (mg/dL)	132 ± 31	115 ± 33
AI	2.6 ± 0.9	3.0 ± 0.7
FPG (mg/dL)	124 ± 39	126 ± 41
IRI (μU/mL)	42.1 ± 47.8	34.7 ± 31.6
HOMA-IR	13.2 ± 16.9	10.8 ± 9.4
GA (%)	17.1 ± 2.7	18.4 ± 5.2
AST (IU/L)	45 ± 21	54 ± 26
ALT (IU/L)	55 ± 33	83 ± 49
γ-GTP (IU/L)	58 ± 40	127 ± 81
Type IV collagen (ng/mL)	141 ± 29	152 ± 60

ChE, cholinesterase; HbA1c, hemoglobin A1c; BW, body weight; BMI, body mass index; W, waist circumference; TG, triacylglycerol; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; AI, arteriosclerosis index; FPG, fasting plasma glucose; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment-insulin resistance; GA, glycoalbumin; AST, aspartate transaminase; ALT, alanine transaminase; γ-GTP, γ-glutamyl transpeptidase.
Data are given as mean ± s.d. (s.d = standard deviation).

2. Materials and methods

2.1. Subjects

Subjects were diagnosed as having NASH by a liver biopsy as well as recognized by its characteristic symptoms. Subjects who were outpatients and agreed to participate in this study were placed under treatment of an alimentotherapy and an exercise therapy at Aichi Medical University, Japan. Thirty eight subjects, which included twenty two {22} males and sixteen {16} females (mean age 50 ± 16 years) with a mean body mass index (BMI) of 29.9 ± 5.0 kg/m^2 participated in the study. Subjects were selected to the following criteria: history of alcohol drinking (for men less than 20 g/day, and for women less than 10 g/day), and confirmed steatohepatitis by liver biopsy diagnosis. Major exclusion criteria were the evidences of other forms of liver diseases such as virus hepatitis, metabolic liver disease, autoimmune liver disease, cirrhosis, and alcoholic fatty liver diseases. Thus, only NASH patients were selected. Other excluded patients were those who had a previous history of hypertension before and after cardiac failure, serious ketosis, diabetic coma, type I diabetes, serious renal dysfunction, severe infection, autoimmune liver disease, possibility of pregnancy and after operation patients. Twenty six NASH patients (13 male and 13 female) were assigned to green tea catechin treatment group whereas, 12 NASH patients (10 male and 2 female) not receiving green tea catechin treatment were assigned to the control (placebo) group. Background history of patients and their blood profiles are shown in Table 1. The study protocol was approved by the Institutional Review Board of Aichi Medical University. The purpose, nature and potential risks of the study were explained to the patients and all patients provided a written informed consent prior to entering in the study.

2.2. Study design

NASH patients who fulfilled all inclusion criteria and agreed for catechin intake were assigned to the catechin treatment group. The control (placebo) group, consisted of those patients who fulfilled all inclusion criteria, but did not consume catechin. Catechin treatment group was given six catechin tablets per day (two tablets after each meal; three times a day). Total catechin consumption was 600 mg (100 mg per tablet) and treatment continued for 6 months together with dietary and exercise therapy. On the other hand, the patients in placebo (control) were subjected to similar dietary and exercise therapy. NASH patients of both group underwent follow-up examinations at baseline and after 3 and 6 months of the study period. The aforementioned dose of 600 mg catechin per day for the NASH patients of the treatment group was set on basis of tested compliance to the intervention wherein the given dose demonstrated a significant effect on the level of catechin in the subjects.

Table 2 – Test tablet composition and catechins concentration.

Components	Dose composition (mg/per tablet; 200 mg)
Cellulose	44.1
Reduced maltose sugar	20.0
Glycerin fatty acid ester	6.0
Soybean polysaccharide	4.0
Caffeine	1.1
Green tea extract (GTE)	125.0[a]
Catechins content in GTE	(%)
(–)-Gallocatechin	2.2
(+)-Catechin	0.9
(–)-Epigallocatechin	2.8
(–)-Epigallocatechin gallate	52.6
(–)-Epicatechin	4.6
(–)-Gallocatechin gallate	1.9
(–)-Epicatechin gallate	14.9
Total catechins content	79.9

Weight per tablet 200 mg.
[a] Catechin content per tablet ~100 mg.

The NASH patients of both groups were given the similar exercise regime and dietary therapy. Since the control (placebo) group constitutes uneven distribution of males and females, therefore identical exercise regime was offered to male and female participants to avoid the influence of greater compliance of exercise regime in the male on the results. Also, the exercise regime focused on moderate intensity activities, with particular emphasis on walking. NASH patients of both groups were asked to perform a walking exercise using pedometers and encouraged to walk about 7000 steps every time for three times every week, i.e. on every alternate day of the trial period. Participants were subjected to consultation with registered dietician to follow up with given dietary advice for adequate dietary regime during the trial period. All participants were assigned a calorie goal based on their starting weight as per recommendation of globally accepted regimes. In addition, the participants were also taught the basic principles of healthy eating and proper exercise regimes.

Green tea catechin tablets used in this study were a commercial product of Taiyo Kagaku Co., Ltd. Japan under the brand name of 'Sunphenon®'. Sunphenon is well known for its complete safety data including acute and sub-acute toxicities. Each tablet contained green tea extract (catechins), cellulose, reduced maltose syrup, glycerin fatty acid esters and soybean polysaccharide. It is worth mentioning that the catechin tablets used in this study contained very low amounts of caffeine. Thus, a caffeine effect on this study could not be assumed. A detailed composition and the dosage of catechins used in the tablets are presented in Table 2.

2.3. *Analytical measurements*

Body weight (BW), waist circumference (W), biochemical analysis data on fasting plasma glucose (FPG), immunoreactive insulin (IRI), glycoalbumin (GA), triacylglycerols (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), type IV collagen, and high-sensitive C-reactive protein (hs-CRP) were analyzed for each subject that participated in this study.

Body mass index (BMI; kg/m^2) was calculated from body weight and the height of each individual. The homeostasis model assessment, as an index of insulin resistance (HOMA-IR), was calculated as FPG level (mg/dL) multiplied by IRI level (mg/mL)/405. The arteriosclerosis index (AI) was obtained by (TC – HDL-C)/HDL-C.

2.4. *Body fat distribution (determined by CT)*

NASH patients of both groups were subjected to quantitative abdominal CT at umbilical level to estimate the cross-sectional abdominal subcutaneous fat area (S) as well as visceral fat area (V) prior to and during the 3 and 6 months of the trial period The V/S ratio was then determined. Abdominal CT was also used to determine the size of each patient's liver and spleen. Liver attenuation and spleen attenuation measured at different sites correspond to different hepatic segments and were further used to calculate the ratio of the CT attenuation value of the liver to that of the spleen (L/S ratio) as a measure for quantitative estimation of the hepatic contents and compared before and after green tea consumption for both groups, wherein an L/S ratio <1 was considered as a representation of fatty liver condition (Ricci et al. (1997).

2.5. *Statistical analysis*

For all measures, descriptive statistics were calculated and graphs drawn to look for outliers and all results were analyzed for their significance. Values reported were expressed as mean ± standard deviation. Association between the both NASH patients groups at baseline and at fixed intervals during the trial period was assessed as *between subject group*, and association within each group were compared using analysis of variance (ANOVA). Data were analyzed by ANOVA with time as a repeated measure and treatment as a fixed factor. The significances were established at the 95% confidence level ($P < 0.05$) for all comparisons without adjusting the significance levels for testing hypothesis. Abdominal CT findings including the L/S ratios and V/S ratios were compared between patients with NASH using the aforementioned significance levels obtained from ANOVA. Also, an independent two-tailed student *t*-test was performed to verify the statistical significance.

3. Results

3.1. *Baseline characteristics and anthropometrics*

Thirty eight NASH patients completed the screening evaluation and underwent the study (Fig. 1). The baseline parameters and anthropometric laboratory determinations of NASH patients in both green tea catechin treatment group ($n = 26$; 13m and 13f) and control (placebo) group ($n = 12$, 10m and 2f) are listed in Table 2. At the baseline, the comparable parameters were observed for patients in both groups, therefore statisti-

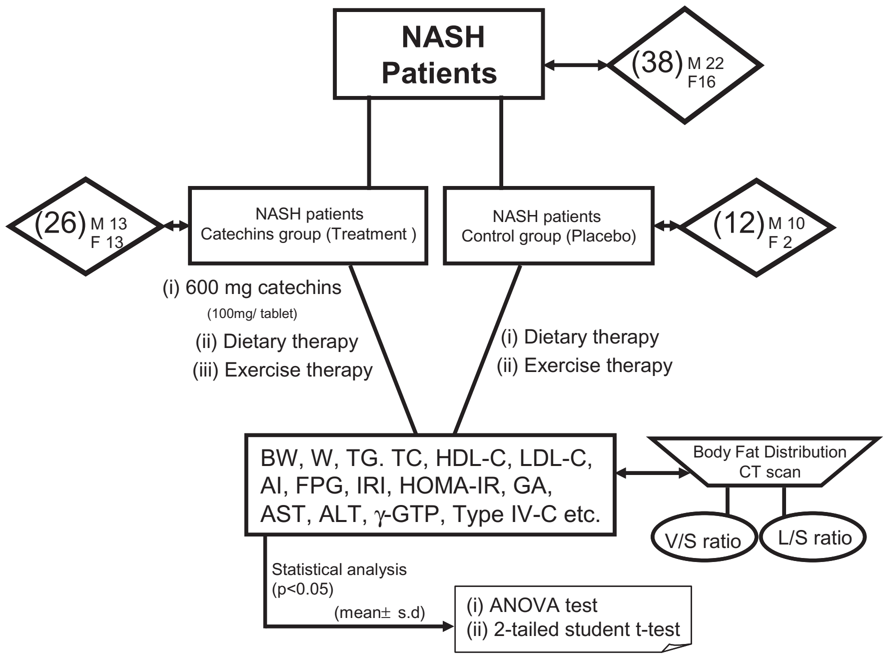

Fig. 1 – Enrollment of trial participants of NASH patients and description of clinical parameters for its significance evaluation.

cal significance levels were not determined as no particular parameter might differ at this stage. Further, a significant decrease ($P < 0.05$) in the anthropometric measurements including body weight, BMI, and waist circumference was observed with green tea catechin treatment group of NASH patients after treatment of 3 and 6 months, compared to NASH patients of control (placebo) group, who received only dietary and exercise therapy (see Table 3). BW changed about 2.7% from the baseline after 3 months and the difference was further increased to 4.3% after 6 months of treatment. Similarly, the changes in BMI were 3.4% and 5.1% and changes in waist circumference were 1.7% and 2.5% after 3 and 6 months of treatment, respectively. These results support the findings related to effectiveness of green tea catechins and demonstrate the compliance to the intervention dose in reducing body fat and its anti-obesity effect. However, NASH patients of control (placebo) group showed an improvement in waist circumference only after 6 months ($P = 0.03$), and this was mainly attributable to the dietary and exercise therapy regimes. Otherwise, there was no level of significance in the percentage changes of BW and BMI.

3.2. *Serum lipid estimation (blood biochemical studies)*

Considerable changes were observed in the serum lipid profiles of the NASH patients within both groups; the results are shown in Table 4. The concentration of TG in the blood that usually provides energy for different metabolic processes was effectively controlled among the catechin treatment group, whereas the TG level was higher for the control (placebo) group even after 6 months of dietary and exercise therapy. An increase in HDL-C level was observed in catechin treatment group, which is a good indicator of the improvement in NASH patients because its high level seems to be associated with low incidence of coronary heart diseases. While at the same time, HDL-C level was somewhat reduced among the control (placebo) group of NASH patients. On the other hand, the LDL-C level

Table 3 – Anthropometric results of green tea catechins treatment group and control (placebo) group of NASH patients.

		BW (kg)	BMI (kg/m^2)	W (cm)
Catechins treatment group	At baseline	78.1 ± 13.2	30.3 ± 4.7	93.6 ± 12.7
	After 3 months	75.6 ± 13.2	29.3 ± 4.5	91.8 ± 12.7
	After 6 months	74.4 ± 13.0	28.8 ± 4.4	90.7 ± 13.0
	[a]*P* value (*ANOVA*)	(<0.01)	(<0.01)	(<0.01)
Control (placebo) group	At baseline	84.6 ± 9.4	30.2 ± 2.9	88.3 ± 6.1
	After 3 months	84.1 ± 10.2	30.1 ± 3.3	88.3 ± 6.3
	After 6 months	84.9 ± 10.3	30.4 ± 3.1	89.1 ± 6.1
	[a]*P* value (*ANOVA*)	(0.35)	(0.39)	(0.03)

[a] *P* value (after 6 months).

Table 4 – Changes in serum lipids concentrations in green tea catechins treatment group and control (placebo) group of NASH patients.

		TG (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	AI
Catechins treatment group	At baseline	167 ± 70	206 ± 32	52 ± 17	132 ± 31	2.6 ± 0.9
	after 3 months	139 ± 46	197 ± 31	54 ± 13	127 ± 29	2.4 ± 0.9
	after 6 months	170 ± 97	206 ± 36	56 ± 16	123 ± 31	2.6 ± 1.0
	(ANOVA) [a]P value	(0.018)[b]	(0.027)[b]	(0.011)	(0.219)[b]	(0.045)[b]
	F value	2.51	3.85	3.95	1.56	3.29
Control (placebo) group	At baseline	182 ± 62	201 ± 27	50 ± 15	115 ± 33	3.0 ± 0.7
	after 3 months	197 ± 48	211 ± 41	46 ± 8	126 ± 38	3.5 ± 0.7
	after 6 months	249 ± 111	224 ± 39	46 ± 9	129 ± 45	3.8 ± 0.8
	(ANOVA) [a]P value	(0.006)	(0.092)	(0.21)	(0.196)	(0.016)
	F value	4.81	2.31	1.55	1.87	3.92

Abbreviations key as illustrated in Table 1.
Data are given as mean ± s.d. (s.d = standard deviation).
[a] P value was significantly different from value before treatment in both green tea catechins treatment group and control (placebo) group (after 6 months).
[b] After 3 months.

decreased among green tea catechin treatment NASH patients, whereas an increasing trend was observed for the control (placebo) group of NASH patients, as LDL-C considered to be bad cholesterol whose levels of less than 130 are normally recommended. The TC of catechin treatment group could be maintained very close to its recommended level of 200 throughout the treatment duration, while TC was found to be slightly increased among the control (placebo) NASH patients. The arteriosclerosis index (AI) calculated using the total cholesterol level and HDL-C level is actually a measure of hardening of the arteries due to plugging by cholesterol plaque, was very well maintained during the green tea catechin treatment among the catechin treatment group. Whereas, AI level was significantly higher among the control (placebo) patients (see Table 4) which might indicate a potential risk of atherosclerosis development by accumulation of cells containing excess lipids within the arterial wall that are closely related to NASH development.

3.3. *Assessment of hepatic parameters*

Several markers related to inflammatory and dominant characteristics of NASH such as AST, ALT, γ-GTP, type IV collagen and hs-CRP were determined. Although most of them have fairly low specificities and sensitivities (Conigrave et al., 2002), their combination could provide significant information compared with single serum enzyme estimations. The test results of the aforementioned markers which are helpful to determine the liver function are illustrated in Fig. 2. As is evident from Fig. 2, catechin treatment group had little change in levels of AST and ALT from baseline, however there was a progressive rise in AST and ALT levels for the control (placebo) group NASH patients. While, in catechin treatment group NASH patients both AST and ALT levels remained constant throughout the green tea catechin intake period. It is worth mentioning that almost 70% of the NASH patients of catechin treatment group remained under the upper limit of AST (~40 IU/L) during the trial period, while all of control (placebo) group of NASH patients were above the upper limit of AST throughout the trial. On the other hand, nearly 62% of the catechin treatment group NASH patients were under the upper limit of ALT (~56 IU/L) while only 16% of the control (placebo) group of NASH patients maintained ALT levels under the limit. A significant positive ANOVA correlation ($P < 0.01$) was found between green tea catechin intake versus the control (placebo) groups, while no similar correlation was observed in the AST or ALT level in the individual group of the NASH patients. This could be attributed to an anti-inflammatory effect of the green tea catechins, which in turn helps in controlling the progression of NASH related symptoms. Further, the control (placebo) group of NASH patients had higher γ-GTP levels than the catechin treatment group of NASH patients, and remained higher throughout the trial. On the other hand, the levels of γ-GTP in the catechin treatment group of NASH pa-

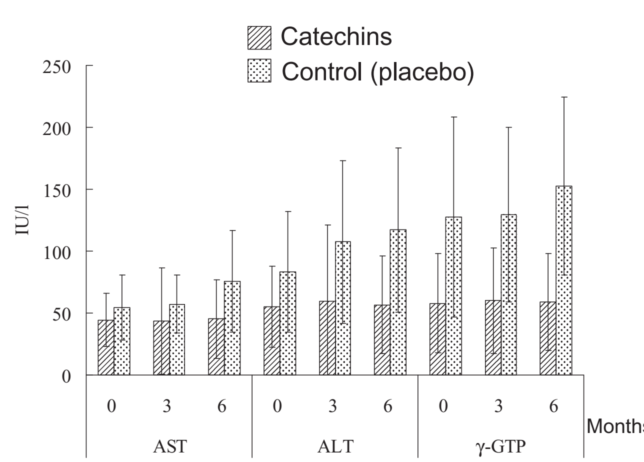

Fig. 2 – Changes in serum activities of enzymes AST and ALT, γ-GTP during trial period in green tea catechins treatment group and control (placebo) group of NASH patients. Data are given as mean ± standard deviation. $P < 0.01$ was significantly different from value at baseline in green tea catechins treatment group.

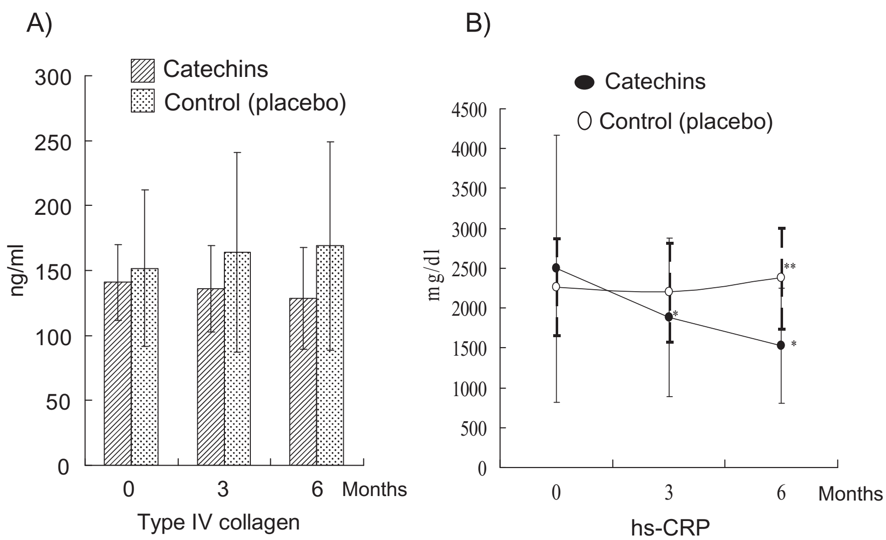

Fig. 3 – Variation in serum level concentrations of (A) type IV collagen, and (B) hs-CRP during trial period in both green tea catechins treatment group and control (placebo) group NASH patients. Data are given as mean ± standard deviation. *P < 0.05 was significantly different from value before treatment in green tea catechins group NASH patients. **P < 0.05 was significantly different from value before treatment in control (placebo) group NASH patients.

tients could be well controlled during green tea catechin treatment period. Additional information could be extracted from the AST/ALT ratio, wherein the value of <1 is suggestive for non-alcoholic liver disease. A significance ANOVA correlation for AST/ALT ratio was observed among the catechin treatment group NASH patients ($P = 0.032$; $F = 3.69$) compared to the control (placebo) group of NASH patients ($P = 0.119$; $F = 2.31$). Further details about AST/ALT are given in the subsequent discussion section.

An estimation of serum type-IV collagen, which is a highly sensitive marker for active fibrosis measured by enzyme immunoassay using monoclonal antibodies, and hs-CRP as a liver inflammation marker measured by a highly sensitive particle-enhanced immunoturbidimetric assay, is presented in Fig. 3. The liver function marker serum type-IV collagen was significantly decreased ($P < 0.01$; Fig. 3A) in catechin treatment group, while hs-CRP showed a progressive increase in control (placebo) group of NASH patients (during the treatment period with a significant level of difference could be attributed to excessive consumption of alcohol ($P < 0.03$; Fig. 3B). For both groups of NASH patients, the estimated initial admission values (see Table 1) of cholinesterase (ChE) were further regularly analyzed for the trial period. ChE levels exhibited little change during the trial period (data not shown) suggesting that it should be necessary and highly recommended to ingest the green tea catechins as a long term treatment particularly in view of the significance of ChE marker. Also, its correlation with visceral fat accumulation could not provide any sufficient information on its significance levels.

3.4. *Body fat distribution*

According to CT results recorded for the control (placebo) group of NASH patients, a significant decrease in V/S ratio and an increase in L/S ratio were noticed at significant levels (Fig. 4). The results indicated that even moderate alcohol consumption correlated with abdominal distribution of body fat could enlarge the visceral fat area. Further, we tried to correlate the L/S ratio with AST and ALT levels of the patients to quantify their impact on these markers, which was found to increase over the trial period. We believe that such necessary information would be helpful to elucidate the exact physiological role of L/S ratio and its contribution to the progression of NASH.

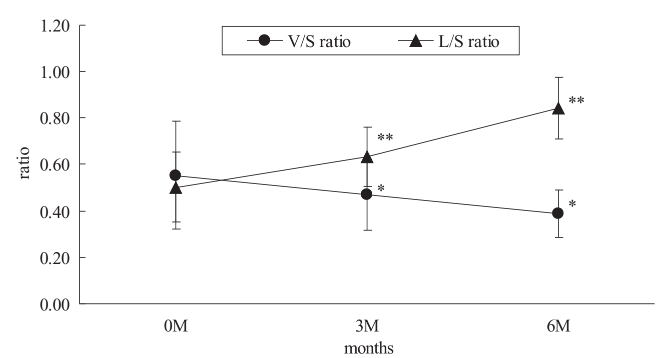

Fig. 4 – Significant changes in body fat distribution ratio measured by computed tomography (CT) in green tea catechins intake group. V/S ratio is expressed as circle and L/S ratio as triangle. Liver to spleen (L/S) CT attenuation ratio was significantly improved in green tea catechins treatment group NASH patients relative to those in control (placebo) group after 6 months of trial period. Data are given as mean ± standard deviation. *P < 0.05 was significantly different from value before treatment in V/S ratio. **P < 0.05 was significantly different from value before treatment in L/S ratio.

Table 5 – Changes in serum levels in green tea catechins treatment group and control (placebo) group of NASH patients.

		FPG (mg/dL)	IRI (μU/mL)	HOMA-IR	GA (%)
Catechins treatment group	At baseline	124 ± 39	42.1 ± 47.8	13.2 ± 16.9	17.1 ± 2.7
	after 3 months	111 ± 16	33.1 ± 31.3	9.1 ± 8.9	15.7 ± 2.8
	after 6 months	113 ± 22	21.6 ± 11.8	6.0 ± 3.7	15.5 ± 2.9
	(ANOVA)	(0.037)[b]	(0.007)	(0.007)	(<0.001)
	[a]P value				
	F value	3.51	5.53	5.39	12.04
Control (placebo) group	At baseline	126 ± 41	34.7 ± 31.6	10.8 ± 9.4	18.4 ± 5.2
	after 3 months	150 ± 51	37.5 ± 30.0	15.6 ± 15.6	20.1 ± 7.8
	after 6 months	164 ± 51	48.3 ± 34.3	21.3 ± 19.1	23.5 ± 11.2
	(ANOVA)	(0.011)	(0.038)	(0.011)	(0.076)
	[a]P value				
	F value	5.44	3.74	5.51	2.87

Abbreviations key as illustrated in Table 1.
Data are given as mean ± s.d. (s.d = standard deviation).
[a] P value was significantly different from value before treatment in both green tea catechins treatment group and control (placebo) group (after 6 months).
[b] After 3 months.

3.5. *Blood sugar evaluation*

Regardless of the age and gender of the NASH patients of this study, green tea catechins were found to have a favorable impact on their blood plasma levels. Since NASH could also be related with complicated diabetes disease known for high blood sugar levels in plasma diabetes parameters such as FPG, GA, IRI and HOMA-IR are also rising in NASH patients. In order to have additional information, the aforementioned diabetes parameters were estimated for the NASH patients in both groups and a positive correlation could be observed (see Table 5). In the catechin treatment group of NASH patients, the values of FPG level, IRI, HOMA-IR, and GA were considerably reduced to significance levels of $P < 0.037$, $P < 0.007$, $P < 0.007$ and $P < 0.001$, respectively, over the period of therapy. While, the levels of these aforementioned diabetes parameters were continuously increased in the control (placebo) group of NASH patients.

4. Discussion

In the present contribution, we have tried to justify the needs of human clinical trials to evaluate green tea catechins for treatment and/or prevention of NASH development. Epidemiological evidence have indicated that green tea catechins consumption is associated with lower circulating levels of TG, aminotransferases, and atherogenic lipoproteins. It could also be correlated to a lower cardiovascular risk factors and causes of mortality as well as basic factors that are closely related with NASH. It is believed that the present study is the first ever report on the hepatoprotective effects of green tea catechins in human NASH. Further, the safety of green tea catechins has been extensively studied and a number of case studies of idiosyncratic hepatoxicity have been published in humans using green tea extracts or its catechins as a part of active matrix formulation (Master-John & Bruno, 2012; Weisburger, 2003). In this clinical study, we have investigated diet and exercise therapy induced models of NASH while examining the hepatoprotective effects of green tea catechins. A common diet model was used with a balanced energy and fructose level. As fructose decreases lipoprotein lipase activity (Stanhope et al., 2009), this may explain why plasma TG are greater in NASH patients despite impaired hepatic TG secretion. Overall, the restricted diet and exercise therapy showed a synergistic effect in NASH patients of catechin treatment group, as most of the serum lipid markers, hepatic markers and blood sugar levels were significantly improved after the catechin treatment. Moreover, the effects of aforementioned diets and exercise therapy appear to have been achieved with moderate restrictions of energy intake without energy starvation. In this study, diet and exercise therapy along with green tea catechin treatment are considered very important as levels of serum enzymes AST, ALT, and TG in blood can be significantly correlated with NASH parameters. This is because the TG synthesis in hepatocytes is up-regulated by the increased delivery of dietary carbohydrates, protein and fat to the liver. The elevated levels of AST, ALT and TG occur when steatosis enhances in liver and resulted in glucose tolerance of heptatocytes and possibly a release of cytokins which produces an accumulation of free fatty acids in the liver leading to hepatic fibrosis. Additionally, the diet and exercise therapy can improve BMI along with biochemical hepatic markers and may therefore be helpful in preventing the progression of NASH upon green tea catechin treatment. Aminotransferase enzymes (ALT and AST) normally reside inside cells i.e. in cytoplasm, so raised levels usually represent hepatocellular damage. ALT is more specific to the liver, while AST is also found in cardiac and skeletal muscle and red blood cells. Very high levels (>1000 IU/L) suggest drug-induced hepatitis (e.g. paracetamol), acute viral hepatitis (A or B), ischemic, or rarely, autoimmune hepatitis. Further, the ratio of ALT/AST could suggest the further insights on the NASH development, even though the individual serum aminotransferase levels alone are not conclusive. Usually, lower ALT/AST ratios (less than 1) could be found in patients whose NASH disease is very much advanced. The diagnostic significance of meaningful ALT/AST scores has already been established as a practical guideline by the American College of Gastroenterology

(McCullough, 2004). In chronic liver disease the level of ALT is greater than AST (ALT > AST), however, once cirrhosis is established the level of AST turns higher than ALT (AST > ALT). The ratio of ALT/AST > 1.0 suggests nonalcoholic liver disease (Conigrave et al., 2002; Nyblom, Berggren, Balldin, & Olsson, 2004). The observed levels of the ALT were higher than AST for most of the cases of NASH patients of both groups in this study. However, the levels of both aminotransferase enzymes could be effectively controlled among the NASH patients of green tea catechin treatment during the trial period, whereas, the levels of both ALT and AST were continuously raised during this study among the control (placebo) group of NASH patients. The ALT/AST ratios were >1.0 indicated that no cirrhosis developed among the NASH patients of both groups. Furthermore, the ALT/AST ratio may depend on other coexisting factors with multiple conditions and could be less useful in such scenarios (Nyblom et al., 2004). Therefore, to find further support for the direct green tea catechin effect on NASH prevention the γ-GPT levels were correlated with ALT/AST ratio. γ-GPT is a membrane-bound glycoprotein enzyme which is also related to the bile ducts typically elevated in cholestasis. Its action is to catalyze the transfer of the γ-glutamyl moiety of glutathione to various peptide acceptors. Chronic ethanol consumption is known to readily induce a rise in serum γ-GPT, however due to lack of specificity the γ-GPT is a poor marker when alcohol consumption needs to be screened in patients with nonalcoholic liver disease. Since γ-GPT could increase in all forms of liver disease, particularly in cases of intra- or posthepatic biliary obstruction, interpretation of γ-GPT with respect to ALT and AST is very useful. An observation of mild liver disease revealed among the NASH patients of both groups of this study from the ALT/AST > 1.0 could further support the effectiveness of green tea catechin treatment as γ-GPT levels were effectively controlled in catechin treatment group of NASH patients. Meanwhile, the γ-GPT levels were raised among the NASH patients of the control (placebo) group during the trial period. Thus, a positive correlation between the ALT/AST ratio and serum γ-GPT was found in the catechin treatment group of NASH patients, but not in the control (placebo) group of NASH patients. A hypothetical explanation for this difference between the groups might be due to induced significant cholestasis without corresponding cytolytic changes in the hepatocytes, reflected in the serum aminotransferases of catechin treatment group of NASH patients subjected to green tea catechin treatment along with diet and exercise therapy. Moreover, the magnitude of weight loss has been correlated strongly with improvements in serum ALT levels as disease markers of NASH, wherein improvement could partly be attributed to the phenomenon of regression to the mean values.

The estimation of glycohemoglobin (HbA1C) levels along with fasting plasma glucose could be the effective marker associated with NASH development; however the derangement of insulin regulation that is often associated with BMI issues, which may play a casual role in the pathogenesis of NASH, are also very important. Since HbA1C measures the amount of glucose chemically attached to red blood cells which live about 3 months, HbA1C indicates average glucose levels for the last 6–8 weeks only. Therefore, the HbA1C levels were almost unchanged from baseline during the trial period. Further, markers of insulin resistance such as serum TG, HDL-cholesterol and fasting blood plasma glucose were in the following order TG < FPG < HDL-C suggesting that features of insulin resistance (IRI and HOMA-IR) observed in the present study could be consequences of hepatic insulin resistance and considered in line with earlier studies supporting the association between insulin resistance and NASH patients that have been correlated with fibrosis and steatohepatitis. As per observed transition of HOMA-IR and GA in the present study, an improvement was evident in insulin resistance and GA among catechin treatment group of NASH patients, whereas it was found worsening among control (placebo) group of NASH patients. Such phenomenon could be positively attributable to an inhibitory action of green tea catechin intake along with controlled diet and exercise therapy. Also, since the results demonstrate the inhibitory effect of green tea catechins on lowering the plasma LDL-C concentration among the catechin treatment group of NASH patients, it is thought to be attributed to a reduction of oxidized LDL concentration by plasma concentrations of catechins. Further the combined decreasing trend of LDL-cholesterol and LDL-C/HDL-C ratio confirm an improvement in the lipid profiles following the green tea catechin treatment in catechin treatment group of NASH patients. Thus, green tea catechins could demonstrate several protective functions that would be expected to prevent the progression from steatosis of NASH, which include its direct and indirect anti-oxidative activities along with its anti-inflammation characteristics (Nakagawa et al., 1999).

In general, the ability of green tea catechins to protect against oxidative stress (Namal Senanayake, 2013), as evidenced by significant lipid peroxidation levels, including reduced dietary lipid and carbohydrate absorption, thermogenic responses, decreasing lipogenesis along with increasing insulin sensitivity and energy expenditure, could be labeled as a centrally implicated pathogenic event contributing to the progression of liver steatosis to NASH (Chung, Yeung, Park, Volek, & Bruno, 2010). It is thought that catechins reduces oxidative stress in hepatocytes through its potent antioxidant activity. More precisely, it has been shown that green tea catechins may decrease oxidative stress either directly, by scavenging reactive oxygen species (ROS) (Jovanovic, Hara, Steenken, & Simic, 1995) and reactive nitrogen species (RNS) (Pannala, Rice-Evans, Halliwell, & Singh, 1997), or indirectly by activating the nuclear factor erythroid 2 (Nrf2) pathways, which usually regulates the transcription of genes related to the cellular antioxidant defense (Shen et al., 2005). Given that reasonable plasma bioavailability of epigallocatechin gallate (EGCg), one of major components of green tea catechins, it has been proposed that EGCg protects against NASH in the adipose sterol regulatory element-binding protein-1c (SREBP-1c) over expression model by decreasing the expression of hepatic phosphorylated Akt, phosphorylated inhibitor of nuclear factor kappa-B (NFkB), and phosphorylated NFkB (Ueno et al., 2009). Furthermore, the anti-inflammatory effects of green tea extract (GTE) for NASH diagnosis may result through interaction between galloylated green tea catechins and the 67-kDa laminin receptor (67LR) wherein EGCg plays an important role in binding and to activate 67LR (Byun, Fujimura, Yamada, & Tachibana, 2010). Furthermore, catechins have inhibition effect on lipase, enzymes related to glucose and fat absorption, and also inhibit α-amylase and α-glucosidase (Nakai et al., 2005).

Usually the mean L/S attenuation ratio is considered normal if it is 1 or greater (Ricci et al., 1997). In the patients with NASH, the initial mean L/S ratio was nearly 0.49 and tended to increase (L/S ratio >0.80 ± 0.17; after 6 months) during the catechin treatment along with dietary control and exercise therapy. This indicates a sign of improvement in NASH patients under the green tea catechin treatment. This could be attributable to green tea catechin intake that inhibits the lipid absorption in small intestine and help reducing the blood lipid contents. EGCg has also been reported to protect fatty liver and liver damages due to ischemia-reperfusion injury (Bursill, Abbey, & Roach, 2007; Fiorini et al., 2005). Since there are limited reports on the use of CT features to stage NASH, the CT findings are not possible to correlate with grade of necroinflamation or fibrosis. Although the CT parameters do not seem to be helpful in diagnosing or stratifying patients with NASH, the evaluation of the ratio of V/S ratio with the help of CT evidently indicates a tendency of decreasing internal fat distribution. In contrast, Oliva et al. (2006) reported an increase of V/S ratio and reversely the decrease of L/S ratio with NASH. Anti-flammatory parameters such as hs-CRP significantly improved among NASH patients of green tea catechin treatment, while the control (placebo) group of NASH patients was reported with the aforementioned parameters which were less favorable and recognized to be gradually accelerating chronic hepatitis. Regardless of a decreasing trend in the type IV collagen values, no significant differences could be observed, however, an effective combinational V/S ratio and L/S ratio in the abdomen CT significantly improved among NASH patients of catechin treatment group. This suggests the effect of green tea catechins on liver function by promoting lipid metabolism in the liver and thus inhibiting the progress of NASH. In addition, green tea catechins may restrict iron absorption in the liver and therefore be effective in the treatment of NASH.

Furthermore, no side effects during the green tea catechin treatment have been observed, indicating that it is safe to use green tea catechins in NASH patients (Lambert et al., 2010). The amount of green tea catechin intake (600 mg) in this study was equivalent to five to six cups of tea infusion with almost no caffeine content. Furthermore, since both green tea catechin treatment and the control (placebo) group of NASH patients were under similar controlled dietary and exercise (physical) therapy without changing their lifestyle, only a gradual reduction in the body weight in green tea catechin treatment group was observed, which could be attributable to a weight loss effect of green tea catechins, thus a positive anti-obesity effect of green tea catechins in NASH patients (Rains, Agarwal, & Maki, 2011).

5. Conclusion

Conceptualizing the anti-obesity characteristics of green tea catechins which are logically associated with an improvement of body fat distribution, green tea catechins could effectively improve the NASH related parameters including recovery of insulin resistance and anti-inflammatory, and thus are capable of inhibiting NASH. This study demonstrated the beneficial effects of green tea catechins on NASH risk factors. Although, the conventional method of controlled diet and physical exercise is very important for the general public, it is rather difficult to continue it effectively due to busy lifestyles, unhealthy eating and irregular exercise. Therefore, it could be suggested that green tea catechins are a simple and affordable treatment for NASH patients, however further investigation is clearly warranted to determine the mechanisms of NASH prevention with green tea catechins. In general, the catechin consumption decreases the fat accumulation in the liver along with oxidative stress markers and improves the liver inflammation and blood biochemistry that are necessary for NASH treatments. Larger trials are required to define the optimal dose of green tea catechins for NASH prevention via their antioxidant and anti-inflammatory characteristics.

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