Studies on the Development of a Non-genetic Obese Type 2 Diabetic Rat Model: Amelioration by (+)-Catechin Hydrate

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Abstract

This study was planned to investigate the effect of pure catechin, a green tea flavonoid on the levels of fasting plasma glucose, plasma insulin, plasma and tissue lipids in high fat high sucrose diet fed rats for a period of 12 weeks. The results indicate that feeding the rats with high fat and high sucrose diet resulted into the development of an obese type 2 diabetic rat model and (+)-catechin hydrate to some extent prevented the weight gain, rise in blood glucose, cholesterol and Triglyceride levels. Insulin levels were also high in high fat high sucrose fed rats as compared to rats fed high fat, high sucrose and administered (+)-catechin hydrate. Catechin also prevented the weight gain in control group thereby limiting its role only in obesity. Catechin supplementation in rats fed high fat and high sucrose diet was also found to prevent increase in cholesterol, triglycerides and glycolipid levels in liver and cholesterol and glycolipid levels in heart. In conclusion high fat and high sucrose diet is useful in the development of an obese type 2 diabetic rat model and catechin to some extent is beneficial.

Keywords: (+)-catechin hydrate; liver; heart; triglyceride; cholesterol.

1. Introduction

We have previously developed an insulin resistant rat model by chronic feeding of low magnesium and high sucrose diet. This was a non-genetic non obese model [1]. The major cause of insulin resistance in this model was high sucrose

diet. It was presumed that a combination of high fat and high sucrose diet will lead to the development of an obese type 2 diabetic rat model as it is well known that refined sugars and fats have a number of detrimental effects. High sucrose feeding stimulates hepatic lipogenesis by increasing the activity of some enzymes like acetyl CoA carboxylase [2] which contributes to

increase in plasma triglycerides, total cholesterol, LDL-Cholesterol and decreases HDL-Cholesterol. Hyperlipidemia is one of the major risk factors in the development of atherosclerosis and cardiovascular diseases. Constant hyperlipidemia caused by high fat diet and hormonal imbalance can cause increased thickening of blood leading to blocked blood vessels, obesity, stiffness of heart muscle lining, glycosylation of hemoglobin increasing the risk of heart attack [3] and oxidation of LDL causing atherosclerosis [4].

Green tea obtained from the leaves of *Camellia sinensis* is a widely consumed beverage in Asia and is known for its health promoting effects [5]. Epidemiological studies have shown a significant inverse relationship between the consumption of green tea beverages and plasma lipid concentration [6, 7, 8]. It has been suggested that green tea consumption prevents type 2 diabetes [9]. The amelioration of insulin resistance by green tea is associated with increased expression levels of glucose transporter IV in fructose fed diabetic rats [10].

Green tea extract contains polyphenols especially flavanols and flavonols which can constitute 30% of fresh leaf dry weight [5], teanins and caffeine. Catechins (e.g., catechin, epicatechin, epigallocatechin and their gallates) are predominant form of flavanols present in green tea and have been shown to cause hypoglycemia by enhancing insulin stimulated glucose uptake in rat adipocytes [11], by reducing serum glucose levels in alloxan diabetic rats [9] and by inhibiting glucose uptake in the intestine by inhibition of sodium-dependent glucose transporter in rabbit intestinal epithelial cells [12]. Catechins are particularly known for their anti-diabetic [11], anti-inflammatory [13], anti-oxidative [14], anticancerous [15] and neuroprotective [16] effects and it is known that hypolipidemic effect of most abundant catechin (-)-epigallocatechin gallate (EGCG) is due to enhanced removal of intravenously injected ¹⁴C-Cholesterol from plasma of rats [17], inhibition of cholesterol absorption [18], or increase in LDL receptor expression [19].

Despite a preponderance of data on the effect of green tea and its active components in combating hyperlipidemia, there is virtually no

information regarding the effects of pure catechin (+)-catechin hydrate. So we focused our study in exploring *in vivo* effects of (+)-catechin hydrate on body weight and plasma parameters viz. glucose, triglycerides, cholesterol, insulin and liver and heart tissue lipids in control and high fat high sucrose diet fed rats.

2. Materials and Methods

2.1 Reagents

(+)-Catechin hydrate was purchased from Sigma-Aldrich Company, USA for *in vivo* experiments. All other analytical grade laboratory chemicals and reagents were purchased from Merck (Germany) or SRL Chemicals (India). Ultra pure water prepared by labPURE-series Analytical & Ultrapuruf (BIO-AGE, Mohali, India) was used throughout the experiment. The preparations were made fresh every time before the commencement of the experiment.

2.2 Animals

Studies were carried out in Male Wistar rats, each weighing approximately 100-120 g. These animals were obtained from central animal house, Panjab University, Chandigarh. The animals were housed in polypropylene cages in hygienic conditions with rice husk bedding and, had free access to tap water. Before giving experimental diet, rats were provided a standard rodent lab chow for a 2 week baseline period. Throughout the experiment, all the procedures and care of the animals were conducted in accordance with institutional guidelines and CPCSEA policies (Committee For The Purpose Of Control and Supervision of Experiments on Animals).

2.3 Experimental design

After the completion of the baseline period, rats were given respective assigned diet prepared in the laboratory (Table 1). Rats were randomly divided into the following groups: **Control diet (CD)**, **Control diet & (+)-Catechin hydrate (CD + CH)**, **High fat & high sucrose diet (HFHS)**, **High fat & high sucrose along with (+)-catechin hydrate (HFHS + CH)**. (+)-Catechin hydrate was solubilized in hot distilled water (70 °C) and this solution at room temperature was orally

administered to respective experimental groups with the help of canula at a dose of 110mg/kg

body weight. Rats were provided free access to experimental diet for the period of 12 weeks.

Table 1. Composition of experimental diet

Ingredient	Control diet (CD) (g)	High fat & High Sucrose diet (HFHS) (g)
Starch	658	Nil
Sucrose	Nil	562
Casein	188	188
Methionine	1.9	1.9
Gelatin	14.1	14.1
Safflower oil	41.4	82.4
Bran	37.6	37.6
Vitamin Mix ^a	9.4	9.4
Mineral Mix ^b	49.7	49.7

^a Supplied per kilogram of vitamin mix: 3 g thiamine mononitrate, 3 g riboflavin, 3.5 g pyridoxine hydrochloride, 15 g nicotinamide, 8 g D-calcium pantothenate, 1 g folic acid, 0.1 g d-biotin, 5 mg cyanocobalamine, 12.5 g cholecalciferol, 25 mg acetomenaphthone, 600 mg vitamin A acetate, 22 g d- α -tocophenyl acetate and 10 g choline chloride.

^b Supplied per kilogram of mineral mix: 65.2 g NaCl, 105.7 g KCl, 200.2 g KH_2PO_4 , 40.0 g $\text{FeCH}_3\text{O}_2.5\text{H}_2\text{O}$, 512.4 g CaCO_3 , 0.8 g KI, 0.9 g NaF, 1.4 g $\text{CuSO}_4.5\text{H}_2\text{O}$, 0.4 g MnSO_4 , 0.05 g CoNO_3 and addition of $\text{MgSO}_4.7\text{H}_2\text{O}$ to provide 507 mg of Mg.

2.4 Plasma preparation and estimation of glucose

At the start of the experiment and after every 4 weeks, animals were fasted overnight and blood samples were drawn by puncturing the orbital sinus of the animals under light anesthesia. Blood samples were drawn in vials containing anticoagulant namely potassium oxalate and sodium fluoride. Blood was centrifuged at 3000 g for 10 minutes. Plasma was aspirated and stored at 4 °C till further use for various estimations except glucose, which was estimated immediately. Plasma glucose was measured using GOD/POD method by Trinder [20] (Enzopak kit).

2.5 Analysis of Plasma lipid Profile

Plasma total lipids were estimated by the method of Frings and Fendley [21], triglycerides by GPO-Trinder enzymatic method (PAP) of McGowan *et al* [22] (Erbamannheim kit), plasma Cholesterol by CHOD-PAP method of Roeschlau's [23] (Erbamannheim kit) and plasma HDL-Cholesterol by phosphotungstic method of Burstein [24] (Erbamannheim kit).

VLDL and LDL were calculated by the formulae of Friedwald *et al* [25] according to which

$\text{VLDL-Cholesterol (mg/dl)} = \text{Concentration of triglycerides}/5$

$\text{LDL-Cholesterol (mg/dl)} = \text{Total Cholesterol} - (\text{VLDL} + \text{HDL})$

2.6 Analysis of tissue lipids

At the end of the stipulated period of 12 weeks animals were sacrificed and liver and heart organs were removed. Liver and heart tissue lipids were extracted using the standard Folch [26] extraction technique. Tissue cholesterol was estimated by the method of Zlatkis *et al* [27], tissue triglycerides by the method of Van Handel and Zilversmith [28].

The method of Bartlett [29] was used for the determination of tissue phospholipids phosphorous. Total tissue lipids and tissue glycolipids were measured by the method of Fringes [21] and Dubois *et al* [30] respectively.

2.7 Assay of plasma insulin

Plasma insulin was assayed using double antibody radioimmunoassay technique of Berson and Yellow [31].

2.8 Statistical Analysis

Results were expressed as Mean $\pm$ S.D. Statistical analyses was performed by using one-

way ANOVA followed by Fischer's least significance difference test. The statistical analysis was done using Jandel Sigma Stat Statistical

Software version 2.0. Statistical significance of the results were calculated at least at $p < 0.05$.

Figure 1. Weekly body weight gain of experimental animals. Values are mean $\pm$ SD with 6 animals per group. * $p < 0.05$ vs. CD; # $p < 0.05$ vs. HFHS.

3. Results

3.1 Effect of (+)-catechin hydrate on the body weight gain and organ weight of control and high fat high sucrose diet fed rats

Body weight gain was significantly affected in rats given (+)-catechin hydrate (Figure 1). In CD group, the body weight gain was 119% in 12 weeks, the increase in weight was only 55% in rats of CD + CH group during the same period. In rats of HFHS group, the weight gain was 291% indicating high fat high sucrose induced obesity as compared to CD group. Body weight gain in HFHS + CH group was 215% indicating that (+)-catechin hydrate significantly prevented body weight gain of rats of this group compared to HFHS group ($p < 0.001$).

Administration of (+)-catechin hydrate resulted in smaller increase in heart and liver weights and hence, attenuated hypertrophy in rats

fed control diet as well as high fat and high sucrose diet (Table 2). Liver weight increased 8% less in CD + CH group compared to the CD group ($p < 0.0001$) and 6.3 % less in HFHS + CH group compared to the HFHS group ($p < 0.0001$). Heart tissue weight on the other hand increased 12% less in CD + CH group compared to CD group ($p < 0.0001$) and 14 % less in HFHS + CH group as compared to the HFHS group ($p < 0.001$).

3.2 Effect of (+)-catechin hydrate on the fasting plasma glucose levels

The level of fasting plasma glucose was 10.2% less ($p < 0.0001$) in CD + CH group at the end of 12th week as compared to CD group. Plasma glucose levels in HFHS group increased by 57% at the end of 12th week compared to CD group ($p < 0.0001$) (Table 3). However (+)-catechin hydrate caused 9.6% less increase in glucose

levels in HFHS + CH group during 12th week (p<0.0001).

3.3 Effect of (+)-catechin hydrate on plasma total lipids

The levels of plasma total lipids in CD + CH group were 6% (p<0.01), 7% (p<0.001) and 10% (p<0.001) lower during 4th, 8th and 12th week

respectively compared to the CD group (Figure 2). However, these levels were significantly higher in HFHS group by 23%, 60% and 52% during 4th, 8th and 12th week respectively as compared to the CD group (p<0.0001 in each case) but significantly lower by 11%, 25% and 20% (p<0.001 in each case) during 4th, 8th and 12th week respectively in HFHS + CH group.

Table 2. Organ weight of experimental animals

Organ weight of experimental animals (g)				
ORGAN	GROUP 1 (CD)	GROUP 2 (CD + CH)	GROUP 3 (HFHS)	GROUP 4 (HFHS + CH)
LIVER	6.59 ± 0.17	6.06 ± 0.05*	8.88 ± 0.13*	8.32 ± 0.14 [#]
HEART	0.68 ± 0.02	0.60 ± 0.02*	1.36 ± 0.09*	1.17 ± 0.05 [#]

Values are mean ± SD with 6 animals per group. *p<0.05 vs. CD; [#]p<0.05 vs. HFHS.

Table 3. Levels of fasting plasma glucose in experimental rats.

Plasma glucose levels (mg/dl)				
	GROUP 1 (CD)	GROUP 2 (CD + CH)	GROUP 3 (HFHS)	GROUP 4 (HFHS + CH)
START	99.13 ± 5.97	95.11 ± 6.27	98.09 ± 11.25	97.54 ± 11.67
4 WEEKS	94.86 ± 0.78	93.91 ± 0.65*	128.47 ± 3.91*	113.87 ± 6.44 [#]
8 WEEKS	96.43 ± 10.08	90.86 ± 4.10 ^{NS1}	155.51 ± 23.44*	126.08 ± 17.04 [#]
12 WEEKS	93.89 ± 1.17	84.32 ± 3.37*	148.02 ± 10.36*	133.75 ± 7.96 [#]

Values are mean ± SD with 6 animals per group. *p<0.05 vs. CD during same week; [#]p<0.05 vs. HFHS during same week; ^{NS1} non-significant change vs. CD during same week.

3.4 Effect of (+)-catechin hydrate on the plasma Triglyceride levels

As shown in figure 3, (+)-catechin hydrate administration significantly decreased plasma triglycerides in CD + CH group compared to CD group during the 4th, 8th & 12th week period by 40%, 30% and 12.5% respectively (p<0.001 in each case). In HFHS group, the plasma triglyceride levels showed a marked elevation of 52%, 172% and 143% during 4th, 8th and 12th week compared to CD group (p<0.0001 in each case). Administration of (+)-catechin hydrate in HFHS + CH group significantly lowered plasma triglyceride levels during 4th (p<0.05), 8th (p=0.001) and 12th week (p<0.01) period compared to the HFHS group during which the levels were

found to be 49.07 ± 7.36, 63.6 ± 22.53 & 65.92 ± 14.81 mg/ dl respectively.

3.5 Effect of (+)-catechin hydrate on the plasma cholesterol levels

In CD + CH group there was significant reduction in cholesterol levels during 12th week only compared to CD group (p<0.0001) (Figure 4). Cholesterol levels increased by 39.5% in HFHS group during 12 weeks as compared to CD group (p<0.0001). In HFHS + CH group cholesterol levels showed non-significant change till 4 week period but the levels were significantly lower during 8th and 12th week by 12% (p<0.05) and 7% (p<0.05) respectively as compared to HFHS group.

Figure 2. Effect of (+)-catechin hydrate on plasma total lipid levels in control diet fed and high fat high sucrose diet fed animals. Values are mean $\pm$ SD with 6 animals per group. * $p<0.05$ vs. CD during same week; # $p<0.05$ vs. HFHS during same week.

Figure 3. Effect of (+)-catechin hydrate on plasma triglyceride levels in control diet fed and high fat high sucrose diet fed animals. Values are mean $\pm$ SD with 6 animals per group. * $p<0.05$ vs. CD during same week; # $p<0.05$ vs. HFHS during same week.

Figure 4. Effect of (+)-catechin hydrate on plasma cholesterol levels in control diet fed and high fat high sucrose diet fed animals. Values are mean $\pm$ SD with 6 animals per group. * $p < 0.05$ vs. CD during same week; # $p < 0.05$ vs. HFHS during same week; ^{NS1} non-significant change vs. CD during same week; ^{NS2} non-significant change vs. HFHS during same week.

Figure 5. Effect of (+)-catechin hydrate on plasma HDL-Cholesterol levels in control diet fed and high fat high sucrose diet fed animals. Values are mean $\pm$ SD with 6 animals per group. * $p < 0.05$ vs. CD during same week; # $p < 0.05$ vs. HFHS during same week; ^{NS1} non-significant change vs. CD during same week; ^{NS2} non-significant change vs. HFHS during same week.

3.6 Effect of (+)-catechin hydrate on plasma HDL-cholesterol, VLDL-Cholesterol and LDL Cholesterol

Administration of (+)-catechin hydrate caused significant reduction of 20% ($p<0.0001$) during 12th week only compared to the CD group (Figure 5). Though HDL-cholesterol levels remained unaffected in HFHS group compared to CD group, these levels significantly increased by 24.5% ($p<0.01$) and 28% ($p<0.01$) in HFHS + CH group during 8th and 12th week respectively. During 12th

week VLDL-Cholesterol levels were 12.6% lower ($p<0.001$) and LDL-Cholesterol levels were 25% lower ($p<0.001$) as compared to CD group (Table 4 and 5). Both VLDL and LDL Cholesterol levels increased in HFHS group during 12th week ($p<0.0001$ in each case) as compared to the CD group. Administration of (+)-catechin hydrate in HFHS + CH group significantly decreased VLDL-Cholesterol and LDL-Cholesterol during 8th and 12th week ($p<0.01$).

Table 4. Plasma VLDL-cholesterol levels in experimental animals

	Plasma VLDL-Cholesterol (mg/dl)			
	GROUP 1 (CD)	GROUP 3 (CD + CH)	GROUP 2 (HFHS)	GROUP 4 (HFHS + CH)
0 weeks	8.74 ± 0.58	8.72 ± 0.52	8.88 ± 0.53	8.67 ± 1.10
4 weeks	8.92 ± 1.19	5.30 ± 0.53*	13.58 ± 3.69*	9.81 ± 1.97 ^{NS2}
8weeks	8.74 ± 0.52	8.08 ± 1.86 ^{NS1}	23.78 ± 3.93*	12.79 ± 4.51 [#]
12weeks	8.62 ± 0.36	7.53 ± 0.34*	20.88 ± 4.42*	13.18 ± 2.96 [#]

Values are mean ± SD with 6 animals per group. * $p<0.05$ vs. CD during same week; [#] $p<0.05$ vs. HFHS during same week; ^{NS1} non-significant change vs. CD during same week; ^{NS2} non-significant change vs. HFHS during same week.

Table 5. Plasma LDL-Cholesterol levels in experimental animals

	Plasma LDL-cholesterol (mg/dl)			
	GROUP 1 (CD)	GROUP 3 (CD + CH)	GROUP 2 (HFHS)	GROUP 4 (HFHS + CH)
0 weeks	15.78 ± 0.72	15.82 ± 1.38	15.72 ± 1.18	15.90 ± 1.40
4 weeks	15.88 ± 1.69	21.22 ± 1.34*	20.99 ± 1.66*	21.62 ± 1.08 ^{NS2}
8weeks	16.30 ± 1.69	15.53 ± 1.67 ^{NS1}	20.60 ± 2.48*	14.98 ± 2.24 [#]
12weeks	15.72 ± 0.76	11.80 ± 1.60*	21.45 ± 1.15*	16.07 ± 1.28 [#]

Values are mean ± SD with 6 animals per group. * $p<0.05$ vs. CD during same week; [#] $p<0.05$ vs. HFHS during same week; ^{NS1} non-significant change vs. CD during same week; ^{NS2} non-significant change vs. HFHS during same week.

Table 6. Content of tissue lipids in the liver homogenate (mg / g liver tissue)

	GROUP1 (CD)	GROUP2 (CD + CH)	GROUP3 (HFHS)	GROUP4 (HFHS + CH)
Total lipids	45.5 ± 2.05	39.5 ± 1.75*	52.77 ± 3.10*	47.61 ± 1.54 [#]
Phospholipids	26.08 ± 0.97	24.38 ± 0.88*	27.81 ± 0.86 ^{NS1}	25.31 ± 1.17 [#]
Cholesterol	2.62 ± 0.07	2.17 ± 0.13*	2.82 ± 0.06*	2.55 ± 0.11 [#]
Triglycerides	8.18 ± 0.62	7.85 ± 0.72 ^{NS1}	21.55 ± 2.95*	14.91 ± 0.87 [#]
Glycolipids	4.31 ± 0.09	2.32 ± 0.24*	4.86 ± 0.12*	3.94 ± 0.09 [#]

Values are mean ± SD with 6 animals per group. * $p<0.05$ vs. CD during same week; [#] $p<0.05$ vs. HFHS during same week; ^{NS1} non-significant change vs. CD during same week.

3.7 Effect of (+)-catechin hydrate on plasma insulin levels

In CD + CH group, plasma insulin was significantly lower than the CD group during the 4th week (Figure 6). In HFHS group plasma insulin levels were found to be significantly higher than the CD group ($p<0.0001$) after 4 weeks. Administration of (+)-catechin hydrate in HFHS + CH group significantly lowered the plasma insulin levels during the 8th and 12th week by 14% and 26.5% as compared of HFHS group ($p<0.0001$ in each case).

3.8 Effect of (+)-catechin hydrate on tissue lipids

In liver of HFHS group, total lipids increased by 15.98% ($p<0.01$), cholesterol by 7.6% ($p<0.001$), triglycerides by 163.45% ($p<0.0001$) and glycolipids by 12.76% compared to CD group while phospholipids showed non-significant

change (Table 6). (+)-catechin hydrate decreased total lipid levels by 13.2% in CD + CH group compared to CD group ($p<0.001$) and 9.8% in HFHS + CH group compared to the HFHS group ($p<0.01$). Phospholipid content in CD + CH group was 6.5% lower than CD group ($p<0.01$) and 9% lower in HFHS + CH group compared to HFHS group ($p<0.01$). (+)-catechin hydrate also significantly decreased liver cholesterol in both CD + CH and HFHS + CH group by 17% ($p<0.0001$) and 9.5% ($p<0.01$) as compared to the CD and HFHS group respectively. Though liver triglycerides showed non-significant change in CD + CH group, there was significant 30% reduction in HFHS + CH group compared to the HFHS group ($p<0.01$). (+)-catechin hydrate also inhibited the accumulation of liver glycolipids by 46.17 % in CD + CH group and 18.93% in HFHS + CH group compared to the CD and HFHS group respectively.

Figure 6. Levels of plasma insulin in experimental animals. Values are mean $\pm$ SD with 6 animals per group. * $p<0.05$ vs. CD during same week; # $p<0.05$ vs. HFHS during same week; ^{NS1} non-significant change vs. CD during same week.

In heart of HFHS group, total lipids rose by 26.43% ($p<0.0001$), cholesterol by 15.64% ($p<0.001$) and glycolipids by 15.92% ($p<0.0001$) compared to CD group while phospholipids and Triglycerides showed a non-significant change (Table 7). Supplementation of (+)-catechin hydrate significantly reduced heart total lipids and cholesterol content only in CD + CH group by 6.83% and 5.21% compared to the CD group

($p<0.01$ in each case). (+)-catechin hydrate supplementation decreased Triglyceride content in both CD + CH as well as HFHS + CH group by 29.97% and 14.64% compared to CD and HFHS group respectively ($p<0.01$ in each case). In HFHS + CH group, the decrease in glycolipid content was 34.33 % and significantly different from the HFHS group ($p<0.0001$).

Table 7. Content of tissue lipids in the heart homogenate (mg / g heart tissue)

	GROUP1 (CD)	GROUP2 (CD + CH)	GROUP3 (HFHS)	GROUP4 (HFHS + CH)
Total lipids	28.53 $\pm$ 1.03	26.58 $\pm$ 0.96*	36.07 $\pm$ 2.38*	35.19 $\pm$ 2.13 ^{NS2}
Phospholipids	14.52 $\pm$ 0.66	13.81 $\pm$ 1.05 ^{NS1}	13.28 $\pm$ 1.98 ^{NS1}	12.17 $\pm$ 1.04 ^{NS2}
Cholesterol	2.11 $\pm$ 0.10	2.00 $\pm$ 0.09*	2.44 $\pm$ 0.15*	2.30 $\pm$ 0.09 ^{NS2}
Triglycerides	13.48 $\pm$ 0.87	9.44 $\pm$ 0.50*	13.25 $\pm$ 1.27 ^{NS1}	11.31 $\pm$ 0.62 [#]
Glycolipids	4.02 $\pm$ 0.12	4.29 $\pm$ 0.38 ^{NS1}	4.66 $\pm$ 0.21*	3.06 $\pm$ 0.12 [#]

Values are mean $\pm$ SD with 6 animals per group. * $p<0.05$ vs. CD during same week; [#] $p<0.05$ vs. HFHS during same week;

^{NS1} non-significant change vs. CD during same week; ^{NS2} non-significant change vs. HFHS during same week.

4. Discussion

This study clearly shows that high fat and high sucrose diet produces an obese insulin resistant type 2 diabetes model as the results show that even when there is hyperinsulinemia in HFHS group, there is concomitant hyperglycemia. We have previously shown that high sucrose diet given to Mg²⁺ deficient rats produce type 2 diabetes like condition [1, 32, 33]. Increased triglyceride level is a hallmark of type 2 diabetes which is very clear from the results of this study as triglyceride levels are very high in HFHS group as compared to CD group.

The results of the study show that (+)-catechin hydrate decreases body weight gain in both CD + CH and HFHS + CH groups. Dullo *et al* have shown that green tea catechins stimulated O₂ consumption and energy expenditure in humans which may be the prime reason for the decrease of weight [34]. They also showed that these compounds are associated with sympathetic stimulation of fat oxidation as these inhibit the activity of Catechol-O-methyl transferase (the enzyme that degrades noradrenaline). Murase *et al* have shown that tea catechins in a dose dependent

manner decrease body weight in rats [35]. Comparing decrease in body weight gain in CD + CH and HFHS + CH groups reveal that (+)-catechin hydrate has deleterious effect on rats of CD + CH group as body weight gain was less than halved as compared to CD group making rats lean and weak. (+)-catechin hydrate had more effect in reducing heart weight gain than liver weight gain in both CD + CH and HFHS + CH group.

The study further shows that administration of (+)-catechin hydrate had a beneficial effect on insulin sensitivity of high fat high sucrose fed rats as the glucose levels were found to be consistently lower in HFHS + CH group as compared to the HFHS group. Reports in literature have shown green tea extract has anti diabetic effects in rats [36, 37]. *In vitro* studies have shown that EGCG, has the capacity to decrease glucose production in H4IIE rat hepatoma cells [38].

Previous studies have shown that dietary sucrose supplementation increases gene expression of several enzymes including acetyl-coenzyme A carboxylase and fatty acid synthase which lead to increase in triglyceride concentration in blood [39]. Green tea consumption has been associated with decreased

serum levels of triglycerides [40]. Studies have shown that jasmine green tea epicatechins are hypolipidemic in hamsters fed a high fat diet and their hypolipidemic effect is due to decreased intestinal absorption of dietary lipids [41]. In the present study (+)-catechin hydrate also showed its hypolipidemic effect by significantly lowering plasma triglycerides during 4th, 8th and 12th week.

(+)-catechin hydrate decreases the ratio of very low and low density lipoprotein cholesterol concentrations to high density lipoproteins concentrations. This is very important indication of the role (+)-catechin hydrate plays in preventing atherosclerosis and cardiovascular diseases. Thus our study showed a close association between consumption of (+)-catechin hydrate and normalization of plasma parameters which reflect cardiovascular diseases.

Previous studies have shown that green tea extract has insulin like effect [42]. Anderson *et al* have shown that EGCG can potentiate in vitro insulin activity [43]. Infact epigallocatechin gallate is regarded to have a modulating effect on the endocrine system and it is found to have improving effects on diabetes [44]. In our study persistent hyperinsulinemia which was found in rats fed high fat and high sucrose diet was improved by the administration of (+)-catechin hydrate.

5. Conclusion

The present study clearly shows that high fat and high sucrose diet produces an obese rat model which is hyperglycemic and hyperlipidemic and (+)-catechin hydrate is beneficial to some extent in reducing obesity and in improving plasma parameters.

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