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Studies on Antioxidant Role of (+)-Catechin Hydrate in High Sucrose High Fat Diet Induced Oxidative Stress

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Abstract

In the present investigation, effect of pure green tea catechin, (+)-catechin hydrate was studied on plasma uric acid, plasma nitrite, cardiac lipid peroxidation, reduced glutathione and cardiac antioxidant enzymes in high sucrose high fat diet fed rats for a period of 12 weeks. Our results indicate that feeding high sucrose high fat diet resulted in the development of oxidative stress in rats and (+)-catchin hydrate has antioxidant effect. High sucrose high fat diet decreased plasma uric acid and increased plasma nitrite levels. Further lipid peroxidation increased and reduced glutathione levels decreased in heart of high sucrose high fat diet fed rats. Activity of antioxidant enzymes (SOD, GST, GR and GPx) was significantly reduced in high sucrose high fat fed rats. Catechin supplementation improved plasma uric acid and nitrite levels and reduced lipid peroxidation. Further there was normalization in reduced Glutathione levels and activities of cardiac antioxidant enzymes upon administration of (+)-catechin hydrate. It can be concluded that high sucrose high fat diet produces oxidative stress in rats and administration of catechin is helpful to combat oxidative stress.

Keywords: (+)-catechin hydrate; lipid peroxidation; nitrite; uric acid; glutathione; antioxidant enzymes

1. Introduction

In this contemporary world, there has been drastic change in lifestyle and human population has got engulfed by many diseases due to changing eating habits. Foods rich in sucrose and fat are resulting in many health complications which include imbalance in carbohydrate and lipid metabolism. Anti-oxidant defense mechanism is affected in animals fed high sucrose diet leading to enhanced generation of

reactive oxygen species [1]. High sucrose diet has been believed to affect the balance between production of radicals and anti-oxidant defense mechanism and thus can cause oxidative stress. It has also resulted in causing high plasma lipid peroxidation and greater susceptibility to peroxidation in various tissues [2]. Many nutritionists consider sucrose particularly added sucrose in the diet as the most important factor promoting biochemical changes in plasma and different tissues.

Elevated levels of circulating glucose after a high fat diet can produce permanent chemical alterations in proteins and increase lipid peroxidation [3]. According to a study, formation of malondialdehyde, a lipid peroxidation end product, is increased by about 60% in high fat diet fed rats as compared to the normal control rats [4]. In rats fed a high fat diet, overproduction of reactive oxygen species (ROS) also occurs along with antioxidant depletion which marks the onset of diabetes [5]. Although information about the biochemical changes occurring in rats after feeding them sucrose or fat separately is available in literature but there is little information regarding the changes due to combined feeding of high sucrose high fat diet.

Several herbal dietary supplements are known for their potential benefits on metabolic health. In the past few years green tea has attained considerable attention due to its beneficial health effects and antioxidant functions [6]. Green tea which is a widely consumed beverage in Asia, is a rich source of polyphenol content. The latter include flavanals and flavanols which make up around 30% of the dry weight of green tea leaves. Catechins are the predominant flavanols and are mainly composed of EGCG (Epigallocatechin gallate), EGC (Epicatechin gallate) and EC (Epicatechin). Out of all the catechins, EGCG is most abundant in green tea.

Catechins are also known for their antioxidant activity. They upregulate antioxidant enzymes [7] and scavenge ROS (Reactive oxygen species) [8] like superoxide (O_2^-), hydroxyl (OH^-) and peroxyl radicals. A study has shown that green tea catechins increase activities of SOD (Superoxide dismutase) and Catalase in fruitfly by upregulating the genes producing these enzymes [9].

Despite a preponderance of data on the effect of green tea and its active components in combating oxidative stress, there is virtually no information regarding the effects of pure catechin namely (+)-catechin hydrate. The present study was therefore designed to develop a rat model of oxidative stress by chronic feeding of high sucrose and high fat diet. (+)-catechin hydrate was orally administered in this model to observe effects of this pure flavanol against the combined high sucrose high fat diet induced oxidative stress in plasma and cardiac tissue.

2. Materials and Methods

2.1 Reagents

DTNB, NBT, GSH, NADPH and CDNB were purchased from Sisco Laboratories Pvt. Ltd, Mumbai, India. (+)-Catechin hydrate (99.9% purity) 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 & Ultraplusuf (BIO-AGE, Mohali, India) was used throughout the experiment. All preparations were made fresh every time before the commencement of the experiment.

2.2 Animals

Male Wistar rats weighing around 100-150 g 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. Rats were acclimatized by providing standard rodent chow for a two week baseline period. This was followed by feeding of experimental diet for 12 week period. 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) throughout the experiment.

2.3 Experimental design

Experimental diet was prepared in the laboratory (Table 1). Rats were randomly divided into the following groups: **Control diet (CD), Control diet & (+)-Catechin hydrate (CD + CH), High sucrose & high fat diet (HSHF), High sucrose & high fat along with (+)-catechin hydrate (HFHS + CH).** (+)-Catechin hydrate was solubilized in hot double 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 sucrose high fat diet (HSHF) (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, 8g D-calcium pantothenate, 1 g folic acid, 0.1 g d-biotin, 5mg cyanocobalamine, 12.5 g cholecalciferol, 25 mg acetomenaphthone, 600 mg vitamin A acetate, 22 g d-1α-tocophenyl acetate and 10 g choline chloride.

[b] Supplied per kilogram of mineral mix: 65.2 g NaCl, 105.7 g KCl, 200.2g KH_2PO_4, 40.0 g $FeCH_3O_2.5H_2O$, 512.4 g CaCO3, 0.8 g KI, 0.9 g NaF, 1.4 g $CuSO_4.5H_2O$, 0.4 g $MnSO_4$, 0.05 g $CoNO_3$ and addition of $MgSO_4.7H_2O$ to provide 507 mg of Mg.

Table 2 Levels of plasma uric acid (mg/dl)

	GROUPS			
	(CD)	**(CD + CH)**	**(HSHF)**	**(HSHF + CH)**
START	1.04 ± 0.12	1.01 ± 0.10	1.02 ± 0.11	1.01 ± 0.12
4 WEEKS	1.00 ± 0.32	1.14 ± 0.30^{NS1}	0.91 ± 0.08^{NS1}	1.05 ± 0.15^{NS2}
8WEEKS	1.12 ± 0.35	1.50 ± 0.24^{NS1}	0.90 ± 0.05^{NS1}	1.10 ± 0.09$^{\#}$
12WEEKS	1.05 ± 0.11	1.41 ± 0.11*	0.88 ± 0.04*	1.20 ± 0.21$^{\#}$

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

2.4 Plasma preparation and estimation of uric acid

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 potassium oxalate. Blood was centrifuged at 3000 g for 10 minutes. Plasma was aspirated and stored at 4°C for estimation of uric

acid and nitrite. Plasma uric acid was estimated by enzymatic uricase method of Thefeld [10].

2.5 Analysis of plasma nitrite levels

Plasma nitrite levels were estimated using Griess reagent according to method given by Green *et al.* [11].

2.6 Analysis of tissue lipid peroxidation and reduced glutathione levels

At the end of the stipulated period of 12 weeks, rats were sacrificed and heart was carefully removed. It was adequately washed in ice cold normal saline (0.9% NaCl) followed by washing with homogenizing buffer. A 10% (w/v) tissue homogenate was prepared in 0.1M Tris-HCl buffer (pH 7.4) for determination of MDA levels in heart by method of Wills [12]. Reduced glutathione levels in heart were measured by the method described by Ellman [13].

2.7 Assay of tissue antioxidant enzymes

Post mitochondrial supernatant (PMS) obtained from the homogenate of heart organ was used for the estimation of various antioxidant enzyme activities. SOD (Superoxide dismutase), GST (Glutathione-S-transferase) and Catalase activity was estimated by the method of Kono [14], Habig *et al.*[15] and Luck [16] respectively. For the estimation of GPx (Glutathione Peroxidase) , method of Paglia and Valentine [17] as modified by Lawrence and Burk [18] was used. Further GR (Glutathione Reductase) activity was assessed by the method of Horn [19].

2.8 Statistical Analysis

Results were expressed as Mean ± 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 $p<0.05$.

3. Results

3.1 Effect of (+)-catechin hydrate on plasma uric acid levels in control and high sucrose high fat diet fed rats

Table 2 shows that rats of HSHF group had significantly ($p<0.01$) lower plasma uric acid levels compared to CD only at the end of experimental period. Administration of catechin in HSHF + CH group had beneficial effect on plasma uric acid during 8^{th} and 12^{th} week when the levels increased significantly ($p<0.001$) by 22% and 36% respectively compared to HSHF group. On the other hand CD + CH group showed significant ($p<0.01$) increase only at the end of 12 weeks compared to CD group.

Table 3 Levels of plasma nitrite **(**μmol/dl)

	GROUPS			
	(CD)	**(CD + CH)**	**(HSHF)**	**(HSHF + CH)**
START	3.20 ± 0.18	3.28 ± 0.27	3.21 + 0.19	3.20 + 0.18
4 WEEKS	3.23 ± 0.30	3.04 ± 0.32 ^{NS1}	4.32 ± 0.22*	3.68 ± 0.19$^{\#}$
8WEEKS	3.17 ± 0.19	2.98 ± 0.13 ^{NS1}	4.74 ± 0.39*	3.79 ± 0.21$^{\#}$
12WEEKS	3.11 ± 0.15	3.01 ± 0.11 ^{NS1}	4.24 ± 0.20*	3.98 ± 0.09 ^{NS2}

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

3.2 Effect of (+)-catechin hydrate on plasma nitrite levels in experimental groups

HSHF group had significantly higher plasma nitrite levels compared to CD group throughout the study. At the end of 12 weeks, the levels were 36% higher ($p<0.001$). (+)-catechin hydrate supplementation in HSHF + CH group reduced plasma nitrite levels by 14.8% ($p<0.05$) and 20%

($p<0.05$) during 4th and 8th week respectively compared to HSHF + CH group while the change was non-significant at the end of study (Table 3). (+)-catechin hydrate had no effect on rats fed control diet.

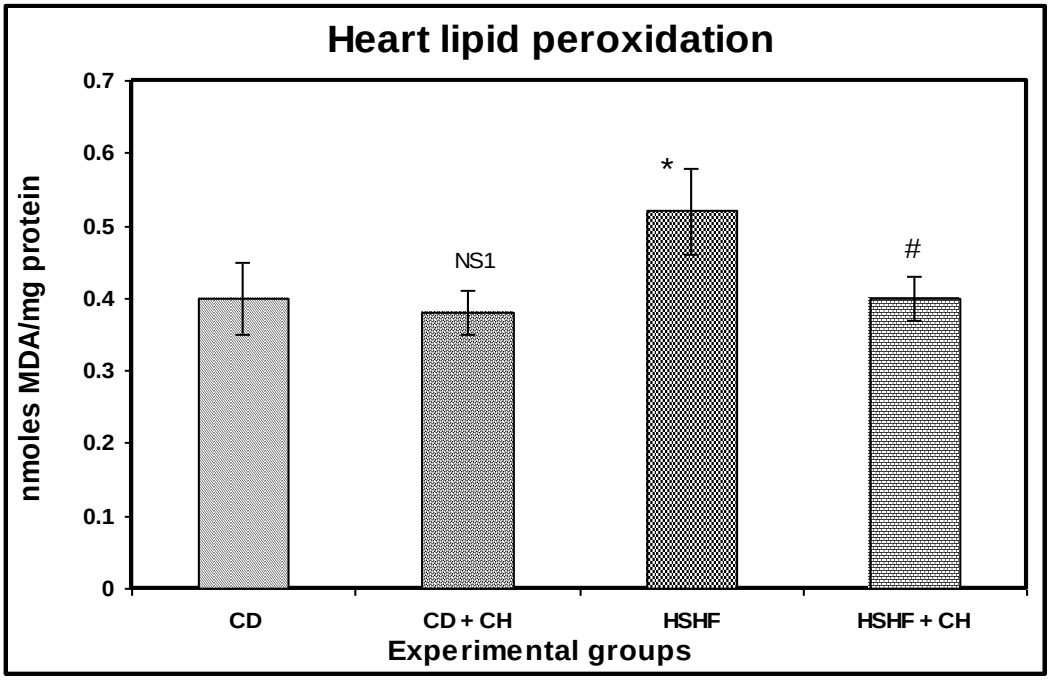

Figure 1 Effect of (+)-catechin hydrate on heart lipid peroxidation in control diet fed and high sucrose high fat diet fed rats. Values are mean ± SD with 6 animals per group. *$p<0.05$ vs. CD; #$p<0.05$ vs. HSHF; NS1 non-significant change vs. CD.

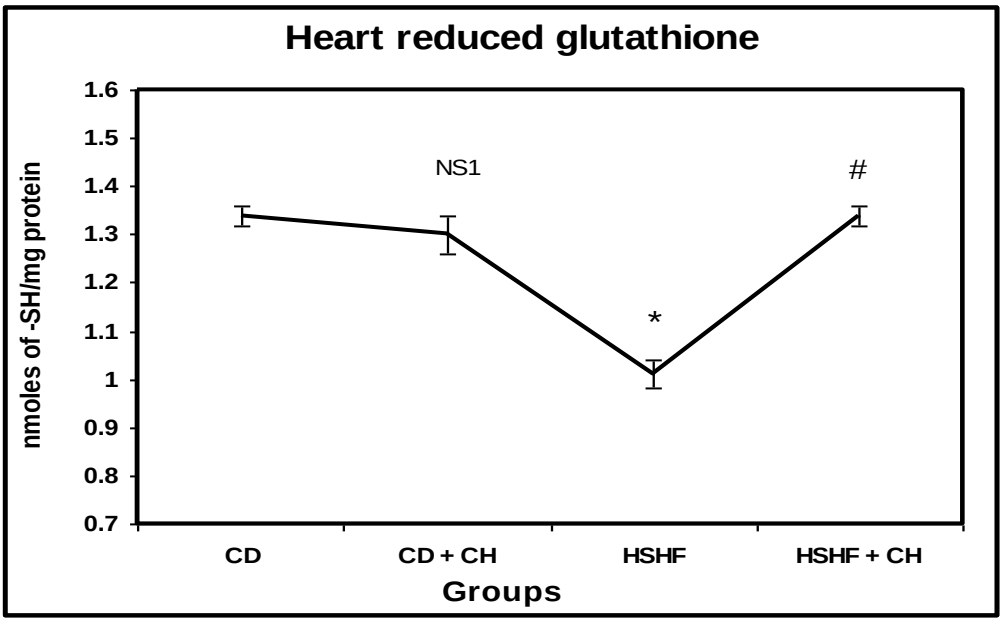

Figure 2 Effect of (+)-catechin hydrate on liver and heart glutathione levels in control diet fed and high sucrose high fat diet fed rats. Values are mean ± SD with 6 animals per group. *$p<0.05$ vs. CD; #$p<0.05$ vs. HSHF; NS1 non-significant change vs. CD.

3.3 Effect of (+)-catechin hydrate on cardiac lipid peroxidation and reduced glutathione levels

HSHF group rats showed increased MDA levels in heart by 30% ($p<0.001$) compared to CD group (Figure 1). (+)-catechin hydrate reduced heart lipid peroxidation by 20 % ($p<0.05$) in HSHF + CH group compared to HFHS group. However the effect was non-significant in control diet fed rats. Reduced glutathione (GSH) levels which decreased by 20% ($p<0.05$) in HSHF group compared to CD were restored by 37.5% ($p<0.001$) in rats co administered (+)-catechin hydrate and high fat high sucrose. The administration of (+)-catechin hydrate had non significant effect in rats fed control diet (Figure 2).

3.4 Effect of (+)-catechin hydrate on cardiac antioxidant enzymes

In heart, activities of SOD, GST, GR and GPx in HSHF group decreased by 43.5% ($p<0.0001$), 49% ($p<0.01$), 49.4% ($p<0.0001$) and 51.6% ($p<0.0001$) respectively compared to CD group. GST and GPx activity showed significant increase of 22.1% ($p<0.05$) and 33.1% ($p<0.01$) respectively in HSHF + CH group compared to HSHF group while activities of other antioxidant enzymes remained unaltered. Administration of (+)-catechin hydrate in CD + CH group showed increase in activity of SOD and GST only compared to CD group ($p<0.01$ in both cases). Catalase activity showed non-significant change on administration of (+)-catechin hydrate in CD + CH and HSHF + CH group (Table 4).

Table 4 Cardiac antioxidant enzyme activity in experimental groups

	GROUPS			
	(CD)	**(CD + CH)**	**(HSHF)**	**(HSHF + CH)**
Catalase (nmoles of H_2O_2 decomposed/min/mg protein**)**	16.15 ± 1.33	15.36 ± 1.00 ^{NS1}	10.14 ± 1.99*	9.84 ± 1.49 ^{NS2}
SOD (units/mg protein)	12.80 ± 1.56	15.68 ± 2.65*	7.25 ± 0.91*	7.68 ± 0.84 ^{NS2}
GST (nmoles of GSH-CDNB conjugate formed/min/mg protein)	55.10 ± 6.47	64.76 ± 7.61*	28.06 ± 4.94*	34.26 ± 1.35*, $^{\#}$
GR (nmoles of NADPH oxidized/min/mg protein)	29.75 ± 1.62	27.61 ± 1.86 ^{NS1}	15.05 ± 1.05*	12.46 ± 2.02*, $^{\#}$
GPx (nmoles of NADPH oxidized/min/mg protein)	19.04 ± 0.68	18.37 ± 1.62 ^{NS1}	9.20 ± 0.57*	12.25 ± 1.96*, $^{\#}$

Values are mean ± SD with 6 animals per group. *$p<0.05$ vs. CD; $^{\#}p<0.05$ vs. HSHF; ^{NS1}non-significant change vs. CD; ^{NS2}non-siginicant change vs. HSHF.

4. Discussion

Effect of high sucrose high fat diet on the development of an obese type 2 diabetic rat model has previously been studied in our laboratory [20]. In this model, persistent hyperglycemia was observed. It was presumed that high sucrose high fat diet causes oxidative stress as consistent hyperglycemia can result in the formation of reactive oxygen species [21]. This study clearly shows imbalance in oxidative stress parameters in plasma and heart on administration of high sucrose and high fat diet.

In this study, depletion of plasma uric acid levels was observed in high sucrose high fat fed rats. One study has shown decreased plasma uric acid in adult rats fed high sucrose low magnesium diet [22]. Administration of (+)-catechin hydrate increased levels of uric acid which may be one of the reasons for protection against diet induced oxidative stress. According to a study, administration of EGCG in healthy

human individuals increased plasma antioxidant activity which was not due to changes in EGCG concentration but due to changes in plasma urate concentrations, which might have interfered with the effect of EGCG to promote antioxidant activity [23].

A previous study has shown that sucrose fed male rats display higher plasma nitrite and nitrates levels [2]. Most of the cytotoxicity attributed to NO is rather due to peroxynitrite production [24]. The latter is formed when NO reacts with superoxide ions. Peroxynitrite interacts with lipids, DNA, and proteins via direct oxidative reactions or via indirect, radical-mediated mechanisms. These reactions can trigger cellular response causing oxidative injury, committing cells to necrosis or apoptosis which can be crucial in metabolic conditions such as myocardial infarction, coronary heart diseases, diabetes and stroke [25]. A study has shown that green tea catechins are more effective than Trolox (synthetic antioxidant) in protecting amino acids such as tyrosine or amino acids of apolipoprotein B in LDL-C against nitration damage [26]. These results are consistent with our study as (+)-catechin hydrate decreased level of plasma nitrite in rats fed high sucrose and high fat.

In our study, heart was affected in terms of lipid peroxidation and development of oxidative stress upon administration of high sucrose high fat diet. Increased oxidative stress leading to damage such as lipid peroxidation could be due to oxygen free radical production or due to decreased protection by enzymatic and non-enzymatic antioxidants [27]. Short term consumption of a high sucrose diet negatively affects the balance of free radical production and antioxidant defence in rats which leads to increased susceptibility of lipids to peroxidation [2]. Administration of (+)-catechin hydrate reduced cardiac lipid peroxidation and hence oxidative stress in rats fed high sucrose high fat diet. Reports in literature have shown that green tea extract reduces level of lipid peroxides in heart of streptozotocin diabetic rats [28]. Yang *et al.* have shown that catechins prevent LPO by scavenging lipid peroxyl radicals and by deactivation of oxidative stress sensitive transcription factor NF-kB [29]. In work carried out by Parvez *et al.* in 2006, it was shown that Drug induced oxidative stress is ameliorated by catechins administration [30]. In the same study, catechins showed significant decrease in LPO levels, H_2O_2 generation and protein carbonyls in liver and kidney of tamoxifen-induced oxidative stress.

Glutathione deficiency in high sucrose high fat diet fed rats contributes to oxidative stress in these animals which may play a key role in pathogenesis of many diseases like diabetes. The observed significantly increased levels of GSH in heart might be due to antioxidant properties of bioactive (+)-catechin hydrate. Studies have reported that increase in green tea consumption increases the index of total antioxidant status along with slight increases in serum GSH content [31]. Studies have shown that polyphenols present in green tea may offer an indirect protection by activating endogenous defense systems [30]. One recent study suggests dietary polyphenols can stimulate antioxidant transcription and detoxification defense systems linked to glutathione and its related enzymes through antioxidant response elements (ARE) [32].

Assessment of antioxidant enzymes demonstrate that heart showed significant reduction in activities of SOD, GST, GR, and GPx on feeding high sucrose high fat diet which is an indication of high sucrose high fat induced oxidative stress. A study reported decrease in heart SOD activity in rats fed sucrose compared with starch fed group [33]. However, they did not find any difference between glutathione peroxidase and catalase in these two groups. In our study, there was a significant reduction in heart Catalase activity in rats fed high sucrose high fat diet compared with control diet fed rats.

Out of above antioxidant enzymes discussed administration of pure (+)-catechin hydrate increased GST and GPx activity while GR activity significantly decreased in rats fed high sucrose high fat. Catechins are known for their antioxidant activity. They upregulate antioxidant enzymes [34] and scavenge ROS [35] like superoxide (O_2^-), hydroxyl (OH^-) and peroxyl radicals. It has been reported that green tea

catechins increase the activities of SOD and Catalase in fruitfly by upregulating the genes producing these enzymes [36]. Some studies have shown that feeding rats with green tea leaves significantly increases liver GST activity and providing mice with green tea polyphenols in their drinking water also significantly increases GST activity in liver and small intestine [34]. On the contrary, one study showed that green tea administration to diabetic rats reduced lipid peroxides and activity of antioxidant enzymes [28].

5. Conclusion

The present study clearly shows that high sucrose high fat diet causes an imbalance in oxidative stress parameters. (+)-catechin hydrate administration seems to be beneficial in restoring plasma and tissue antioxidants.

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