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EFFECT OF B - SITOSTEROL SUPPLEMENT ALONG WITH FAT MODIFIED DIET FOR THE MANAGEMENT OF DYSLIPIDEMIA

Smeea Fatima[1], Momina Shahid[2]*, Mahnoor Naseer Ahmed[3], Saad Shafi[4], Sadia Sabir[5], Noor Ul Ain Shah[6], Wajiha Arooj[7], Ribka Sabahat[8]

[1]Smeea Fatima (smeeafatima21c@gmail.com) Department of Nutrition & Dietetics, University of Management and Technology, Lahore, Pakistan
[2]*Momina Shahid (mominamalik14@yahoo.com) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan
[3]Mahnoor Naseer Ahmed (Mahnoornaseer077@gmail.com) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan
[4]Saad Shafi (Saadimehar125@gmail.com) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan
[5]Sadia Sabir (sadia.sabir@superior.edu.pk) Department of Human Nutrition and Food Technology, Faculty of Allied Health Sciences, Superior University Lahore, Pakistan
[6]Noor Ul Ain Shah (Noorulain.shah@superior.edu.pk) Department of Human Nutrition and Food Technology, Faculty of Allied Health Sciences, Superior University Lahore, Pakistan
[7]Wajiha Arooj (Wajeha.arooj@dnsc.uol.edu.pk) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan
[8]Ribka Sabahat (ribka.sabahat@dnsc.uol.edu.pk) University Institute of Diet & Nutritional Sciences, The University of Lahore, Pakistan

***Corresponding Author:** Momina Shahid
*Email: mominamalik14@yahoo.com

Abstract:
β – Sitosterol supplements have been one of the important Phytosterols. β – Sitosterol is used to lower the cholesterol in the blood which leads to a decrease in the risk of cardiovascular diseases. It is also recognized to lower the incidence of dyslipidemia. Objective of the study was to evaluate the effect of β- sitosterol supplement along with fat modified diet for the management of dyslipidemia. Randomized control trial was conducted. Total of 36 participants successfully completed the trial. Targeted population was patients suffering from dyslipidemia aged 35 to 55 years. All enrolled participants were allocated randomly in 2 groups. Treatment group (T1) was allocated to consume 900 mg beta-sitosterol supplement twice a day along with the fat-modified diet plan. The fat-modified diet plan was containing 1500 – 1600 kcal per day, with AMDR for carbohydrates 202.5 g, Protein 67.5 g, and fats 46.7 g. In the fat group, mostly MUFAs and PUFAs were prescribed. The control group was on a regular basal diet. Duration of study trial was 6 weeks. The results indicate significant difference between the pre and post-interventional treatment group (T1). In the treatment group, post-interventional serum total cholesterol, triglycerides, LDL and HDL are 140.55 ± 35.17 mg/dl ($p \leq 0.002$), 137.22 ± 36.86 mg/dl ($p \leq 0.00001$), 41.72 ± 7.39 mg/dl ($p \leq 0.0001$) and 107.22 ± 23.74 mg/dl ($p \leq 0.00002$) respectively. Furthermore, the treatment group also showed a significant difference if compared with the control group. Independent sample t-test shows p-value of serum total cholesterol ($p \leq 0.00007$), triglycerides ($p \leq 0.014$), and LDL ($p \leq 0.04$) show significant change. The

findings of the current study support the cholesterol-lowering effect of β- sitosterol supplements along with fat modified diet. It is also concluded that the intake of β - sitosterol supplements along with fat modified diet is helpful for the management of dyslipidemia and also lowers the risk of cardiovascular diseases. It is concluded that the current research study shows significantly positive results and supports our alternate hypothesis. It is suggested that the β – Sitosterol should be fortified in the food products to prevent and treat dyslipidemia. In diet-based therapies, beta-sitosterol supplements or beta-sitosterol-enriched food products should be recommended to treat various ailments.
Keywords: β – Sitosterol, Cholesterol, Dyslipidemia, Fat Modified Diet, Lipoproteins, Phytosterol, Supplement

1. Introduction:

Plant sterols were chemically reported in 1922 (Bishnoi *et al.,* 2017) Phytosterol is a phytonutrient (Wahlqvist *et al.,* 2020) class of lipid molecules found in the various plants and similar in structure to the cholesterol. (Gachumi *et al.,* 2021) These phytonutrients are not formed inside the human body. (Wang *et al.,* 2018) In 1951, plant sterol and stanols were recognized as cholesterol lowering agents. (Fumeron *et al.,* 2017) More than 250 types of plant sterols were recognized in the recent past. (Moreau *et al.,* 2018) Most commonly known phytosterols are β- sitosterol, stigma sterol, campesterol, avenasterol and brassica sterol. (Vezza *et al.,* 2020)

β- Sitosterol is a naturally occurring plant sterol with the steroidal moiety. (Patel *et al.,* 2017) Plant sterols are present in the cell membrane of the plants. Phytosterols are the biologically active component which constitute a diverse group of triterpenes. (MS U *et al.,* 2018). Plant sterols and stanols are having same structure as cholesterol but different from the side chain at carbon number 24 and in the configuration & position of double bond structure. Stanols are formed due to the hydrogenation of sterols without any double bond at carbon-5. (Fumeron *et al.,* 2017) Having hydrophobic steroidal skeleton, one hydroxyl group at the carbon-3 and a side chain is present on the D ring. (Feng *et al.,* 2020) PS is a compound having main structure with cyclopentane poly-hydro-phenanthrene. It is widely present in different plants and important component of plant cell biofilm. Its molecular structure is same as cholesterol. (Yuan *et al.,* 2019)

Pharmacokinetics is very important in order to understand the health promoting effect of phytosterols. Phytosterol's bioavailability relies on several factors for example genetic factors, intestinal transporters and their different molecular types. Although phytosterol have same chemical structure like cholesterol, but has lower rate of intestinal absorption as compared to that cholesterol. Less than 5 % of phytosterols (Feng *et al.,* 2021) and 50 to 60 % of the cholesterol intestinal absorption occurs. (Salehi *et al.,* 2021) Phytosterol enter into the digestive system along with the food. After entering, it will bind with the NPC1L1 (Niemann pick C1 like 1) which is found in the apical membrane of cells present in intestinal lumen and intestinal epithelial cells absorb it. (Ticho *et al.,* 2021) Moreover some free plants sterols are also released into the lumen of intestine by the ATP Binding Cassette G5/8 (ABC G5/8) present in the cells of intestine. There are some molecules of phytosterols that bypass the above mechanism and enter into the blood circulation with the lipoproteins. Quantitative distribution of the lipoprotein is same as cholesterol's, due to this reason 70 to 80% molecules of phytosterol circulate in the low-density lipoprotein (LDL) particles. Another known mechanism of phytosterol's metabolism, in which phytosterol molecules bind with the ATP Binding Cassette A1 transporter (ABC A1) in the basolateral membrane of the cells which belong to intestine and then move to become a part of high-density lipoproteins (HDL) particles. After transporting into the liver, phytosterols also come back in intestine through ABC G5/8 protein at hepatobiliary interface. (Scolaro *et al.,* 2019)

Beta-sitosterol is also present in different non-dietary plant sources. BS is a natural micronutrient present in the higher plants. It is mainly present in the tissues and serum of healthy individual with a conc. of 800 to 1000 times less than the endogenous cholesterol levels. Glycoside and sitosterol in it, are also found in the serum but in minor concentrations. Beta – sitosterol are generally considered as naturally safe and effective dietary supplements. BS has been indicated to have many potential benefits. (Rashed, 2020) such as decrease the levels of cholesterol in the human body. β- Sitosterol competes with the intestinal absorption of cholesterol due to this property of phytosterol it is

considered as cardiovascular protective agent. (Patel *et al.,* 2017) Plant sterols and stanols play beneficial roles beyond lowering the LDL-C (low density lipoprotein Cholesterol). (Plat *et al.,* 2019) Cholesterol is a term which is originated from the antique words "Chole" meaning "for bile", "Stereos" meaning "for solid" and having suffix (chemical) "ol" meaning "for an alcohol". Mainly, it is an organic molecule known as sterol or a modified sterol. It is also classified under the molecules of lipids. (Obge *et al.,* 2015) Cholesterol is a lipid or fat which take part in different prominent functions in various biochemical and biophysical activities. Cholesterol is also working as a precursor of various steroidal hormones, bile salts & vitamin D. Cholesterol is necessary for the maintenance of cell membrane rigidity, (Yuan *et al.,* 2020) permeability and fluidity. Cholesterol is a well-known and major sterol in the animals and humans. There are various types of sterols are present in the plants known as phytosterols. (Ogbe *et al.,* 2015)

Homeostasis of cholesterol is important for cellular and systemic functions. Cholesterol levels in the cells also reflects the dynamic balance in the uptake, synthesis, export & esterification. Esterification is a procedure in which cholesterol is transformed into the neutral cholesteryl esters. It may be for the storage in the form of lipid droplets or for the secretion of various constituents of lipoproteins. Imbalanced cholesterol levels in the serum underlies various cardiovascular disorders. It is also a leading cause of other diseases such as cancers and neurodegenerative diseases. (Luo *et al.,* 2020) Arteriosclerosis is also interlinked with the change in plasma concentration of lipoproteins. Circulating plasma levels of cholesterol is also dependent on the biosynthesis through the liver and on the absorption of cholesterol through the intestine. (Yuan *et al.,* 2020)

β- Sitosterol also function as an immunomodulators, tumor cell growth inhibitor, and also having antioxidant capacity. In the near future, it will be considered as a good chemoprotective component for treating different kinds of cancer such as breast cancer and prostate cancer. (Patel *et al.,* 2017) Moreover, various studies were undertaken that explains the β- sitosterol as anti-inflammatory (Corrêa *et al.,* 2017) & antipyretic effects. (Vilahur *et al.,* 2019) It is helpful for the activity of central nervous system (CNS), good analgesic effect, anti-viral and anti-diabetic effects. β- Sitosterol act as good wound healer, protective in pulmonary fibrosis and helpful for COVID-19. (YadaV *et al.,* 2002) Beta-sitosterol is a major compound present in plants. Various evidences were reported in the studies that beta- sitosterol having numerous biological activities for example sedative and anxiolytic effects, anti-microbial, cholesterol reducing effects, hepatoprotective effects. (Babu & Jayaraman, 2020)

Dyslipidemia is a disorder related to metabolism of lipids. Lipid metabolism disorder leads to the abnormal lipid in blood. Such as abnormal increase in levels of triglycerides, total cholesterol, low density lipoprotein cholesterol (LDL) and reduction in high density lipoprotein (HDL). It mostly leads to the series of cardiovascular diseases for example, MI (Myocardial infarction), atherosclerosis, diabetes (DM), hypertension (HTN) and cardiac death. (Yuan *et al.,* 2019). Cardiovascular Diseases (CVD) are included in the most leading causes of mortality and morbidity in US (United States). Increase in level of LDL cholesterol are mainly linked with the high incidence rate of CVD. NCEP (National Cholesterol Education Panel) built a dietary therapy as primary cornerstone of strategies to reduce the LDL levels and risk of cardiovascular diseases. NCEP also added phytosterols (beta - sitosterol) in the Dietary guidelines of TLC (therapeutic lifestyle changes). (Devaraj *et al.,* 2004)

Various researches on the beta sitosterol give us the indication that we can use it as a nutritional supplement to compete with several diseases. (Yadav *et al.,* 2022) β- Sitosterol is the most common phytosterol, which is approved by the Food and Drug Administration (FDA) as vital cholesterol lowering nutraceutical agent. (Babu *et al.,* 2020) In the several countries almost 1.5 – 3 g/ day phytosterols are recommended to lower the risk of CHD (coronary heart disease). (Jones *et al.,* 2018) Different population-based surveys indicates that the average intake of plant sterol in 170 milligrams per day in United States. (Witkowska *et al.,* 2021) Chinese consumes on average 392.3 milligram of phytosterols a day. (Yang *et al.,* 2019)

USFDA establishes a health claim that food having stanols and sterols is effective for lowering the risk of cardiovascular diseases. Plant sterol and stanols are present in the fat-soluble fraction of plants. Foods containing sterol and stanols chemically resembles with cholesterol and action as cholesterol

lowering agent by reducing the intestinal absorption of cholesterol. Sterol specifically decreases the serum LDL and total cholesterol. (Devaraj *et al.,* 2004)
Phytosterols (beta-sitosterol) are available in the market in the form of tablets and capsules with or without other added multivitamins. (Patel *et al.,* 2017) Trend to use functional foods and supplements for controlling the cholesterol concentration is rapidly increasing in the European areas. Among all these, phytosterol (beta- sitosterol) recently achieve attraction due to its cholesterol reducing effect by staying in the frame of a healthy lifestyle by the EFSA (European Food Safety Authority). According to the European Union these products are categorized as food and also recommended for public to be freely purchased under self-prescription. (Poli *et al.,* 2021)
Phytosterols are present in many functional foods which are the part of our diet. (Plat *et al.,* 2019) Naturally occurring in various plants and plant derived foods such as fruits, vegetable oils, seeds, (Yang *et al.,* 2018) cereals, legumes, (Wang *et al.,* 2018) nuts, grains, black and green olives, avocado, cabbages, cauliflowers, sprouts and some other non-vegetable food items including mammalian liver and egg yolks. Highest phytosterol counts 4000 – 4130 milligrams per kilograms were reported in the wheat germs and sesame seeds whereas lowest phytosterol counts 950 milligrams per kilograms in the Brazil nuts (Feng *et al.,* 2020).

2. Rationale:

According to American Heart Association (AHA), Hypercholesterolemia is defined as elevated levels of High-density lipoproteins cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) in the blood which leads to excessive deposition of fat molecules inside the arteries and increases the risk of blockages. (McGowan, *et al.,* 2019) According to World Health Organization (WHO), rate of prevalence of elevated total cholesterol (TC) among adult population is 39%. (Gebreyes *et al.,* 2018) In the Pakistan almost 39.3 % population is diagnosed with hypercholesterolemia from which 90% are women and 83.9% are men. (Basit *et al.,* 2020). Major Phytosterols in the diet are 66% sitosterol, 22% campesterol, 8% stigma sterol and 4% sitostanol plus campestanol, mainly found in the cereal, bread, fruits vegetables and vegetable oils. They are important due to there inhibitory effect on the intestinal absorption of cholesterol. (Plat *et al.,* 2019) According to the European Food Safety Authority (EFSA), recommended dosage of phytosterol is 1.5 to 2.4 grams per day, to lower the cholesterol in the blood which leads to decrease the risk of cardiovascular diseases and it is also recommended and approved by the Food and Drug Administration (FDA). (Babu *et al.,* 2017) Therefore the main purpose of the current study is to evaluate the effect of β- sitosterol supplement along with fat modified diet for management of dyslipidemia.

3. Materials and Methods:

3.1 Study Design: To check the effectiveness of β- Sitosterol supplementation along with fat modified diet for the management of dyslipidemia randomized control trial was done in outdoor patient department, Farooq Hospital, Westwood Colony, Lahore.

3.2 Sample Size: Non-Probability Purposive Sampling technique were used. Sample size was calculated which is 15 for each group. After 20 % dropout sample size was 18 for each group.

3.3 Subjects: To check the eligibility criteria 59 Individuals were assessed. Their demographics history was taken (gender, education, occupation/ profession and contact/address). For anthropometric data participant's age, height, & weight were measured and BMI was calculated. After screening, 41 participants were selected who were meeting the inclusion criteria. Subjects suffering with dyslipidemia were included. Men and women of age 35 – 55 years were enrolled. Patients with fasting LDL level ≥ 62 mg/dl were included. Patients with good general health on the basis of medical and dietary history. According to the exclusion criteria, participants with Body Mass Index (BMI) of 40 kg/m^2 or above, patients with other concomitant disease, active infections or malignancy were not enrolled for the trial. Individuals with extreme dietary habits, eating disorders, alcoholism and cancer were also excluded. Individuals using any medications, dietary supplements or fortified foods with lipid-altering effects, such as phytosterol products were excluded for at least 4 weeks before the start of trial. People currently participated in any

other research study, Pregnant and lactating women were also excluded. Informed consent was taken. 4 Participants declined to participate after initial scrutiny. Total 37 participants were enrolled in the study for the intervention. Randomly, 18 participants were enrolled in Control group (T_0) and 19 participants were enrolled in the treatment group (T_1). In the 2nd week, 1 participant from the treatment group refused to continue the intervention. 36 participants successfully completed the trial.

3.4 Study Product and Diet Instruction: Participants of treatment group were allocated to consume 900 mg of beta-sitosterol (phytosterol) supplements twice a day along with fat modified diet plan for the duration of 6 weeks. Participants of the control group were on the regular basal diet. Fat modified diet plan was contained 1500 – 1600 kcal per day, with 54% carbohydrates of total energy (containing 202.5 g carbohydrate), 18 % protein of total caloric intake (containing 67.5 grams protein) and 28 % fats from the total calories (containing 46.7 grams fats). In the fat, mostly monounsaturated fatty acids and polyunsaturated fatty acids were included. General dietary guidelines were also provided to the participants of treatment group. Dietary guidelines provided were such as use multi grain or whole wheat flour, eat plain chapatti instead of paratha, take your dinner 2 hours before sleep, use fresh vegetables and fruits etc., use olive oil /soybean /mustard oil for cooking, drink plenty of water at least 8 to 10 glasses daily, do not eat sweetened candies, jams & jellies, mayonnaise etc. Avoid dining out, avoid all bakery and packet food items like biscuits, cakes etc. Avoid full cream or full fat dairy products such as milk, yogurt & cheese etc. Do not use canned foods. Red meat & fatty foods like French fries, chips, fast food and other fried items plus tran's fats like margarine and clarified butter were not allowed. Carbonated drinks & Sweeteners like sugar and condensed milk were not allowed.

3.5 Tests performed: After completing the intervention duration of 6 weeks. Same protocol of baseline visit was conducted in post-intervention. Age (years), weight (kg), height (m^2) and Body Mass Index (kg/m^2) were calculated. Blood samples were taken by the biochemical lab assistant. To check the effect of intervention, serum lipid profile tests were performed. Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were the main parameters. Pre- and Post-intervention data was compared to test the hypothesis. Control and treatment group data is compared to test the hypothesis.

3.6 Statistical Analysis: SPSS version 25.0 was used to tabulate and analyse the data. Descriptive and inferential statistics will be used to report the data. The quantitative variables such as age, height, weight, BMI, total cholesterol, triglycerides, HDL and LDL were evaluated using mean ± standard deviation ($\bar{x} \pm SD$) and standard errors. Two different parametric tests were performed. Quantitative variables of pre and post study results were compared by paired sample t-test at level of significance $p \leq 0.05$. Quantitative variables of control and treatment group results were compared by independent sample t-test at level of significance $p \leq 0.05$. The qualitative variables such as gender, study group was reported in the form of percentages and frequencies.

4. Results:

β- sitosterols along with fat modified diet were investigated to find out potential effect in the management of dyslipidaemia. Randomized control trial has done carefully by following the said procedure and results are divided into two major categories; Qualitative and Quantitative Results. The results of the studied parameters are convened below:

4.1 Qualitative Results: Gender, Smoking status, marital status and level of education are included as qualitative characteristics of the participants of both groups (treatment and control groups). Data was presented as frequency and percentage *n* (%) of qualitative characteristics of participants as shown in Table -1.

4.1.1 Gender: In the current study control group, participants were predominantly females 13 (36.1 %) and males 5 (13.9 %). In treatment group, participants were predominantly males 10 (27.8 %) and female were 8 (22.2 %).

4.1.2 Smoking Status: In the current study control group (T_0), 16 (44.4 %) participants were non-smokers and 2 (5.6 %) were smokers. In treatment group (T_1), 14 (38.9 %) participants were non-smokers and 4 (11.1%) smoker also shown in Table -1.

4.1.3 Marital Status: In the current study control group 16 (44.4 %) participants are married, 1 (2.8%) unmarried and 1 (2.8%) are widow as shown Table -1. In the current study treatment group, 15 (41.7%) participants are married, 1 (2.8%) unmarried, 1 (2.8%) widow and 1(2.8%) are divorced as shown in Table -1.

4.1.4 Educational Status: In the current study control group, 3 (8.3%) participants are at lower, 8 (22.2%) at middle and 7 (19.4%) are at higher educational status. In treatment group (T_1), 5 (13.9%) participants are at lower, 9 (25%) at middle and 4 (11.1%) are at higher educational status as shown in Table -1.

Table -1 Frequency and Percentage of Qualitative characteristics of Participants.

Characteristics		Sample Size (*n* = 36)	
		Control Group (T_0) ***n* (%)**	**Treatment Group (T_1)** ***n* (%)**
Gender	Male	5 (13.9 %)	10 (27.8 %)
	Female	13 (36.1%)	8 (22.2 %)
Smoking Status	Smokers	2 (5.6 %)	4 (11.1 %)
	Non – Smokers	16 (44.4 %)	14 (38.9 %)
Marital Status	Married	16 (44.4 %)	15 (41.7 %)
	Unmarried	1 (2.8 %)	1 (2.8 %)
	Widow	1 (2.8 %)	1 (2.8 %)
	Divorced	-	1 (2.8 %)
Educational Status	Lower	3 (8.3 %)	5 (13.9 %)
	Middle	8 (22.2 %)	9 (25 %)
	Higher	7 (19.4 %)	4 (11.1 %)

4.2 Quantitative Results:

Participants under study were randomly divided into 2 groups' treatment and control. The results were attained majorly by the anthropometric measurements and lipid profile analysis of blood samples. We analyze the results to compare the pre- and post-interventional difference in both groups (treatment group and control group). All the Quantitative data were reported as mean ± standard deviation as shown in Table -2.

4.2.1 Anthropometric Measurements: Anthropometric measurements of participants were taken before and after intervention. Mean ± Standard deviation of age (years), height (m^2), weight (kg) and body mass index (BMI) in kg/m^2 are displayed in the Table -2.

4.2.1.1 Age: Participants enrolled in the study were 35 to 55 years old as mentioned in the inclusion criteria. Mean ± standard deviation age of control group is 44.61 ± 5.13 years and treatment group 44.94 ± 6.72 years.

4.2.1.2 Height: Height of participants enrolled in the current study is reported as mean and standard deviation. Height of treatment group is 2.80 ± 0.32 m^2 and control group is 2.74 ± 0.27 m^2.

4.2.1.3 Weight: Paired sample t-test was applied to analyze the pre and post interventional weight of treatment group. In treatment group, Pre interventional weight was 83.27 ± 10.49 kg and post-interventional weight is 81.50 ± 9.84 kg as shown in Table -2. *p*- Value of treatment group is 0.031 which is less than 0.05 as shown in Table -2. Significant change was reported in the weight of the treatment group participants. Difference among pre and post interventional weight of treatment group participants is expressed in Figure -1. In control group, Pre intervention (T_0) weight was 77.83 ± 9.93 kg and post-interventional (T_0) weight was 78.08 ± 9.93 kg as shown in Table -2. *p*- Value of control group was 0.349 which is more than the 0.05. Non-significant change was reported in the weight of the control group participants.

Difference among pre and post interventional weight of control group participants is expressed in Figure -2.

An independent sample t- test was also applied to determine level of significance between treatment group (T_1) and control group (T_0). Equal variances assumed *p*- value is 0.307. It shows non-significant change in weight of treatment and control group as shown in Table -3.

Table -2 Distribution of Parameters between pre and post interventional treatment group (T_1) and Control group (T_0).

Parameters	Treatment group (T_1)			Control Group (T_0)		
	Pre-Intervention	Post-Intervention	*p*-Value	Pre-Intervention	Post-Intervention	*p*-Value
Weight (kg)	83.27 ± 10.49	81.5 ± 9.84	0.03*	77.83 ±9.93	78.08 ± 9.93	0.349^{NS}
BMI (Kg/m^2)	29.60 ± 2.97	29.14 ± 3.09	0.106^{NS}	28.39 ± 4.65	28.49 ± 4.60	0.26^{NS}
Total Cholesterol (mg/dl)	157.94 ± 36.99	140.55 ± 35.17	0.002*	227.77 ± 77.51	238.44 ± 85.37	0.24 ^{NS}
Triglycerides (mg/dl)	167.22 ± 41.9	137.22 ± 36.86	0.00001*	189.16 ± 80.53	190.16 ± 78.84	0.449 ^{NS}
HDL (mg/dl)	38.61 ± 7.70	41.72 ± 7.39	0.0001*	44.11 ± 8.5	44 ± 8.4	0.794 ^{NS}
LDL (mg/dl)	142.1 ± 37.85	107.2 ± 23.74	0.00002*	136.94 ± 57.86	138.44 ± 57.54	0.53 ^{NS}

*Significant ^{NS} Non-significant

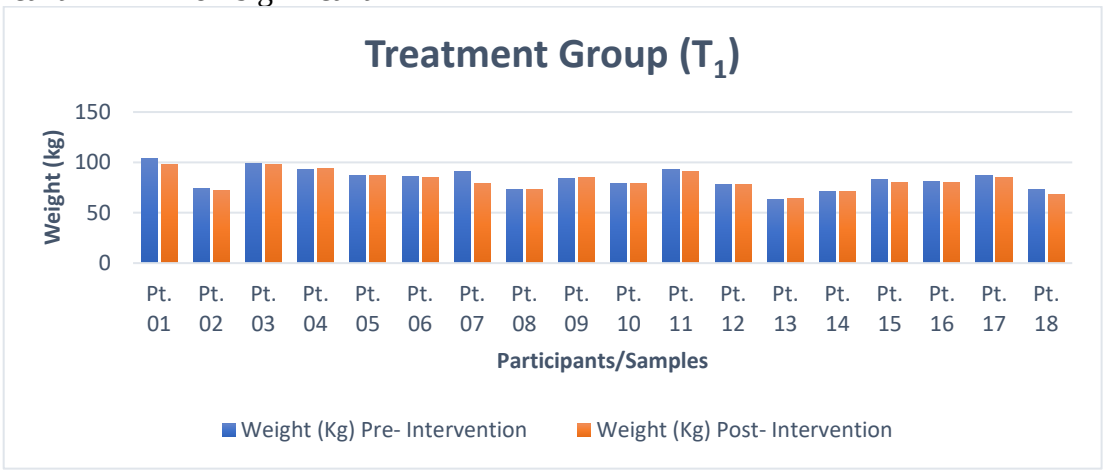

Figure -1 Difference between pre and post interventional weight of Treatment group.

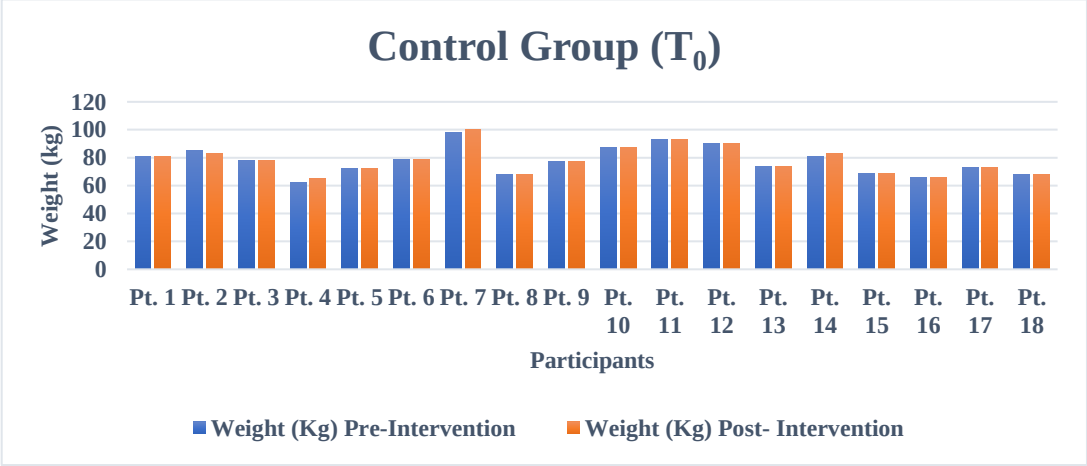

Figure -2 Difference between pre and post interventional weight of Control group.

4.2.1.4 Body Mass Index (BMI): Body mass index is a parameter or a measure which is used to check the weight is healthy or not. Paired sample t-test was applied to analyze the pre and post interventional BMI of treatment group. In treatment group, pre intervention BMI was 29.60 ± 2.97

kg/m^2 and post-interventional BMI was 29.14 ± 3.09 kg/m^2 as shown in Table -2. Level of significance in treatment group is expressed as *p* value 0.1 which is more than 0.05 as shown in Table -2. In control group, pre intervention BMI was 28.39 ± 4.65 kg/m^2 and post-interventional BMI was 28.49 ± 4.60 kg/m^2 as shown in Table -2. In the control group, there is non-significant difference reported. Level of significance of control group is 0.26 which is more than 0.05 as shown in Table -2. Difference among pre and post interventional BMI of treatment group participants is expressed in Figure -3 and control group in Figure -4. *p*-value of the treatment group and control group is considered as non-significant. According to the results of current research, no effect was observed on the BMI with the intake β-sitosterol supplements along with fat modified diet.

An independent sample t- test was also applied to determine level of significance between BMI of treatment group (T_1) and control group (T_0). Equal variances assumed *p*- value is 0.619. It shows non-significant change in BMI of treatment and control group as shown in Table -3.

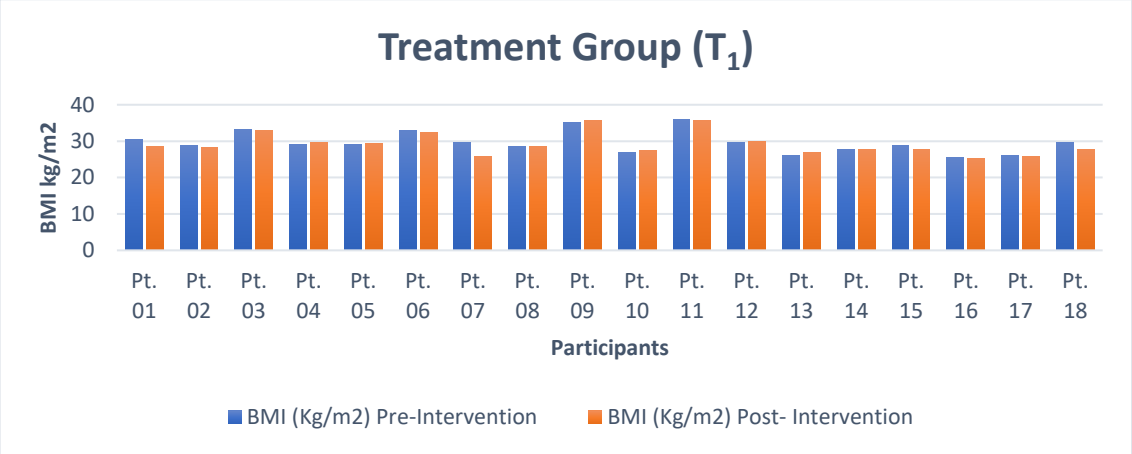

Figure -3 Difference between pre and post interventional BMI of Treatment group.

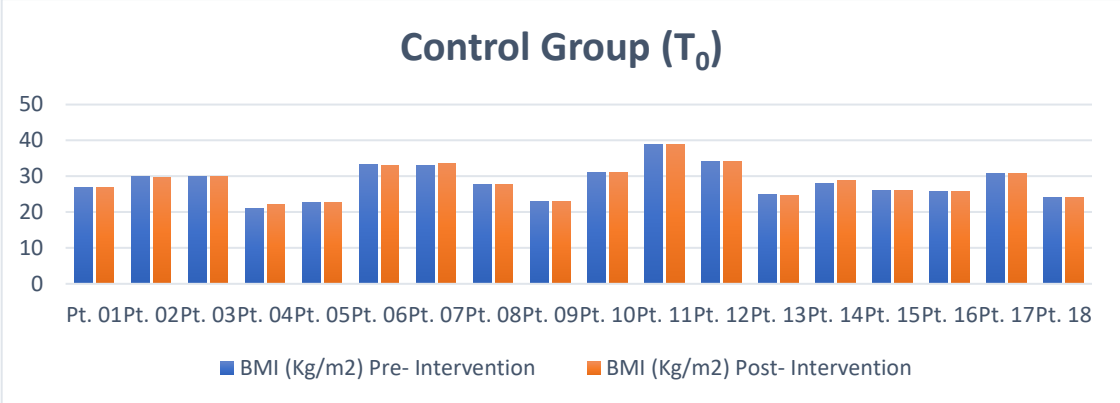

Figure -4 Difference between pre and post interventional BMI of Control group.

4.2.2 Serum Lipid Profile: Mean ± Standard deviation of serum total cholesterol (TC), serum triglycerides (TG), high density lipoproteins (HDL) and low-density lipoprotein (LDL) are displayed in the Table- 2. Statistical analysis expressing significant effect on the cholesterol levels of treatment group with the intake of β- sitosterol supplements along with fat modified diet.

4.2.2.1 Serum Total Cholesterol: Paired sample t-test was applied to analyse the pre and post interventional serum total cholesterol (TC) levels. In treatment group (T_1), pre intervention serum total cholesterol was 157.94 ± 36.99 mg/dl and post-interventional serum total cholesterol is 140.55 ± 35.17 mg/dl. Level of significance (*p* <0.002) of treatment group is lower than 0.05 as shown in Table -2. Hence, there is significant change is reported in the serum TC of the treatment group. In control group (T_0), pre intervention serum total cholesterol was 227.77 ± 77.51 mg/dl and post-interventional serum total cholesterol was 238.44 ± 85.37 mg/dl as shown in Table -2. *p*- value is 0.24

which shows non-significant change. Difference among pre and post interventional Serum TC of treatment group participants is expressed in Figure -5 and control group in Figure -6.
An independent sample t- test was also applied to determine level of significance between treatment group (T_1) and control group (T_0). The results indicate significant change between post interventional treatment (140.55 ± 35.17 mg/dl) and control group (238.44 ± 85.37 mg/dl). Equal variances assumed *p*- value 0.000076. It shows significant change in serum total cholesterol (TC) of treatment and control group shown in Table -3.

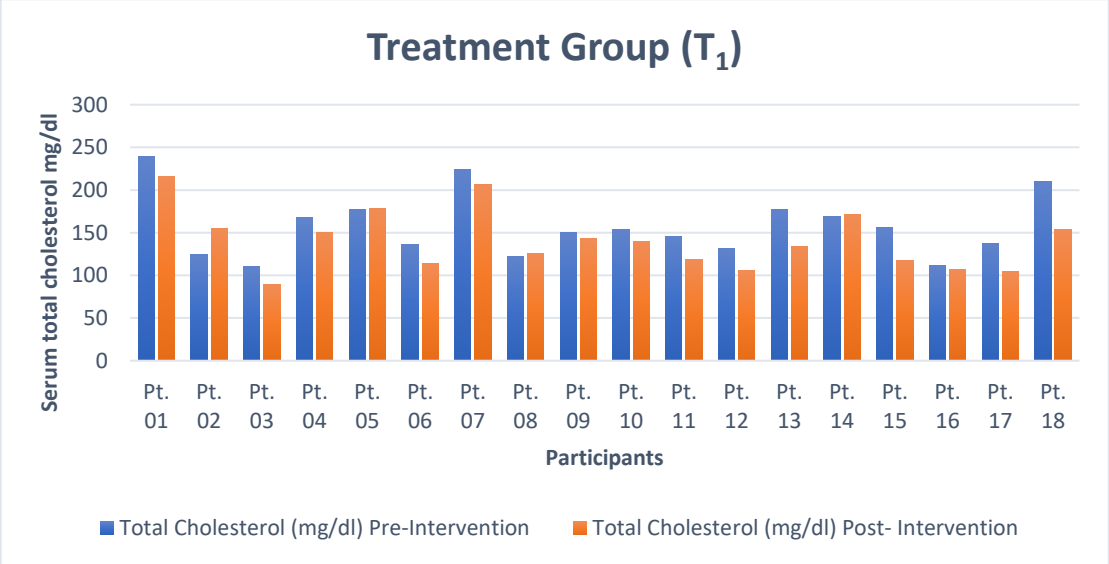

Figure -5 Difference between pre and post interventional serum total cholesterol (mg/dl) of Treatment group.

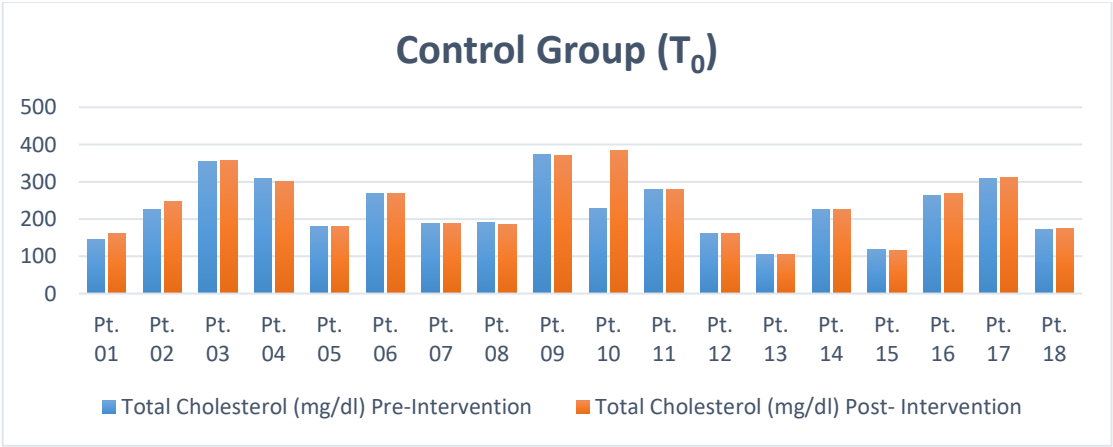

Figure -6 Difference between pre and post interventional serum total cholesterol (mg/dl) of Control group.

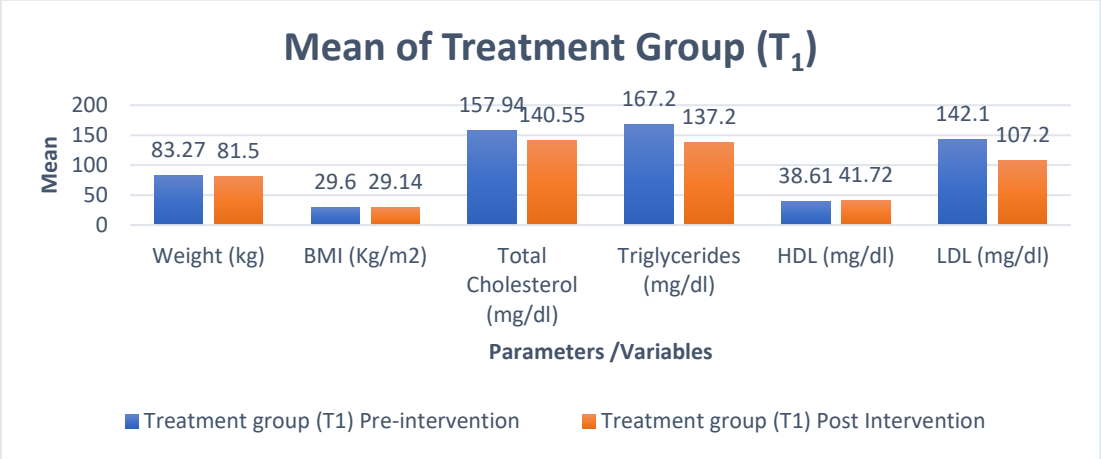

Figure- 7 Mean value indicating the positive change between pre and post intervention data of treatment group.

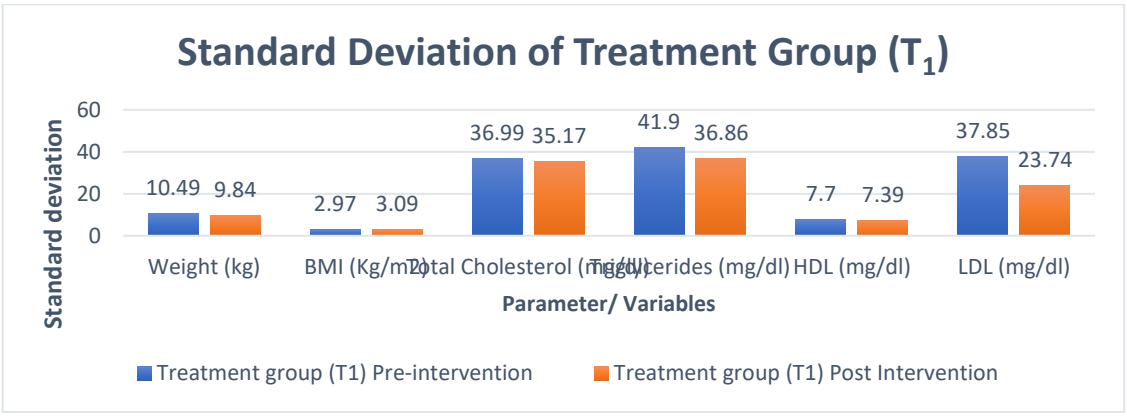

Figure- 8 Standard deviation indicating the significant change between the pre and post intervention data of treatment group.

4.2.2.2 Serum Triglycerides: Paired sample t-test was applied to analyze the pre and post interventional serum triglycerides (TG) levels. In treatment group (T_1), pre intervention serum triglyceride was 167.22 ± 41.90 mg/dl and post-interventional serum triglyceride is 137.22 ± 36.86 mg/dl. As shown in Table -2, *p*- value of treatment group is 0.00001 which is lower than 0.05. So, there is significant change in serum TG of treatment group. In control group (T_0), pre intervention serum TG was 189.16 ± 80.53 mg/dl and post-interventional serum triglyceride is 190.16 ± 78.84 mg/dl. *p* -value is 0.449 which is higher than 0.05 as shown in Table -2. Difference among pre and post interventional serum TG of treatment group participants is expressed in Figure -9 and control group in Figure -10.

An independent sample t- test was also applied to determine level of significance between treatment group (T_1) and control group (T_0). The results indicate significant change between post interventional treatment (137.22 ± 36.86 mg/dl) and control group (190.16 ± 78.84 mg/dl). Equal variances assumed *p*- value 0.014. It shows significant change in serum triglycerides of treatment and control group shown in Table -3.

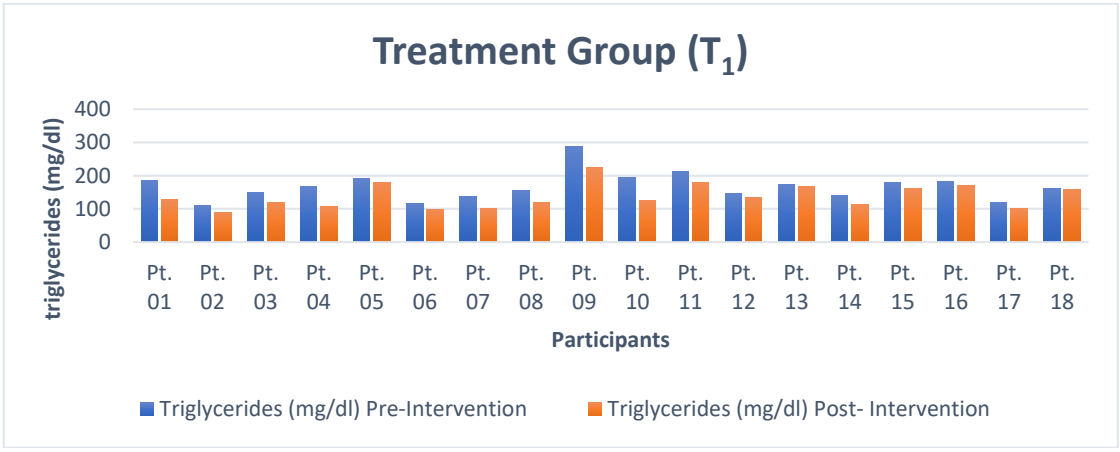

Figure -9 Difference between pre and post interventional serum Triglycerides (mg/dl) of Treatment group.

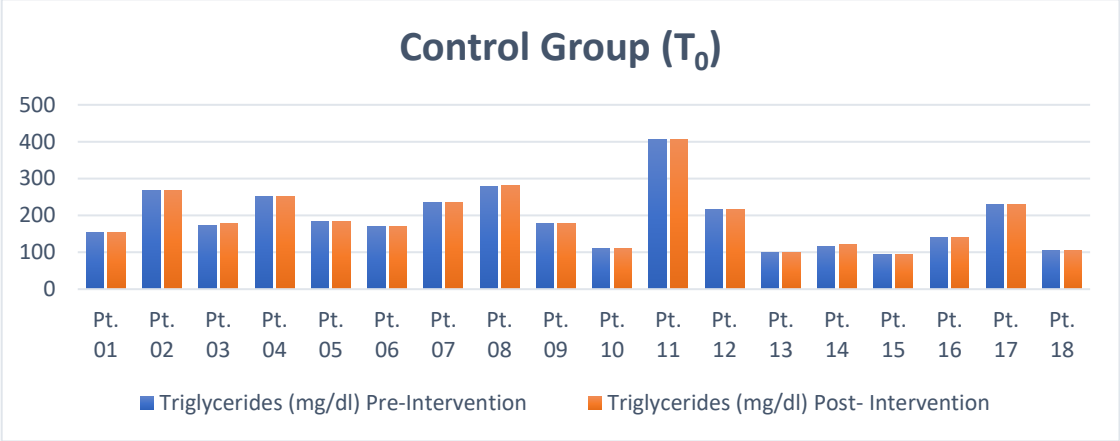

Figure -10 Difference between pre and post interventional serum Triglycerides (mg/dl) of Control group.

4.2.2.3 Serum HDL: Paired sample t-test was applied to analyze the pre and post interventional serum HDL levels. In treatment group (T_1), pre interventional serum HDL was 38.61 ± 7.70 mg/dl and post-interventional serum HDL is 41.72 ± 7.39 mg/dl. Level of significance of treatment group is 0.0001 which is lower than 0.05 as shown in Table -2. Hence, there is significant change is reported in treatment group. In control group (T_0), pre intervention serum HDL was 44.11 ± 8.55 mg/dl and post-interventional serum HDL is 44.00 ± 8.49 mg/dl. *p* -value is 0.794 which is higher than 0.05 as shown in Table -2. There is no significant change in serum HDL of control group. Difference among pre and post interventional Serum HDL of treatment group participants shown in Figure -11 and control group in Figure -12.

An independent sample t- test was also applied to determine level of significance between treatment group (T_1) and control group (T_0). The results indicate significant change between post interventional treatment (41.72 ± 7.39 mg/dl) and control group (48 ± 6.49 mg/dl). Equal variances assumed *p*- value 0.011. It shows significant change in serum HDL of treatment and control group shown in Table -3.

Table -3 Independent sample t-test applied to check the difference between treatment and control group.

Parameters	**Sample Size (*n* = 36)**		
	Post-Interventional (Mean)		***p*- value**
	Control Group (T_1)	**Treatment Group (T_1)**	
Weight (kg)	78.08 ± 9.93	81.5 ± 9.84	0.307 ^{NS}
BMI (kg/m^2)	28.49 ± 4.60	29.14 ± 3.09	0.619 ^{NS}
Total Cholesterol	238.44 ± 85.37	140.55 ± 35.17	0.00007*

(mg/dl)			
Triglycerides (mg/dl)	190.16 ± 78.84	137.22 ± 36.86	0.014*
HDL (mg/dl)	48 ± 6.49	41.72 ± 7.39	0.011*
LDL (mg/dl)	138.44 ± 57.45	107.22 ± 23.74	0.04*

NS Non-Significant *Significant

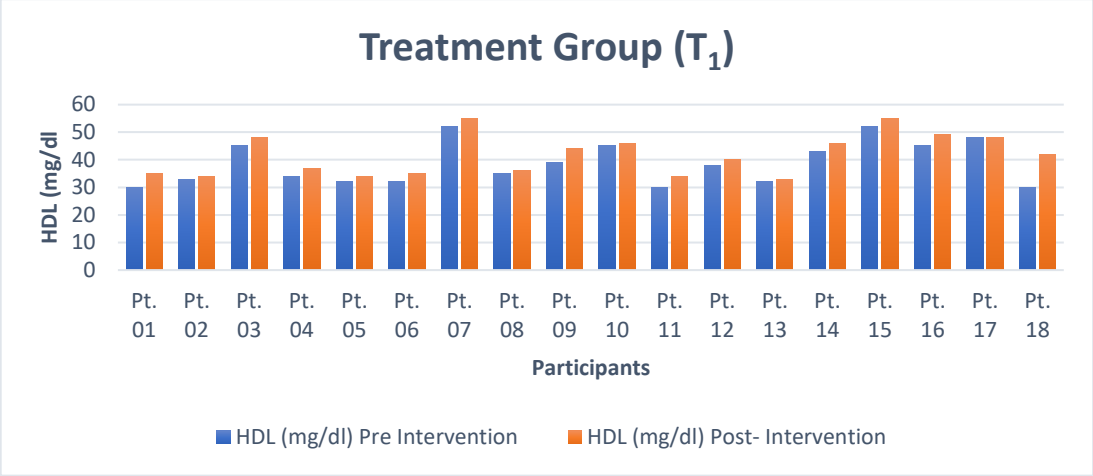

Figure -11 Difference between pre and post interventional serum HDL (mg/dl) of Treatment group.

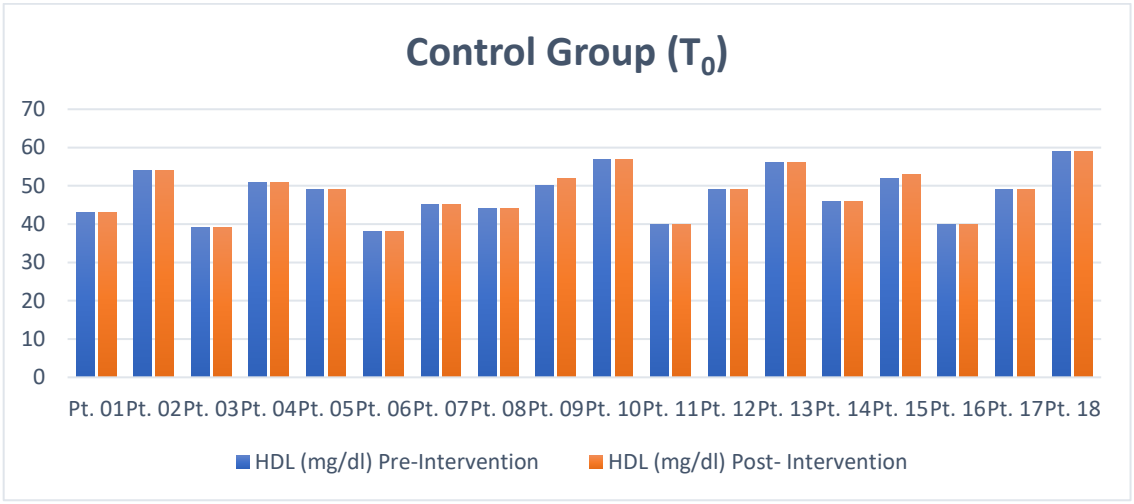

Figure -12 Difference between pre and post interventional serum HDL (mg/dl) of Control group.

4.2.2.4 Serum LDL: Paired sample t-test was applied to analyze the pre and post interventional serum LDL. In treatment group (T_1), pre interventional serum LDL was 142.11 ± 37.85 mg/dl and post-interventional serum LDL is 107.22 ± 23.74 mg/dl. p- value is 0.00002 which is lower than 0.05 as shown in Table -2. Significant Change was reported in the serum LDL levels of treatment group. In control group (T_0), pre intervention serum LDL was 136.94 ± 57.86 mg/dl and post-interventional serum LDL is 138.44 ± 57.45 mg/dl. *p* –value is 0.530 which is higher than 0.05 as shown in Table -2. So, *p* -value of the control group is considered as non-significant. Difference among pre and post interventional Serum LDL of treatment group participants shown in Figure -13 and control group in Figure -14.

An independent sample t- test was also applied to determine level of significance between treatment group (T_1) and control group (T_0). The results indicate significant change between post interventional treatment (107.22 ± 23.74 mg/dl) and control group (138.44 ± 57.45 mg/dl). Equal variances assumed

p- value 0.04. It shows significant change in serum LDL of treatment and control group shown in Table -3.

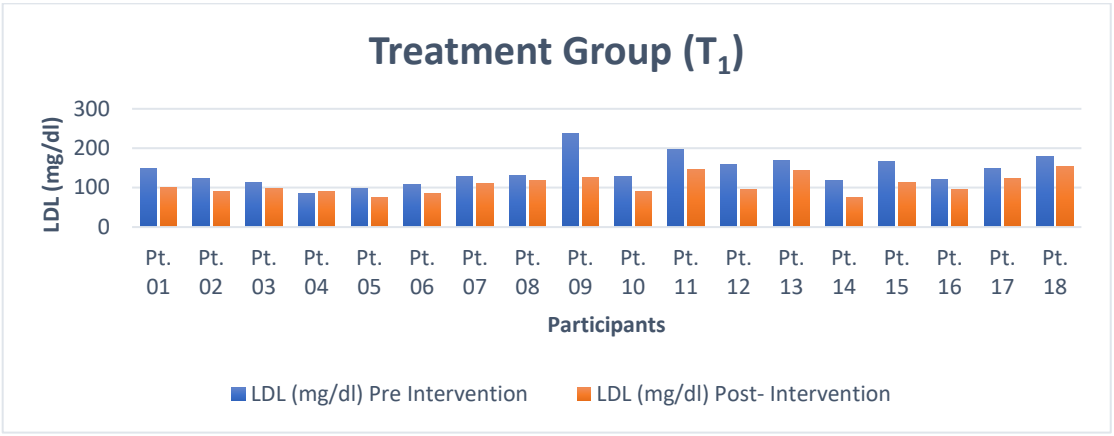

Figure -13 Difference between pre and post interventional serum LDL (mg/dl) of Treatment group.

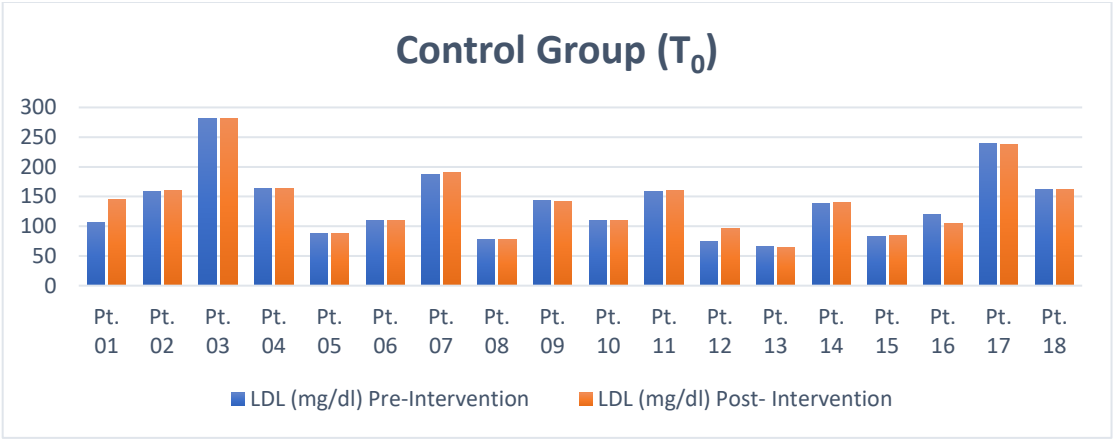

Figure -14 Difference between pre and post interventional serum LDL (mg/dl) of Control group.

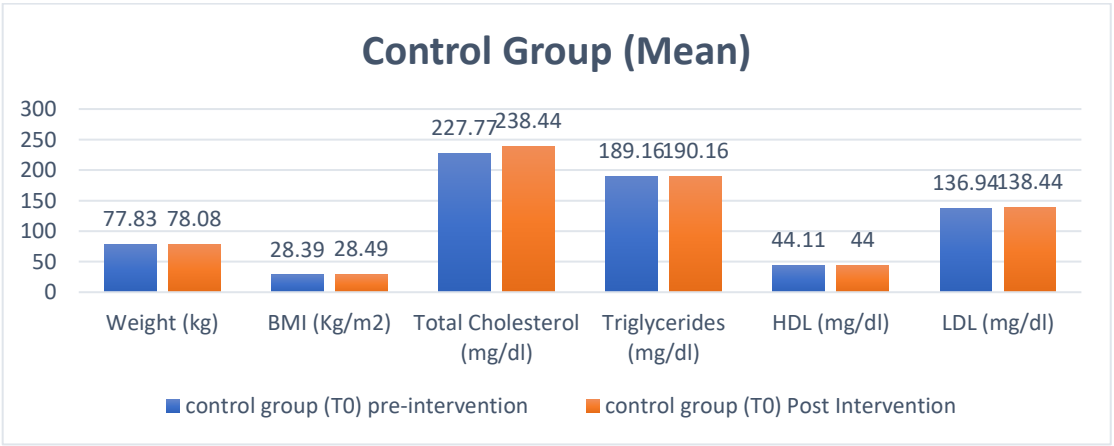

Figure -15 Mean value indicating the positive change between pre and post intervention data of Control group.

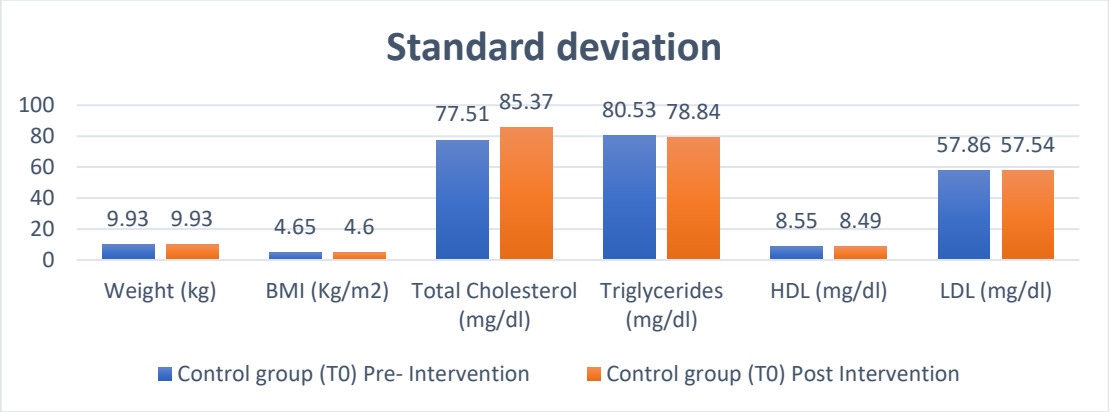

Figure -16 Standard Deviation indicating the positive change between pre and post intervention data of Control group.

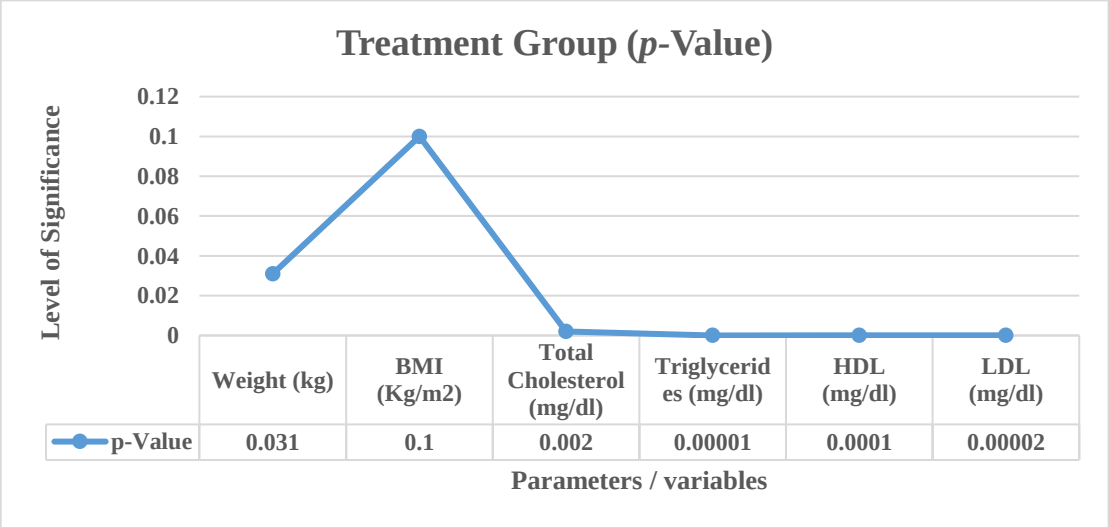

Figure -17 Level of significance (*p*- value) between pre and post interventional treatment group (T_1)

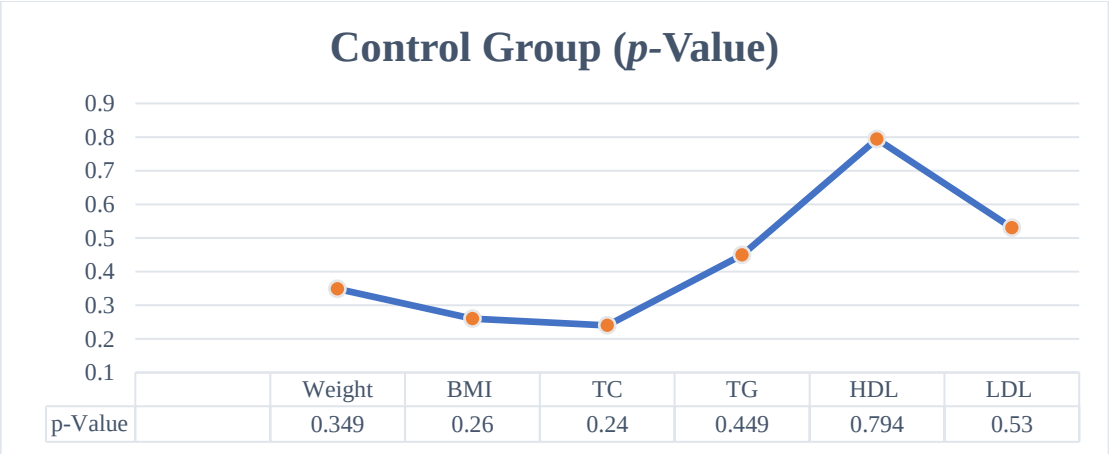

Figure -18 Level of significance (*p*- value) between pre and post Interventional control group (T_0)

5. Discussion:

In the current study, 1.8 g /day β- sitosterols along with fat modified diet shows significant effect in the management of dyslipidaemia. Randomized control trial was done and trial duration was 6 weeks. The results were attained majorly by the biochemical analysis (lipid profile analysis) of blood samples before and after trials. We mainly check the parameters (weight, BMI, Serum TC, TG, LDL & HDL)

to compare the pre- and post-interventional difference in both groups (treatment group and control group).

Chau *et al.,* 2020 directed research in China to investigate the effectiveness of phytosterol fortified soya milk on the levels of LDL as a primary variable. According to the results of referred study, there was significant decrease in serum LDL Cholesterol levels by 5.96% with median of 6.74% as compared to baseline data (2.80±0.75) and *p*- value 0.048. Current study expressing the consumption of beta-sitosterol is helpful for lowering the serum LDL levels. Pre interventional LDL was 142.1 ± 37.85 mg/dl and post interventional LDL is 107.2 ± 23.74 mg/dl. Level of significance is $p \leq 0.00002$. According to the post interventional results of current study, serum total cholesterol $p<0.002$, serum triglycerides $p < 0.00001$, LDL $p < 0.00001$. 1.8 g/day of phytosterol intake along with fat modify diet show significant results. Oliveira *et al.,* 2020 conducted a study in which interventional group was treated with soya milk which is fortified with 1.6 grams per day of phytosterol and current interventional group was treated with 1.8 g /day beta-sitosterol supplements along with fat modified diet. Results of refered study indicate that phytosterol lower the total plasma cholesterol -5.5% and p<0.001, triglycerides level by -8.3 % (p<0.05) & -6.4% LDL-C (p<0.05).

Bloom *et al.,* 2019 conducted research, in which it was reported that the consumption of spread fortified with the 2 grams per day of plant sterols and 1 gram per day of EPA and DHA from the fish oil is beneficial to decrease the serum LDL-cholesterol and triglycerides. Low density lipoproteins show significant change with baseline (LS means + 95% CI) 4.00 (3.92 to 4.08) mmol/L and $p <$ 0.001 and currents study's $p < 0.00002$. Triglycerides was 1.65 (1.58 to 1.73) mmol/L with the $p <$ 0.001 and our current study's $p < 0.00001$. It is concluded that the intake of beta sitosterol along with fat modified diet have significant effect in lowering the LDL & triglyceride.

Current study is also showing significant change in serum LDL with $p \leq 0.00002$. Hence, dietary supplementation of phytosterol is beneficial and effective for the reduction LDL-cholesterol. Reaver *et al.,* 2019, a study was conducted to investigate the impact of 1.5 g/day phytosterol supplementation. Supplementation of phytosterol significantly reduce 10.2 % of low-density lipoproteins cholesterol level and $p = 0.008$.

In current study we treated our interventional group with 1.8 g/day beta-sitosterol supplement along with fat modified diet for the management of dyslipidemia. Significance level of TC $p < 0.024$, TG $p< 0.00001$ and LDL $p< 0.00002$. Trautwein *et al.,* 2018 a double-blind randomized placebo controlled parallel study was directed. Intake of plant sterols significantly reduce the fasting total cholesterol (p = 0.006), triglycerides (p = 0.024) and low-density lipoproteins (p = 0.009). We can conclude that 2 grams per day of plant sterols give us positive health impacts. Ferguson *et al.,* 2018 also conducted a double-blind randomized placebo-controlled study. Volunteers were randomly assigned to consume placebo, 200 mg/day curcumin, phytosterols 2 g/day, and combination of both 200 mg/day curcumin and 2 g/day phytosterol for 4 weeks. There was significant reduction in LDL and TC in the volunteers taking phytosterol supplementation and volunteers taking combination of both. (LDL p=0.004 & TC p=0.004).

Mohammad *et al.,* in 2018 was directed a study. Supplement group was treated with the 1.6 grams phytosterols capsules daily. According to the results of blood samples, there were significant decrease in the LDL-C levels of supplement group as compared to control group. If we compare current study with the above referred study than it is deduced that the current study is supporting the effectiveness of phytosterol supplements on LDL.

Our current study is clear representation of significant change in the serum LDL. Pre interventional serum LDL level was 142.1 ± 37.85 mg/dl and post intervention is 107.2 ± 23.74 mg/dl. Level of significance is equals to or less than 0.00002 which more significant than the referred study. A randomized double blind controlled study, was carried out by Laitinen *et al.,* 2017. 2 g /day of plant stanol ester supplemental intake reduce the 7.6% of LDL-C with baseline difference -0.20 ±0.06 (p value 0.001) as compared to placebo.

According to the results of current study's treatment group expressing significant decrease in serum LDL $p \leq 0.00002$ and TC $p< 0.024$. Cheung *et al*., 2017 directed a study in China. According to the findings, 1.5 g/day phytosterol fortified milk show healthful response for lowering the serum LDL

and total cholesterol in 3 weeks. Results of biochemical analysis expressing that there is significant reduction in the serum LDL-C levels of treatment group with baseline $9.5 \pm 2.0\%$ and $p < 0.0001$. Phytosterol enriched milk also significantly lower the total cholesterol $p<0.0001$. It was concluded that phytosterol enriched milk could be recommend for lowering the cholesterol.

McKenney *et al.,* 2014 directed study trial of effectiveness of soft-gel dietary capsules of plant sterol and stanol. At baseline low density lipoproteins (LDL) was 3.92 ± 0.09 mmol/L (151.6 ± 3.3 mg/dL). According to the results, this study supports the effectiveness of 1.8 g/ day intake of plant sterol and stanols dietary Supplements. Results of our current study also support the 1.8 g/day intake of phytosterol. LDL's level of significance is 0.00002 which is more significant than 0.05.

Maki *et al.,* 2012 a randomized placebo controlled; crossover research was conducted to investigate the lipid lowering effect of 1.8 g/day of phytosterol dietary supplement for 6 weeks along with TLC diet. Results indicated that there was significant decrease in LDL-C (p=0.002) and total cholesterol (0.024). Current research shows significant reduction in serum LDL & TC levels. The baseline of current study's LDL cholesterol was 142.1 ± 37.85 mg/dl and results indicated that there is significant decrease in LDL-C ($p \leq 0.00002$). Current study's total cholesterol at baseline was 157.94 ± 36.99 mg/dl and after intervention period it is 140.55 ± 35.17 mg/dl with $p \leq 0.024$. So, it is deduced that 1.8 g/day of beta-sitosterol (phytosterol) supplementation led to favorable low density lipoprotein changes in patients suffering with hypercholesterolemia and dyslipidemia.

In the previous study Shai *et al.,* 2008 level of significance was $p < 0.001$ & current study's p- value is 0.031 in the treatment group shows significant reduction in the weight by following the interventional plan. It is concluded that fat modified diet plan plus beta-sitosterol supplementation is significantly effective for weight loss and management of Dyslipidemia.

Wang *et al.,* 2019 administered a study with the goal to find out the efficacy of plant sterol alone and in combination with omega 3 fatty acids. According to the results, in comparison to the placebo group, group taking combination of phytosterol and omega 3 FAs showing significant difference -0.1 ± 0.31 mmol/L with post mean standard deviation 1.27 ± 0.29 mmol/L and p value < 0.05. In current study, mean standard deviation at baseline was 38.61 ± 7.70 mg/dl and after treatment period was 41.72 ± 7.39 mg/dl with the p value 0.0001. Level of significance of current study is less than 0.05 means my research study showing significant effects on HDL.

In the current study, we treated participants with beta-sitosterol supplementation along with fat modified diet plan which include fat 28 % of total diet (minimum saturated fat and high level of MUFA & PUFAs). Current study's post interventional results of serum total cholesterol, triglycerides, LDL and HDL are 140.55 ± 35.17 mg/dl, 137.2 ± 36.86 mg/dl, 41.72 ± 7.39 mg/dl and 107.2 ± 23.74 mg/dl respectively. All the previous referred studies and discussion about the results of current research are supporting the lipid lowering & cholesterol lowering effect of β- sitosterol supplements along with fat modified diet. Our research study shows significant results and supports the alternate hypothesis.

6. Conclusion:

β – Sitosterol is a phytosterol which is important due to its inhibitory effect on the intestinal absorption of cholesterol. β – Sitosterol is used to lower the cholesterol in the blood which leads to decrease the risk of cardiovascular diseases. The current study was performed to evaluate the effectiveness of β-sitosterol supplement along with fat modified diet for management of dyslipidemia. Trials were successfully conducted on the dyslipidemia patients and parameter were statistically analyzed. The results indicate significant difference between treatment group (T_1) and control group (T_0). Furthermore, in the treatment group, results indicate significant difference between baseline and post intervention data. Based on the results, it is concluded that the incorporation 1.8 g /day capsule of supplement β – sitosterol along with fat modified diet produced favorable changes in the lipid profile (Total cholesterol, Triglycerides, LDL and HDL) of dyslipidemia patients. It is also concluded that the intake of beta-sitosterol supplement along with fat modified dietary pattern will help in lowering the risk of hypercholesterolemia and other cardiovascular diseases.

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