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EFFECT OF CATECHIN RICH GREEN TEA (*CAMELLIA SINENSIS*) EXTRACTS ON OBESITY TRIGGERED HEPATIC STEATOSIS IN RATS FED WITH HFCS

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ABSTRACT

Tea, the most commonly consumed non-alcoholic beverages is known for its aroma, taste and putative physiological functions. Its richness in flavonoids and other polyphenols had shown a wide range of biological and pharmaceutical benefits. Fatty liver disease is a metabolic syndrome characterized by large vacuoles of triglycerides accumulated in the liver cells by the process of steatosis. Presently weight management is the established treatment advised for fatty liver hepatic steatosisThe present study was designed to evaluate the effects of extracts of *Camellia sinensis* (green tea) on serum lipid profile in high fructose corn syrup (HFCS) induced fatty liver in rat. Green tea extracts were administered orally at various concentrations to the fatty liver induced mice. The study concluded that green tea extracts possesses antiobese, antilipidemic properties and prevents various complications of fatty liver disease with a prophylactic effect to improve the health.

KEYWORDS: ***Camellia sinensis,*** **HFCS, Cholesterol, hepatic steatosis, Fatty Liver**

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INTRODUCTION

Hepatic steatosis is one of the several liver diseases that are collectively termed fatty liver disease or 'Fatty Liver' [1]. It is often characterized by large vacuoles of triglycerides accumulate in the liver cells or excess hepatic lipid accumulation, hepatic injury, and dyslipidemia [2, 3]. Fatty liver can also be associated with excess alcohol consumption or metabolic syndrome [4]. Metabolic syndrome is also often characterized by chronic inflammation and hepatic steatosis. It may even leads to diabetes, hypertension, obesity and dyslipidemia. Invasive liver biopsy remains as the only reliable means to diagnose hepatic steatosis and assess its severity. At present, there are no well-established treatments for hepatic steatosis beyond weight management or comorbidity management. Because weight loss has a poor long-term success rate [5], complementary therapeutic strategies for fatty liver disease are needed. Therefore, the prevention of hepatic steatosis or limiting hepatic lipid accumulation and injury using unique dietary approaches may reduce the incidence and/or likelihood of progressing toward more severe forms of fatty liver disease.

Tea, *Camellia sinensis* (L.) O. Kuntze (syn. *Thea sinensis* L.) belongs to family Theaceae, originated from China, is one of the most commonly consumed non-alcoholic beverages in the world due to its desirable aroma, taste and putative physiological functions [6]. Tea is rich in flavonoids and other polyphenols that have been shown to possess a wide range of biological and pharmaceutical benefits including antioxidant [7], hypocholesterolemic [8,9], antihyperglycemic [10], hepatoprotective [11, 12], chemopreventive [13], anticarcinogenic [14,15] effects. While green tea contains an array of compounds, the putative antiobesity effects have been most commonly attributed to the polyphenolic fraction of green tea, specifically the catechins. Green tea catechins may affect multiple aspects of energy balance that in aggregate, result in body weight and fat loss. Epidemiological data suggest that the consumption of green tea *(Camellia sinensis*) is associated with reduced mortality from all causes and from cardiovascular disease [4]. The mechanisms by which green tea or its catechins protects against chronic disease remain unclear. However, considerable evidence from *in vitro* animal, and human studies suggests the protective effect of green tea may be partly mediated through the antioxidant properties of its catechins. Avena *et al,* (2010) [16] documented that HFCS is considered to be more lipogenic than glucose in the recent studies. HFCS causes obesity, the precursor for fatty liver. People are unaware of the effects of HFCS on their health especially liver. It has thus become vital to identify remedial measure to the damage caused by HFCS and other similar substances. This study was conducted to investigate the effect of dietary green tea extract on the development of obesity- triggered hepatic steatosis and injury in obese rat, a commonly used model for studying the mechanisms leading to the development of hepatic steatosis.

MATERIALS AND METHODS

(i) Preparation of plant extract and biochemical estimation

Samples of green tea were collected from Stanes Amalgamated Co., Coimbatore. Leaves were dried in an hot air oven at 70ºC for 3 hrs and then dried leaves were powdered using a warring blender and methanolic extraction was done using Soxhlet apparatus and fractionated in a separating funnel using ethyl acetate as the solvent. Ethyl acetate fractions were pooled, evaporated finally by using a rotary evaporator. The extract was stored in airtight containers in refrigerator below 10 °C [17] until further use. Total polyphenols content were estimated using folin phenol ciocalteu reagent in the presence of sodium carbonate. The absorbance of blue

colour developed was measured at 700 nm according to Dev Choudhury & Goswami, [18]. Total catechin was estimated using acidified vanillin reagent and the absorbance was measured at 500 nm where (+) Catechin was used as standard [19]. Total polyphenols and catechins were expressed as % in extract.

(ii) Experimental Animals

Thirty male wistar albino rats, weighing (15-30 gm), purchased from Thrissur Veterinary College, Kerala were used for the study. They were housed at ambient temperature of 25±2 ^{o}C and 45-55% humidity, with 12 hrs light dark cycle. Animals were fed with standard laboratory diet and water was given *ad libitum*. The mice used in the study were maintained in accordance with guidelines of the Institutional Animal Ethical Committee of the university .The institutional animal ethical committee approval (IAEC) was obtained under the experiment No. IAEC / KU / BT / 12 / 007 for the present study. All the animals were randomly divided into the five groups with six animals in each group viz., normal control (group I), model group (group II), Green tea extract treated groups at 300, 450, and 600mg/ kg ((group III, IV, V) respectively.

(iii) Experimental design

All rats except normal group is fed with HFCS dissolved in water to a final concentration of 20% through liquid diet in a feeding bottle of capacity 125mL to achieve fatty liver disease, while normal control rats were fed with water. This diet was fed to rats of group II to group V for 20 days from the start of experiment [16]. Tea extracts were given to the rats belonging to group III at a dosage of 300 mg per kg body weight of the animal, group IV was given 450 mg and the group V at 600 mg per kg of the body weight of the animal. The dosages were administered orally using a gastric tube. The extract was dissolved in water to produce an aqueous extract. Concentrations were adjusted such that about 1ml of diluted extract was administered to each animal. The body weights, feed consumed and liquid consumed were documented daily.

(iv) Determination of serum lipid Levels

On day 21, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted mice and serum cholesterol [20], serum triglyceride [21], serum HDL,LDL & VLDL [22] were estimated. The whole liver from each animal was removed after sacrificing the animal and was collected in 10% formalin solution. Sections of 5*μ* thickness were made and stained by haematoxylin and eosin (H & E) for histological examination [23].

The experimental results were expressed as mean of six replicates ± SEM.

RESULTS

Results of the estimation of total poplyphenols and total catechin revealed that the crude extract isolated from the green tea leaves by rotary evaporation have 74.7 % w/w of total polyphenols and 51.8 % w/w total catechins respectively.

1. Effect of Green tea extract on body weight in HFCS treated rats

Animals treated with HFCS had a significant increase in body weight in Group II animals on the 7th, 14th, 21st day, when compared to the normal group (Group I).Administration of green tea extract decreased the body weight significantly in a dose related fashion (300, 450 and 600 mg/kg) when compared to the untreated control group (Group II).The results were depicted in Table 1. Increase in the concentration of extract decreased the body weight significantly.

2. Effect of green tea extract on serum lipid profile

The hypolipidemic effects of green tea extracts (300, 450 and 600 mg/Kg) after administering HFCS in rats is summarized in table 2. Group II animals receiving HFCS showed a significant increase in serum lipids levels on 7th 14th, 21st day when compared to the normal group (Group I). A significant decrease was observed in serum triglycerides, cholesterol, VLDL, LDL except HDL in animals treated with green tea

extract in a dose dependant manner. Highest decrease in biochemical parameters were noticed in the treatment of extract at 600 mg/kg of body wt.

3. Effect of Green tea extract on restoration of shape of Liver

Hepatic histological evaluation is regarded as the 'gold standard" approach to evaluate the presence and severity of fatty liver. Thus, liver sections were evaluated histologically to assess the extent to which green tea extract attenuated the development of hepatic steatosis. HFCS treated rat showed fatty change around the central portal vein and also showed fibrosis**.** The animals treated with green tea extracts of various doses showed normal lobular structure with no fatty change around the central portal vein.

DISCUSSIONS

The green tea extract was found to contain a good percentage of polyphenolic content making it ideal for anti-lipogenic studies. Earlier studies prove that catechins are the best catalysts for fat burning or oxidation [24]. These catechins are known to modulate lipid metabolism by composite protein production such as leptin which is known to modulate the hypothalamus in regard to the reduction in food consumption [25]. The anticancer activity of the polyphenols are already reported [26]. The body weights show that there was gradual and progressive rise in the liquid consumption. The rats belonging to group I showed steady increase in weight, whereas the rats belonging to group II, III, IV, V showed rapid increase in the weight over a period of 7 to 14 days. The feed consumed was modulated by the liquid consumed. Thus HFCS tends to increase the affinity of the animals towards a sugary diet. The group II which was fed with HFCS throughout the experiment showed an exponential increase in the body weight. Experimental group treated with tea extracts tough marginally improved weight, but were able to contain the obese nature. Reduction in the body weight in Group III, IV and V were evident on day 21 of the experiment. This was concluded to be the potential of green tea extracts on weight reduction.The results lipid profiling showed that the blood cholesterol level was the highest in the group fed only with HFCS and was abnormally off the charts when compared to the normal group. Those groups that received the green tea extracts in the diet showed a reduction in the blood cholesterol levels. The bad cholesterol namely LDL and VLDL levels in the blood were pretty high in the second group whereas, in group III, IV and V the levels were significantly reduced. Surprisingly the green tea was able to increase the good cholesterol levels in the blood which is very healthy for an individual. Thereby, proving the positive influence of green tea over lipid metabolism in the body [27]. Histopathology results also supported the influence of green tea over a healthy liver. The group of rats fed with HFCS showed an increase in number of hepatocytes around the central vein thus suggesting possible inflammation in the liver. The group II also displayed fatty change around the portal veins with large circumscribed area of fibrosis confirming the liver damage. The only possible explanation in the scenario would be that HFCS was responsible for the fatty change, fibrosis which possible could worsen with continual ignorance. Groups, III, IV and V which were given green tea extracts along with HFCS proved to have prevented the advent of fatty change and a probable fibrosis [28]. In all the green tea extract treatments, the normal lobular structure of the liver was unaltered. Antidiabetic and antihyperlipidemic effects and their interaction on lipids and lipoproteins in streptozotocin-induced diabetic rats were also reported [29].

Table 1

The effect of Green tea extract on body weight of experimental mice (Values are mean of six observations; n=6).

Group	Treatment	Day 1	Day 7	Day 14	Day 21	
		---------- mg ----------				
Group I	Normal control	93	116	126	138	
Group II	HFCS (20%)	94	128	174	214	
Group III	HFCS+GTE(300mg/kg)		94	136	153	142
Group IV	HFCS+GTE (450mg/Kg)		95	139	153	132
Group V	HFCS+GTE (600MG/Kg)		94	110	142	119
Statistical significance						
	CD (P<0.05)		2.16	7.64	8.76	5.31
	CV		1.21	3.24	3.11	1.89

Table 2

Effect of green tea extract on liquid feed intake in HFCS treated rats (Values are mean of six observations; n=6).

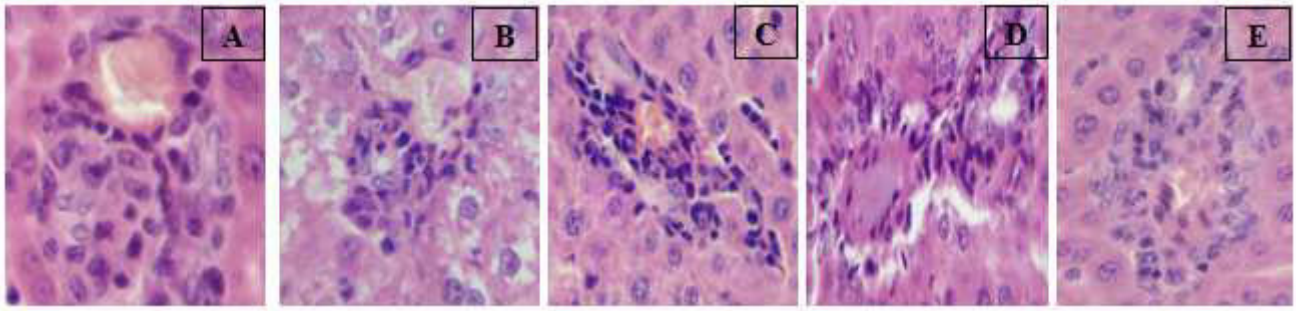
Group	Treatment	Day 1	Day 7	Day 14	Day 21	
		---------- ml ----------				
Group1:	Normal control	30	43	55	68	
Group2:	HFCS (20%)	30	71	96	100	
Group3:	HFCS+GTE(300mg/kg)		38	56	62	78
Group4:	HFCS+GTE(450mg/kg)		35	48	58	60
Group5:	HFCS+GTE(600mg/kg)		39	52	64	70
Statistical significance						
	CD (P<0.05)		4.69	4.82	5.51	7.97
	CV		7.19	4.75	4.36	5.62

Table 3

Biochemical analysis (serum lipid profile) of blood obtained from various experimental groups (Values are mean of three observations; n=3)

Group	Cholesterol	Triglycerides	HDL	VLDL	LDL
Group I	146	50.2	38.4	10.04	97.56
Group II	198	170.3	34.4	34.06	129.54
Group III	127.5	35.7	32.0	7.14	88.36
Group IV	121.5	50.4	37.5	10.08	73.92
Group V	97.5	33.6	67.6	7.72	27.18
Statistical significance					
CD (P<0.05)	7.53	5.92	3.3	1.7	3.79
CV	2.89	4.61	4.16	6.58	2.41

Figure 1
Photomicrographs of rat liver stained by haematoxylin and eosin of various treatments



A: normal control mice (Group I) and B: HFCS treated mice (Group II) and C: Green tea extract treatment (300 mg/kg) (Group III), D: Green tea extract of 450mg/Kg (Group IV), E: Green tea extract 600mg/kg (Group V) at 40x microscopic magnification.

CONCLUSION

The study clearly shows that HFCS can trigger the onset of fatty liver and fibrosis at a very early stage. Green tea extract rich in catechins can prove to be an effective remedy for those suffering from obesity, fatty liver, fibrosis of liver and even liver cirrhosis. Cholesterol which is vital to all the body organs in good concentration lethal in higher levels, on administering green tea has proven in reducing LDL levels and increases the HDL levels. Results concluded that green tea have profound impact on architecture of liver thereby making it ideal for consumption in present day lifestyle with a prophylactic effect to improve the health.

Conflict of Interest
Conflict of interest declared none.

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