International Journal of Scientific Research in Science and Technology
Print ISSN: 2395-6011 | Online ISSN: 2395-602X (www.ijsrst.com)
doi : https://doi.org/10.32628/IJSRST218451

Efficacy of Chocolate on Hypercholestrolemic Rats

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Article Info
Volume 8, Issue 4
Page Number : 332-340

Publication Issue
July-August-2021

Article History
Accepted : 15 July 2021
Published : 20 July 2021

ABSTRACT

Chocolate comprises number of raw and processed foods produced from the seed of the tropical cocoa tree. Chocolate is produced from cocoa mass added with sugar and cocoa butter. Chocolate has been promoted for its health benefits, as it seems to possess substantial amount of antioxidant that reduces the formation of free radicals. Researchers concluded that chocolate containing higher amount of cocoa have higher procyanidins content, so higher antioxidant capacities. Evidence suggests that regular consumption of cocoa products containing flavanol may reduce the risk of cardiovascular diseases. Thus, the present study was carried out to assess the effect of chocolate on lipid profile of hypercholesterolemic rats. The rats were grouped under three categories; Triton 400mg was injected to experimental rats to make it hypercholesterolemia. Based on the objectives a cocoa rich chocolate sample was developed using cocoa seed, cocoa butter, sugar and lecithin and the experimental hypercholesterolemia rates were feed with chocolate sample for duration of 20 days, and the lipid profile was analysed at initial and at final days of supplementation. During the period of supplementation, the Cholesterol and LDL level were decreased gradually which may be due to the supplementation of chocolate. Significant reduction was observed at 5% level. Thus, it was concluded that in the present study there was a significant effect on lipid profile of chocolate supplemented with hypercholesterolemic rats.

Keywords : Chocolate, Rat study, Lipid profile, Procyanidins

I. INTRODUCTION

Cardiovascular disease, including coronary heart disease (CHD) and stroke, remains the leading cause of morbidity and mortality worldwide.A growing body of evidence suggests that chocolate may decrease the risk of CVD via an ability to lower blood pressure, improve endothelial function, and improve insulin sensitivity [1].Evidence is accumulating that some forms of cocoa and chocolate, rich in flavonoids, may have the potential to improve cardiovascular health [2].

Hypercholesterolemia is a lipoprotein metabolic disorder characterized by high serum low density lipoprotein and blood cholesterol. It has been reported by [3]as one of the most important risk factors in the development and progression of atherosclerosis that led to cardiovascular diseases (CVDs). Hypercholesterolemia is a major problem to many societies as well as health professionals because of the close correlation between cardiovascular diseases and lipid abnormalities [4,5]. Clinical trials have demonstrated that intensive reduction of Plasma LDL-C levels could reverse atherosclerosis and decrease the incidence of CVDs [6,7].

Inflammation plays a role in the development of atherosclerosis and coronary heart disease [8]. Elevated markers of inflammation, in particular C-Reactive protein (CRP), are associated with an increased risk of future cardiovascular events in healthy subjects, in patients with stable or unstable coronary artery disease and acute myocardial infarction (9,10). Although the prognostic value of CRP in patients with myocardial infarction has not been tested in large studies, several data indicatethatCRPisan important marker of risk also in this clinical setting [11,12].

Feeding rats with high cholesterol diet will result in increase of triglyceride, serum total cholesterol levels and cardiac biomarkers which are the indicator of cardiovascular diseases. Hypercholesterolemic animals are useful models for studies on cholesterol homeostasis, and drug trials to better understand the relationship between disorders in cholesterol metabolism, atherogenesis as well as possible treatments for the reduction of circulatory cholesterol levels [13,14].

Considering the above literature facts, the investigator planned to evaluate the plasma lipid profile lowering activity of chocolate in rats fed with a high cholesterol diet, thus reducing the risk of cardiovascular diseases.

The objective of the study was to find the lipid lowering effect of cocoa based chocolate in hypercholesterolemic rats.

II. METHODS AND MATERIAL

2.1. EXPERIMENTAL PROCEDURE

Male Wistar Albino rats weighing 150 to 300g were used for study. The animals were procured from the animal house, Nandha College of pharmacy, Erode. The animals were grouped and housed in Metallic cages with 3 animals per cage and kept in a room with controlled condition of 37°C, with 12 hours light/ dark cycle. The animals were acclimatized to laboratory conditions for 2 days before commencement of experiments. The animal care and handling were done according to the regulation of institution, with good laboratory practice on animal experimentation. The protocol of study with Register number: 688/02/C was approved by CPCSEA and experiments were performed in the laboratory according to the ethical guidelines suggested by IAEC.

2.2. GROUPING OF ANIMALS

The studies were carried out using male wistaralbino rats of weight 100 to 300g. The rats were divided into 3 groups of 6 animals each.

Group I: Normal control rats

Group II: Rats induced with hypercholesterolemia

Group III: Hypercholesterolemic rats treated with test sample.

Group I was given only the saline solution 0.8 ml of saline solution per kg of body weight through proper vehicle [15]. Group II and Group III were induced with hypercholesterolemia using Triton 400 mg per kg body weight. The hypercholesterolemia was induced after 72 hours of ingestion of triton, and their initial lipid profile were noted. According to [16] dark chocolate supplementation for three weeks in subjects

significantlyincreasesHDL-cholesterol compared to the unsupplemented counter parts. So the Group III animals were supplemented with test sample of 30g per day for the duration of 20 days. Procyanidins are a class of polyphenolic compounds found in several plant species and may be present as individual monomers or, in some cases, as oligomeric units by ref[17]. The procyanidin content of the chocolate samples were measured using the ORAC assay previously developed by [18]. The procyanidins content of chocolate was obtained from the analytical data given by Adamson [19] and according to their data the quantification of procyanidins in chocolate was 1.7mg/g. A study conducted by [20] incrataegusazarolus has isolated the procyanidins from the fruit and has given procyanidins extract from fruit to the hypercholesterolemic mice in three proportions as 10mg, 50mg and 100mg in which 50mg showed a positive effect on Hypercholesterolemia. Based on this in the present study the procyanidins content of 50mg containing 30g chocolate was given to the hypercholesterolemic rats to find the lipid lowering effect.Group II were given chow diet for the experimental period containing calories 3.1 kcal/g, protein 25g, carbohydrate 58g and fat 17g. The chow diet and triton were brought from Goodwell company, Bangalore.

The composition of chow diet was given in Table 1.

Table 1 : Composition of chow diet

Ingredients (g/kg)	Composition
Ground corn	520.40
Dehulled soyabean meal	240.00
Ground Oats	133.60
Wheat middling	40.80
Soya bean oil	39.20
Corn gluten meal	10.00
Iodized salt	10.00
Brewers dried yeast	0.5
Calcium Carbonate	5.50

2.3. ANALYSIS OF SAMPLE

At the end of experiment the animals were anesthetized with chloroform and the blood sample was collected from the orbital sinus of the rats and the serum was separated by centrifuge by [21] at 3000 rpm for 15 minutes and utilized for various biochemical assays.

2.4. BODY WEIGHT

Rats were weighed and randomly assigned to different experimental groups (Six animal per group) [22]. The body weight of animals was observed using electronic weighing balance in all groups at the beginning and end of the experiment to know the alteration in their body weight.

2.5. BIOCHEMICAL ANALYSIS

The biochemical analysis including triglyceride, HDL, and total cholesterol was performed before and after the period of study from serum of animals. They were determined in the serum of the rats by adopting the protocol outlined in the manufacture's assay with enzymatic tinder kits bought from M/s. Agappe diagnostics, Kerala. The LDL and VLDL was calculated using fried Wald equation: LDL-cholesterol= Total cholesterol – (HDL Cholesterol+ VLDL cholesterol) and VLDL cholesterol = Triglyceride/5 (ref 4).

2.6. STATISTICAL EVALUATION OF EXPERIMENTAL CONSTITUENTS

The data's were expressed as mean and standard deviation and analysed with t-test. The level of statistical significance observed at 5% level.

III. RESULTS AND DISCUSSION

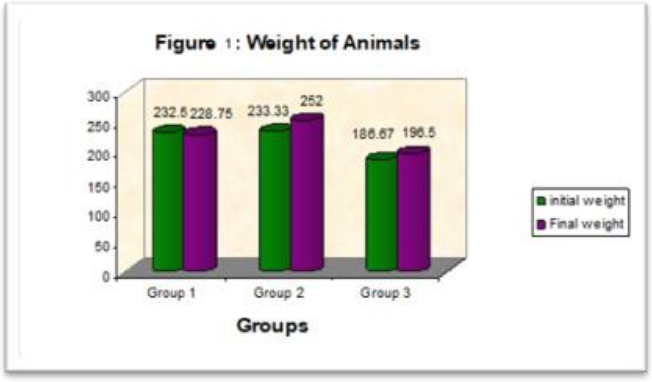
Cocoa and chocolate products have been known to contain high level of flavan-3-ols, polyphenolic compound which have significant antioxidant activity. Studies have shown that the intake of this polyphenolic rich fractions derived from cocoa,

increased the resistance of LDL to oxidation and suppressed the formation of atherosclerosis in hypercholesterolemic rabbits. So a chocolate sample rich in flavanol was developed, evaluated organoleptically and supplemented to rats to find out the effect of cocoa based chocolate on cardiovascular diseases.

3.1 Weight of animals

Weight is an indicator of administration of food or fatty acid deposition. The initial weight was noted and after the period of study the final weight was also noted.

In this study for group I the mean initial weight was 232.5±36.80 and mean final weight was 228.75±44.5 which has decreased from initial weight. For group II the mean initial weight was 233.3± 30.27 and the final weight was 252.0±29.8 which was found to be increased from the initial level. The increase in weight in group II was due to fat deposition in hypercholesterolemic rats which was fed with chow diet. For group III the mean initial weight was 186.67±77.88 and the mean final weight was 196.5±68.5 which has increased than the initial level. Figure 1 shows the initial and final weight of animals.



A study by [23] showed no significant changes observed in weight gain among the control and hypercholesterolemic animals, while[24] reported a linear weight increase in all the animals fed with both experimental and control diets. However, [4 and 25]reported similar weight loss as observed in this study with diet-induced cholesterol for hypercholesterolemic rat models. By study by [26]there were significant differences in the weight gain pattern of the animals fed with hypercholesteremic diet compared to those fed with the control diet. While the animals fed on the control diet maintained a consistent weight gain throughout the period, those fed with hypercholesterolemia diet only gained weight up till the 4th week.

In the present study there was linear weight increase observed in experimental group III fed with chocolate sample compared to control group I, but a significant difference was observed in rats with hypercholesterolemia group II, at 5% significant level. No significant changes were seen for group I at initial and final level. The increase in weight of group II was due to hypercholesterolemia with chow diet. There was no significant change observed in group III, which was supplemented with chocolate sample. So it is inferred that the differences in weight occur in rats during cardiovascular diseases may be due to the deposition of fat in the body.

3.2. THE INITIAL AND FINAL LIPID PROFILE OF ANIMALS

A study by [27] showed that there was a significant elevation of serum total cholesterol (TC), low density lipoprotein (LDLC) and triglycerides in the Hypercholesterolemia induced rats, while there was no such difference in the HDL-C of both groups. Increase in LDL-C has been pointed out as one of the risk factors for the development of atherosclerosis and related cardiovascular diseases (ref 26). The lipid profile of the animals was given in this Table 2.

Table 2 : The Initial and Final Lipid Profile of Control and Experimental Groups

* Significant at 5% level

Lipid profile	Group I		't'-value	Group II		't'-value	Group III		't'-value
	Initial	Final		Initial	Final		Initial	Final	
Cholesterol (mg/dl)	54.91±8.46	43.5±4.10	5.03*	61.54±6.50	59.7±11.46	1.31 ns	69.17±15.87	46.1±13.15	13.34*
Triglyceride (mg/dl)	63.49±7.70	63.0±1.41	0.233 ns	19.52±2.67	16.0±2.828	2.5*	34.62±7.43	43.5±38.89	3.06*
HDL (mg/dl)	13.72±2.11	17.0±1.41	1.89 ns	15.38±1.62	8.0±5.657	5.24*	17.29±3.96	14.0±8.485	1.05 ns
LDL (mg/dl)	28.48±6.85	14.9±4.38	6.56*	42.22±4.69	43.5±1.83	0.76 ns	44.95±12.54	23.4±3.11	7.98*
VLDL (mg/dl)	12.5±1.54	12.6±0.28	0.112 ns	3.94±0.52	3.2±0.56	1.254 ns	6.92±1.48	8.7±27.77	1.369 ns

NS -Not Significant

The above table reveals that the mean initial cholesterol level of group I was 54.91±8.46 and final mean cholesterol level was 43.5±4.10, the initial triglyceride level was 63.49±7.70 and final mean triglyceride level was 63.0±1.41, the initial High Density Lipoprotein (HDL) level was 13.72±2.11 and final HDL level was 17.0±1.41, the initial Low Density Lipoprotein (LDL) level was 28.48±6.85 and final LDL level was 14.9±4.38 and the initial Very Low Density Lipoprotein (VLDL) level was 12.5±1.54 and final VLDL level was 12.6±0.28. For group I the Cholesterol and LDL level was found to be decreased and, but no such difference was observed in triglyceride, HDL and VLDL. This shows that group I getting normal saline has no difference in their lipid profile after a period of 20 days. For group II the initial cholesterol level was 61.54±6.50 and final cholesterol level was 59.7±11.46, the initial triglyceride level was 19.52±2.67andfinaltriglyceridelevelwas 16±2.82, the initial HDL level was 15.38±1.62 and final HDL level was 8.0±5.65, the initial LDL level was 42.22±4.69 and final LDL level was 43.5±1.83 and the initial VLDL level was 3.94±0.52 and final VLDL level was 3.2±0.56. For

group II, the cholesterol, LDL and VLDL have no significant difference and their lipid profile before and after did not differ significantly, but the HDL and triglyceride level found to be decreased from the initial level. This shows group II remained as hyperlipidemic during the experimental period without any change. For group III the initial cholesterol level was 69.17±15.87andfinalcholesterollevelwas 46.1±13.15, the initial triglyceride level was 34.62±7.43 and final triglyceride level was 43.5±38.89, the initial HDL level was 17.29±3.96 and final HDL level was 14±8.48, the initial LDL level was 44.95±12.54 and final LDL level was 23.4±3.11 and the initial VLDL level was 6.92±1.48 and final VLDL level was 8.7±27.77. For group III there was a significant decrease observed in the cholesterol, triglyceride and LDL level from the initial level, while the HDL and VLDL do not have a significant difference. This shows the effect of supplementation to group III for a period of 20 days, with a reduction in their cholesterol and LDL level.

Figure 2 shows the mean initial and final cholesterol level of rats.

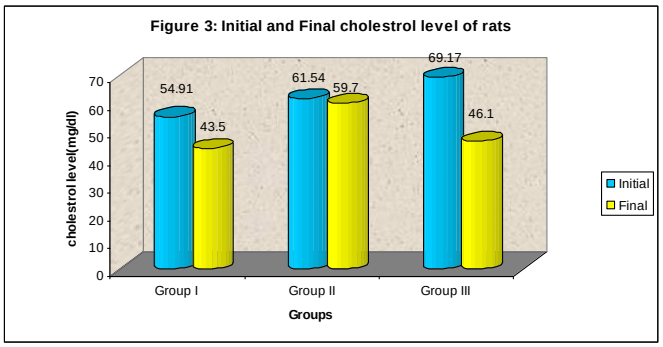


Figure 3 shows the mean initial and final Triglyceride level of rats.

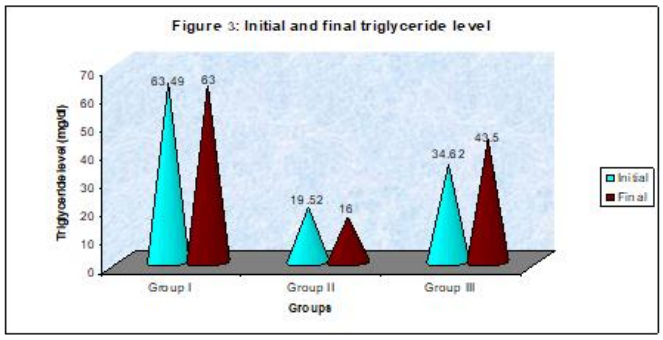


Figure 4 shows the mean initial and final HDL level of rats.

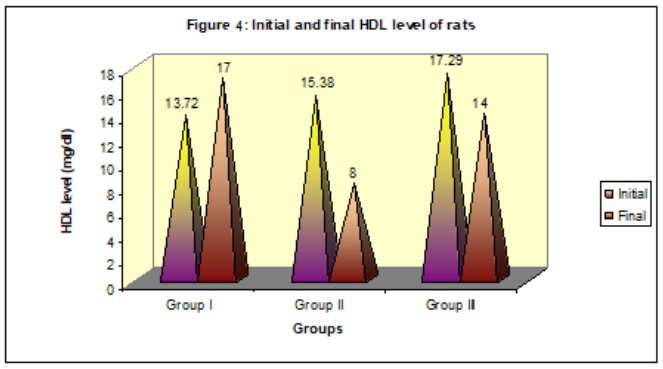


Figure 5 shows the mean initial and final LDL level of rats.

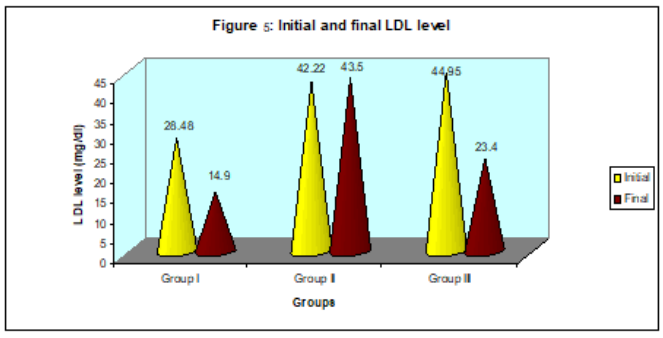
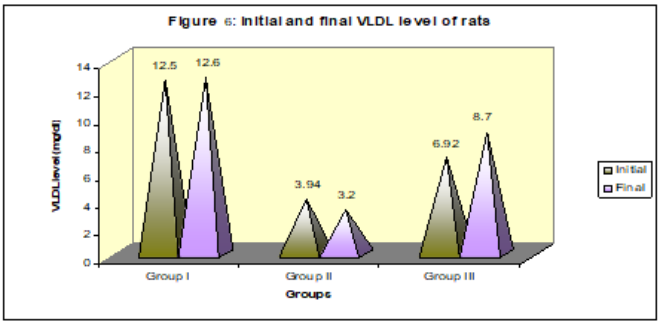


Figure 6 shows the mean initial and final VLDL level of rats.



Many studies have reported high dietary fat and cholesterol induce hypercholesterolemia in animal models like rats [28,29]. Similar results were observed with the hypercholesterolemia fed animals having elevated lipid status than the control in our study. In a study by [30]ingestion of cocoa procyanidin reduced plasma total cholesterol levels in rats fed on a high cholesterol diet. The accumulation of cholesterol and triglyceride in liver of HCD-fed rats was significantly decreased by addition of 1% cocoa procyanidins to the diet.

A significant difference was observed for each group at 5 % level. From the present study it is inferred that group I getting normal saline has not shown any difference in their lipid profile, group II has shown elevated lipid profile and remained hypercholesterolemic and group III showed a reduction in their lipid profile than initial level, when compared to hypercholesterolemic rats.The study by [31] suggested that people who have myocardial infarction tend to have higher levels of triglycerides and Very Low Density Lipoprotein (VLDL) than normal people. A study by Framinham suggested that raised levels of triglycerides are associated with increased cardiovascular health disease risk only in the presence of reduced HDL cholesterol. The present study confirms that the lipid profile was increased for cardiovascular diseases, but a decrease was seen during the supplementation of cocoa based chocolate, which confirm the effect of cocoa based chocolate for cardiovascular diseases.

IV. CONCLUSION AND RECOMENTATION

The weight of the animals was assessed for the three groups at initial and final level. The increase in weight was seen in group II when compared to group I and group III at final level.

The lipid profile of the animals was analyzed for their initial and final level, and significance was observed for each group.

The lipid profile in Group I (control) showed a decrease in cholesterol, LDL and an increase in Triglyceride, HDL and VLDL than the initial level. In Group II, there was no significant difference observed before and after the study period in their cholesterol, LDL and VLDL level, but the HDL and triglyceride was found to be decreased than the initial level. In group III there was a significant difference observed among cholesterol, triglyceride and LDL level as they were decreased from the initial level, but there is slight decrease in HDL and VLDL level, which showed the effect of supplementation.

In conclusion the present study showed a favorable effect of cocoa-based chocolate on lipid profile. The recommendation of study suggests that the regular feeding or intake of cocoa based chocolates may favour cardiac health; the invivo assay on human being may prevent the increased mortality rate due to cardiovascular diseases.

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Cite this article as :

Nisha. S. M, Sujatha. T, Thilagavathi, "Efficacy of Chocolate on Hypercholestrolemic Rats", International Journal of Scientific Research in Science and Technology (IJSRST), Online ISSN : 2395-602X, Print ISSN : 2395-6011, Volume 8 Issue 4, pp. 332-340, July-August 2021. Available at doi : https://doi.org/10.32628/IJSRST218451
Journal URL : https://ijsrst.com/IJSRST218451