

**“Effect of Sunflower seeds on Hypercholesterolemia, Fatty liver, fasting
blood glucose in Diabetes Mellitus type 2 patients”**

A

Thesis

Submitted to



For the award of

DOCTOR OF PHILOSOPHY (Ph.D.)

IN

(Nutrition & Dietetics)

By

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PUNJAB
2019**

DECLARATION

I hereby declare that the report entitled “**Effect of Sunflower seeds on Hypercholesterolemia, Fatty liver and fasting blood glucose in Diabetes Mellitus type 2 patients**” written and submitted by me to the Lovely Professional University, Phagwara in partial fulfilment of the requirements for the degree of PhD in Nutrition and Dietetics is my own and original work. The work has been conducted under the guidance of **Dr. Leena Parihar** former Assistant Professor in the School of Bioengineering at Lovely Professional University and presently working as Head of Department at Seth G.L. Bihani SD PG College, Sri Ganganagar, Rajasthan and **Dr. Vikas Kumar** Assistant Professor, Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Phagwara, Punjab.

I further declare that my work has not been submitted to this or any other university for the award of any other degree, diploma or equivalent course.

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Certificate

This is to certify that the work reported in the Ph.D. thesis entitled “**Effect of Sunflower seeds on Hypercholesterolemia, Fatty liver and fasting blood glucose in Diabetes Mellitus type 2 patients**”, submitted by **Cheenam Bhatia** at **Lovely Professional University, Phagwara, India**, is a bonafide record of her original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

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ACKNOWLEDGMENT:

Although on the cover of the thesis just my name appears but there are number of people that need to be thanked. I owe my deep gratitude to all those wonderful people who have made the completion of this thesis possible and it's because of them that I will forever cherish my roller coaster experience.

My deepest gratitude is to my guide and in real terms my advisor, **Dr. Leena Parihar**. I am deeply indebted to my advisor who gave me not only the immense support to fulfill my endeavors but at the same time the freedom to explore on my own. **Dr. Parihar** enlightened me on how to express ideas and question thoughts. Her motivation, immense knowledge, patience and support aided me to overpower innumerable critical situations and thus complete my thesis.

My co-advisor, **Dr. Vikas Kumar**, had always been there give an ear to all my problems and thus advice. I am immensely indebted to her for lending me her precious time for long discussions to sort out the technical issues of my study. I am also very grateful to her for carefully reading and rectifying my countless manuscript revisions.

My special thanks go to **Dr. Monica Gulati**, Dean, Lovely School of Pharmacy and pharmaceutical sciences. Her judicious remarks along with her constructive criticisms at each and every stage of my thesis preparation and research were thought-provoking and they helped me widen my perspective and always focus on my research objectives. I will always be grateful to for teaching and inculcating high research standard values in me.

My heartfelt thanks go to **Dr Joinder Singh Panwar**, who has always been very kind and helpful towards me even when my guide Dr Leena Parihar was unavailable. His valuable help always helped me get through the obstacles that came my way.

I would also like to thank my hospital staff who always gave me the right assistance, support and the platform to get through the research work. **Dr H.P. Singh**, the Medical Director of Fortis Escorts Hospital needs a big and special thanks to always support me and help me through my thesis, I will always be indebted to you Sir. **Mrs. Guljeet Dang**, Chief Dietician, Fortis Escorts Hospital Amritsar have always pushed me to work even harder for my goal,

hence my heartfelt thanks to her. Also my other colleagues and friends in the hospital especially **Ms Anuradha Sharma** who helped me in my thick and thin time needs to be thanked.

Special thanks to **Dr Harpreet** Asst. Professor Statistics at Sri Guru Ram Das Institute of Health and Medical Sciences for her kind help in data analysis.

None of this however would have been possible without the love and patience of both my families; continuous support from my parents and great motivation from my sister. My father **Mr R.K. Bhatia**, who always motivated me and wanted to see me above all, my mother **Mrs Shashi Bhatia** who although is a housewife herself but taught me to excel in all fields. My elder sister **Mrs Trisha Bhatia** who herself is pursuing PhD from a University in America always believed in me. Most importantly my husband **Mr Jabar Singh Saund** who not just supported me but also made sure I never gave up, no matter how tough the situations might become. My father-in-law **Mr Shamsher Singh Saund** and my mother-in-law **Mrs Jatinder Kaur** need special and heartfelt thanks as without their cooperation this could not have been possible. They made sure that I never deviate from my goal that I had set in my mind even after my wedding.

Last but not the least, I would like to thank almighty God, who has always blessed me and given me the strength to complete my research work.

Cheenam Bhatia

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Table of Contents:

Chapter	Title	Page No
1.	Introduction	3
2.	Review of literature	7
2.1	Studies conducted on sunflower seeds	7
2.2	Effect of food processing (roasting) on the quality attribute of sunflower seeds	10
2.3	Antioxidant potential of sunflower seeds	11
2.4	Relationship between diet and diseases	13
2.5	Product development	17
2.5.1	Cookies	17
2.5.2	Biscuits	18
2.5.3	Physicochemical composition of biscuits and cookies	19
2.5.4	Phytonutrient Composition of biscuits and cookies	20
2.5.5	Organoleptic evaluation	20
3.	Scope of study/hypothesis	22
4.	Objectives	23
5.	Materials and methods	24
5.1	Analysis of tocopherol and phenolic content of sunflower seeds	24
5.1.1	Raw materials	24
5.1.2	Sample preparation	24
5.2.3	Roasting	24
5.2	Analysis of non-roasted and roasted sunflower seeds	25
5.2.1	Free Radical Antioxidant Scavenging Capacity	25
5.2.2	Total phenolic content	26
5.3	Effect of sunflower seeds on various biochemical parameters of sample patients (n=300)	26
5.3.1	Design of study	28
5.3.2	Sample selection	28
5.3.3	Data Collection	28
5.3.4	Pre-supplementation data collection	28
5.3.5	Grouping and supplementation	33

5.4	To prepare and evaluate the sunflower seeds' enriched food product	35
5.4.1	Procurement of raw materials	35
5.4.2	Flour Preparation	35
5.4.3	Experimental Plan	35
5.4.4	Evaluation of functional properties of the sunflower enriched flour	38
5.4.5	Physical evaluation of sunflower seeds enriched cookies	39
5.4.6	Physicochemical composition of cookies enriched with sunflower seeds	39
5.4.7	Total Phenolic Content	42
5.4.8	Antioxidant activity by DPPH Assay	42
5.4.9	Organoleptic evaluation	43
6.	Results and Discussions	44
6.1	Analysis of sunflower seeds	44
6.1.1	Radical Scavenging Capacity (DPPH assay)	44
6.1.2	Total Phenolic content	45
6.2	Effect of sunflower seeds on various biochemical parameters	47
6.3	Comparison of Sunflower Seeds with Regular Medication	63
6.3.1	Cost	64
6.3.2	Side effects	64
6.4	Product Formulation and development of sunflower seeds enriched cookies	65
6.4.1	Functional Analysis of Sunflower seeds enriched flour blends	65
6.4.2	Physicochemical analysis of sunflower seeds enriched flour blends	67
6.4.3	Total phenolic content of sunflower seeds enriched flour blends	69
6.4.4	Antioxidant activity of sunflower seeds enriched flour blends	70
6.5	Analysis of cookies enriched with sunflower seeds	70
6.5.1	Physical analysis of cookies enriched with sunflower seeds	70
6.5.2	Physicochemical analysis of cookies enriched with sunflower seeds	73
6.5.3	Total phenolic content of cookies enriched with sunflower seeds	74
6.5.4	Antioxidant activity of cookies enriched with sunflower seeds	75
6.6	Organoleptic analysis of cookies enriched with sunflower seeds	76
6.7	Formulation of capsule with sunflower seeds powder	77

7.	Conclusions	79
8.	References	82
9.	Index	90
10.	Appendix I – General information questionnaire	91
11.	Appendix II – Dietary recall	95
12.	Appendix III – Questionnaire post capsular supplementation of sunflower seeds	97
13.	Appendix IV – Evaluation performa for hedonic rating test of the food products	98
14.	Appendix V - Reference value for the biochemical parameters	99
15.	Appendix VI – Master Sheet	100

LIST OF FIGURES

<u>Fig No.</u>	<u>Title</u>	<u>Page No.</u>
5.1	Flowchart for preparation and analysis of the sunflower seeds	25
5.2	Flowchart elucidating design of the study	27
5.3	Flowchart showing classification of the Case and the Control Group	34
5.4	Flowchart of cookies' preparation	37
6.1	Serum cholesterol levels in control group (Group 1)	48
6.2	Effect of sunflower seeds on serum cholesterol levels (Group 1)	48
6.3	Serum triglyceride levels in control group (Group 1)	48
6.4	Effect of sunflower seeds on serum triglyceride levels (Group 1)	48
6.5	Serum LDL levels in control group (Group 1)	49
6.6	Effect of sunflower seeds on serum LDL levels (Group 1)	49
6.7	Serum HDL levels in control group (Group 1)	49
6.8	Effect of sunflower seeds on serum HDL levels (Group 1)	49
6.9	Serum FBS levels in control group (Group 2)	50
6.10	Effect of sunflower seeds on serum FBS levels (Group 2)	50
6.11	Serum HDL levels in control group (Group 2)	51
6.12	Effect of sunflower seeds on serum HDL levels (Group 2)	51
6.13	Serum SGOT levels in control group (Group 3)	52
6.14	Effect of sunflower seeds on serum SGOT levels (Group 3)	52
6.15	Serum SGPT levels in control group (Group 3)	52
6.16	Effect of sunflower seeds on serum SGPT levels (Group 3)	52
6.17	Serum cholesterol levels in control group (Group 4)	54
6.18	Effect of sunflower seeds on serum cholesterol levels (Group 4)	54
6.19	Serum triglyceride levels in control group (Group 4)	54
6.20	Effect of sunflower seeds on serum triglyceride levels (Group 4)	54
6.21	Serum LDL levels in control group (Group 4)	55
6.22	Effect of sunflower seeds on serum LDL levels (Group 4)	55
6.23	Serum HDL levels in control group (Group 4)	55
6.24	Effect of sunflower seeds on serum HDL levels (Group 4)	55
6.25	Serum FBS levels in control group (Group 4)	56

6.26	Effect of sunflower seeds on serum FBS levels (Group 4)	56
6.27	Serum SGOT levels in control group (Group 4)	56
6.28	Effect of sunflower seeds on serum SGOT levels (Group 4)	56
6.29	Serum SGPT levels in control group (Group 4)	57
6.30	Effect of sunflower seeds on serum SGPT levels (Group 4)	57
6.31	Serum cholesterol levels in control group (Group 5)	59
6.32	Effect of sunflower seeds on serum cholesterol levels (Group 5)	59
6.33	Serum triglyceride levels in control group (Group 5)	60
6.34	Effect of sunflower seeds on serum triglyceride levels (Group 5)	60
6.35	Serum LDL levels in control group (Group 5)	60
6.36	Effect of sunflower seeds on serum LDL levels (Group 5)	60
6.37	Serum HDL levels in control group (Group 5)	61
6.38	Effect of sunflower seeds on serum HDL levels (Group 5)	61
6.39	Serum FBS levels in control group (Group 5)	61
6.40	Effect of sunflower seeds on serum FBS levels (Group 5)	61
6.41	Serum SGOT levels in control group (Group 5)	62
6.42	Effect of sunflower seeds on serum SGOT levels (Group 5)	62
6.43	Serum SGPT levels in control group (Group 5)	62
6.44	Effect of sunflower seeds on serum SGPT levels (Group 5)	62
6.45	Total Phenolic content of the flour enriched with sunflower seeds	69
6.46	Anti-radical activity of flours enriched with sunflower seeds	69
6.47	Effect of incorporation of sunflower seed flour (SSF) in wheat flour (WF) on weight of Cookies	72
6.48	Effect of incorporation of sunflower seed flour (SSF) in wheat flour (WF) on height of Cookies	72
6.49	Effect of incorporation of sunflower seed flour (SSF) in wheat flour (WF) on diameter of Cookies	72
6.50	Effect of incorporation of sunflower seed flour (SSF) in wheat flour (WF) on Spread Ratio of Cookies	72
6.51	Effect of incorporation of sunflower seed flour (SSF) in wheat flour (WF) on total phenolic content of Cookies	75
6.52	Effect of incorporation of sunflower seed flour (SSF) in wheat flour (WF) on anti-radical activity of Cookies	75
6.53	Pictorial view of the cookies prepared from sunflower seeds enriched flour blends	77
6.54	Consumer preference of capsular administration of sunflower seeds	78

LIST OF TABLES

<u>Table No.</u>	<u>Title</u>	<u>Page No.</u>
5.1	Ideal weight for height chart	30
5.2	Experimental Plan for product formulation	36
3.3	Composition of various flour blends	36
5.4	Ingredients used in sunflower seeds enriched cookies preparation	36
6.1	Free radical scavenging capacity of roasted and non-roasted sunflower seeds	44
6.2	Total antioxidant and phenolic content of roasted and non-roasted sunflower Seeds	45
6.3	Correlation coefficients amongst phenolic content and free radical scavenging activity of sunflower seeds	45
6.4	Nutritional comparison of Roasted and Non Roasted Sunflower seeds	46
6.5	Effect of sunflower seeds on cholesterol, triglyceride, LDL and HDL	47
6.6	Effect of sunflower seeds on FBS and HDL (Group 2)	50
6.7	Effect of sunflower seeds on SGOT and SGPT (Group 3)	51
6.8	Effect of sunflower seeds on Cholesterol, Triglycerides, LDL, HDL, FBS, SGOT and SGPT without medicine (Group 4)	53
6.9	Effect of sunflower seeds on Cholesterol, Triglycerides, LDL, HDL, FBS, SGOT and SGPT without medicine (Group 5) [no medicine]	58
6.10	Comparison of natural products with regular medications	64
6.11	Effect of sunflower seeds on functional properties of the flour blends	67
6.12	Effect of sunflower seeds on proximate composition of the flour blends	68
6.13	Effect of sunflower seeds on physical characteristics of the cookies	70
6.14	Effect of sunflower seeds on physicochemical characteristics of the cookies	73
6.15	Effect of sunflower seeds on organoleptic attributes of the cookies	76

TERMINOLOGY

ABTS	=	2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)
%	=	Per cent
BMI	=	Body Mass Index
°C	=	Degree Celsius
Chol	=	Cholesterol
CVD	=	Cardiovascular Disease
DM-2	=	Diabetes Mellitus Type-2
DPPH	=	2, 2- Diphenyl-1-picrylhydroxyl
EC 50	=	Half Maximal Effective Concentration
<i>et al.</i>	=	And other
FA	=	Fatty acid
FBS	=	Fasting Blood Sugar
g	=	Gram
GAE	=	Gallic acid equivalent
GC-FID	=	Gas Chromatography- Flame Ionization Detector
GC-MS	=	Gas Chromatography- Mass Spectrometry
HDL	=	High Density Lipoprotein
HPLC	=	High Performance Liquid Chromatography
IC 50	=	Half Maximal Inhibitory Concentration
<i>i.e.</i>	=	That is
IHD	=	Ischemic Heart Disease
IPD	=	In Patient Department
Kg	=	Kilogram
LDL	=	Low Density Lipoprotein
LFT	=	Liver Function Tests
mg	=	Milligram
ml	=	Millilitre
mg/100g	=	Milligram per 100 gram
mg/ml	=	Milligram per millilitre
MUFA	=	Monounsaturated Fatty Acids

MS (ESI)	=	Mass Spectrometry (Electro Spray ionization)
ω -3	=	Omega 3
ω -6	=	Omega 6
OAC	=	Oil Absorption Capacity
OPD	=	Out Patient Department
PUFA	=	Polyunsaturated Fatty Acids
Rpm	=	Rotation per minute
SGOT	=	Serum Glutamic Oxaloacetic Transaminase
SGPT	=	Serum Glutamic Pyruvic Transaminase
t	=	Time
T	=	Temperature
Tgl	=	Triglycerides
TEAC	=	Trolox Equivalent Antioxidant Capacity
WAC	=	Water Absorption Capacity
yrs.	=	years

Abstract

Objective: The present study was conducted to assess and analyse the effect of sunflower seeds on Hypercholesterolemia, Fatty liver, Fasting Blood Glucose (FBS) in patients with Diabetes Mellitus Type 2

Method: The seeds of sunflower (roasted and non-roasted) were evaluated for different physico-chemical and phytochemical attributes. 300 patients (n=300) were selected for the study and equally segregated into the control and case group. Anthropometric measurements including Weight, Height, and Blood Pressure were recorded along with certain biochemical parameters (Cholesterol, Triglycerides, Low Density Lipoprotein [LDL], High Density Lipoprotein [HDL], Fasting Blood Glucose [FBS], Serum Glutamic Oxaloacetic Transaminase [SGOT], and Serum Glutamic Pyruvate Transaminase [SGPT]) for 6 months pre and post supplementation of 2 gm of roasted sunflower seeds. The seeds of sunflower were also evaluated to study and quantify the tocopherol, phenolic content and antioxidant potential. A product formulation (cookies) has been carried out for the efficient delivery of the sunflower seeds to the patients using different proportions of wheat flour and sunflower seeds.

Result: In case group where the patients were given the intervention of sunflower seeds showed a significant ($P < 0.01$) and a faster decrease in their respective deranged levels as compared to the control group. The total Cholesterol reduced from 254.26 ± 23.40 to 183.40 ± 3.01 mg/dl; LDL from 155.26 ± 8.48 to 122.70 ± 2.94 mg/dl; triglyceride from 236.10 ± 23.18 to 143.20 ± 7.30 mg/dl; FBS from 179.40 ± 19.36 to 109.50 ± 5.19 mg/dl; SGOT from 87.33 ± 13.11 to 39.81 ± 2.10 (u/l), and SGPT from 139.90 ± 25.20 to 46.66 ± 5.24 (u/l) were significantly reduced. In addition, a good increase was also observed in the HDL (the good cholesterol) which is known to be a heart healthy lipoprotein; it increased from 34.56 ± 3.8 to 42.30 ± 1.5 mg/dl. A formulation of 80:20 (Wheat: sunflower seeds flour) revealed good score for the physico-chemical, phyto-chemical and organoleptic parameters.

Conclusion: The study showed that the sunflower seeds can be used as an adjuvant in treating the biochemical parameters like Lipid Profile, Liver Function tests and blood sugar levels in patients with diabetes type-2. The recipe of the developed product can be used for the effective delivering of the sunflower seeds.

CHAPTER-1 **INTRODUCTION**

Sunflower is a tall, erect, efflorescent plant grown annually. It belongs to the genus, *Helianthus* and family *Asteraceae*. Not just the flower has been put to use for the decorative or ornamental purpose since the earlier times but its' seeds also consist of an array of benefits (Albert *et al.* 1997). The head of the flower consists of numerous oils seeds which are edible and energy dense (National Sunflower Association 2011).

The crusty seeds of sunflower are an eminent source of calories, certain minerals and vitamins along with EFAs i.e. essential fatty acids. The seeds are used worldwide for the edible oil extraction. They can also be eaten as it is like tasty and delicious snack (Ensminger *et al.* 1983). The external surface of the seed i.e. hull has grey white coloured stripes on a black coat. Inside the seed is a kernel present that is edible. As a result of the presence of high oil content sunflower seeds are an opulent source polyunsaturated oil (Ensminger *et al.* 1983). The seeds are energy dense; around 584 calories are present in 100 g of the seeds. Also, they supply good quantities of various nutrients; vitamins, minerals and antioxidants.

The seeds have been known to be a great source of the fat soluble vitamins like Vitamin E that acts as a major antioxidant for the body. Vitamin E helps in preventing damage from the free radicals by neutralizing them that otherwise would damage molecules and structures containing fat, as like cell membranes, brain cell, and cholesterol (Zabaniotou *et al.* 2008). In addition to being an excellent source of some essential fatty acids like linoleic acid, the eminent seeds also provide tryptophan an essential amino acid, dietary fiber, certain B Vitamins. Additionally, they are also a rich source of certain phytosterols which are known for their cholesterol lowering action. Apart from these, the glycaemic index of the sunflower seeds have also been found out to be low, making it fit as a snack for the diabetic patients (Arcangelo *et al.* 2002).

Some of the important poly-phenols present in sunflower seeds that are supposed to be beneficial are chlorogenic acid, quinnic acid and caffeic acid. They help to remove the harmful free radicals (oxidant molecules) from the body being the natural antioxidants and hence help the body to get rid of them (Krimmer *et al.* 2011). The sunflower seeds also are a

phenomenal source of certain essential minerals. Copper, Zinc, Iron, Selenium, Manganese, Calcium and Magnesium are widely present in the sunflower. Most of the minerals present in the seeds help in RBC production, bone mineralization, production of hormones, enzyme synthesis, skeletal regulation, metabolic activities of cardiac muscle (Wood *et al.* 1988).

In the relative study, the sunflower seeds' effects have been analyzed and evaluated on patients with diabetes type 2, Hypercholesterolemia and fatty liver grade I. The action of the sunflower seeds on the blood sugar levels, lipid profile, and liver function tests of the patients have been evaluated. The blood glucose evaluations include – Fasting Blood Glucose (FBS); LFT (Liver Function Test) include –Serum Glutamate Oxaloacetic Transaminase (SGOT), Glutamic Pyruvate Transaminase (SGPT). The lipid profile evaluations include – Total Cholesterol, Triglycerides, LDL (Low Density Lipoprotein), HDL (High Density Lipoprotein).

Diabetes Mellitus type-2 (non-insulin dependent diabetes; NIDDM) is one very common ailment of metabolism which has been most commonly identified by hyperglycaemia (high blood sugar) due to deficiency of insulin or insulin resistance (Kumar *et al.* 2005). The main indicators of NIDDM are Polyuria (recurrent urination), Polydipsia (excessive thirst) and Polyphagia (constant hunger). Apart from these, there also occur headache, blurring of vision, tiredness, delayed wound healings, and pruritus in this metabolic disorder. Prolonged hyperglycaemia can lead to complications like diabetic nephropathy, diabetic retinopathy, diabetic neuropathy, stroke, cardiovascular diseases etc. There are specific types of skin rash known as diabetic dermadromes occurring sometimes in diabetes. Being overweight or obesity are two major reasons that lead to Diabetes mellitus (Smyth *et al.* 2006).

According to the 2010 census there has been a massive increase in the people suffering from diabetes than as compared to the census of 1985. Very precisely around 285 million people were found diabetic in 2010 in comparison to the 30 million diabetic patients in 1985. The patients suffering from diabetes also exhibit a poor flow of blood to its extremities which in some cases result in the surgical removal of the affected area. Apart from all the above mentioned ketoacidosis is another major complication taking place in patients with diabetes (Fasanmade *et al.* 2008).

Similar to diabetes, cardiovascular disease (CVD) has now become the leading cause of mortality in India. Premature mortality in terms of years of life lost because of CVD in India has increased by 59%, from 23.2 million to (1990) to 37 million (2010). Despite wide heterogeneity in the prevalence of cardiovascular risk factors across different regions, CVD is emerged as a leading cause of death in all parts of India (Niharika 2016). CVD occur as a result of deranged serum lipid levels also known as dyslipidemia for long periods of time. Hence if the serum lipid levels are taken care of the prevalence of CVD can also be brought under check (Dorairaj *et al.* 2016).

Cholesterol: the main culprit, is a fat like substance of waxy consistency; that is not just present in certain foods (like meat, egg yolk, poultry, dairy products, fish) but also produced in the body itself. Some amount of cholesterol is required by the body to make some hormones, build membranes of cells and produce some compounds that take part in fat digestion. Accumulation of excessive amounts of cholesterol in blood leads to an increased risk of development of any cardiac disease in that person (Austin *et al.* 2004). The hypercholesteraemic patients pose a higher risk of suffering from any cardiac disorder or any cardiovascular disease. The accumulation of cholesterol particularly occurs in the coronary arteries that supply blood to the heart. The abnormal cholesterol build-up in the artery walls narrows and hardens the arteries as it forms clumps known as the plaque in it. With the increase in the size of the clumps, clogging occurs in the arteries restricting the blood flow to the heart leading to angina a form of acute chest pain and proceeds to a condition called myocardial infarct commonly known as the heart attack (Austin *et al.* 2004). The cholesterol being insoluble in water needs to be transported in the blood plasma with lipoproteins which are specific protein particles. The lipoproteins can be classified into four types by their existing density: HDL, IDL, LDL and VLDL which can be elaborated as high, lipoprotein, low and lipoprotein respectively (Wooten *et al.* 2004).

Fatty liver disease which is commonly known as fatty liver is a health condition that can be reversed, if in its initial stages. Fatty liver disease (NAFLD) is a distinct hepatic condition and one of the most common causes of chronic liver disease globally. Prevalence of the disease is estimated to be around 9-32% in the general Indian population, with a higher incidence rate amongst obese and diabetic patients (Kalra *et al.* 2013). In this condition molecules of triglyceride or fat get deposited in the cells of liver as a result of steatosis, a process which includes abnormal deposition of fats in the cell. The condition also relatively

influences the metabolism of fat in the body (Reddy *et al.* 2006). As a result of fat deposition in the liver a condition known as steatohepatitis occurs in some patient's i.e. relative inflammation of the liver (hepatitis). In cases where fatty liver occurs as a result of alcohol intake, the health condition is known as alcoholic fatty liver disease (AFLD) or steatosis. The other forms of fatty liver are termed as Non-alcoholic steatohepatitis (NASH) (if it is not due to alcohol) or alcoholic steatohepatitis (Reddy *et al.* 2006).

The early symptoms of fatty liver include: fatigue, anorexia, loss of weight, weakness, lethargy, nausea, poor concentration and confusion. If not taken care on time it may proceed to critical conditions like cirrhosis of liver, Hepatic Coma, Hepatic Encephalopathy etc. These diseases possess clusters of fatal symptoms in it as it is (Gramlich *et al.* 2004).

Talking about the general medicines which are used to treat such metabolic disorders they accompany an array of side effects. The most commonly used such medicines include Avas, Lovastatin, Glycomet etc. The side effects may include abdominal distension, gastritis, headache, sexual problems, nausea in certain cases and most importantly dependency. The word dependency can be elaborated as- the blood sugar or lipid profile remain under control till the time medicine is being consumed or otherwise the levels become deranged and hence in some cases the medicines have to be continued lifelong. Hence, countering the epidemic requires development of strategies like the formulation and effective implementation of evidence based policy, reinforcement of health system, and treatment with the use of both conventional and innovative techniques.

As per the plan of the study, using the available strategies in the nutritional sciences and a food-based approach, a product (Cookies) was formulated using sunflower seeds in combination with other integral products for its easy dispersion to the patients. Keeping in mind the nutritional attributes of sunflower seeds, different flour blends were used to design and develop healthy sunflower based cookies made from these blends. The result obtained was put to use for the nutritional therapeutic purpose.

Helianthus annus the botanical name for sunflower seeds; it is a member of the genus *Helianthus* and family *Asteraceae* (National Sunflower Association 2011). The seeds produced by sunflower are widely considered as seeds with a good nutritive value and are easily available all over India (Philips *et al.* 2005). The metabolic disorders like diabetes, CVD, fatty liver etc. these days are creating a menace Hence, countering the epidemic requires development of strategies like the formulation and effective implementation of evidence based policy, reinforcement of health system, and treatment with the use of both conventional and innovative techniques. Many studies have been done on sunflower seeds, its health benefits, processing, and nutritive value. Thus the topic of the present study has been reviewed under the following headings

2.1 Sunflower seeds and its composition

Sunflower (*Helianthus annus* L.) with a good stability and an opulent PUFA content makes it world's leading oilseed crops. After the soybean oil it's the sunflower oil that ranks second in world in the vegetable oil production. Whole sunflower kernels can be incorporated into human food formulations (Robertson *et al.* 1975). Tocopherols are the most important compounds having antioxidant activity in sunflower seeds (Velasco *et al.* 2002). Amongst the oilseed crops sunflower has been claimed to be very important and in the world ranks amongst one of the best vegetable oils with a very novel nutritional quality. Generally about ninety percentile of fatty acids are unsaturated in the general sunflower oil composition. They are linoleic and oleic fatty acids. Palmitic, stearic and small quantities of myristoleic, myristic, arachidic, behemic, palmitoleic along with other fatty acids contribute to remaining 10%.

A research was conducted by Katherine *et al.* (2001) on the usually consumed seeds and nuts in United States regarding the quantification of phytosterols. Acid hydrolysis followed by alkaline saponification of the lipid extracts was done. With the help of capillary GC-FID and GC-MS the free sterols were analysed as trimethyl derivatives. Amongst all, the phytosterol content was found to be highest in wheat germ and sesame seeds about (400-413

mg/100g) whereas Brazil nuts were reported with the lowest (95 mg/100 gm). Pistachios and sunflower seed kernels the most commonly consumed snack foods in US were also reported with good amounts of ranging from 270-289 mg/100gm.

The sunflower genotypes and the content of tocopherol and phenols was investigated in the same by Zilic *et al.* (2010). In this study, the tocopherol (α , β , γ) and the phenolic compound content along with the DPPH radical scavenging activity were analyzed in the seeds and kernels of 3 sunflower hybrids. With the help of HPLC method, 6 different phenolic compounds were identified. The most opulent phenol was found out to be Chlorogenic acid which showed a strong correlation with the total phenols ($r=0.93$). Other major phenolics found have been ferulic acid, rosmarinic acid, caffeic acid, rutin, and myriceti. Now as compared to the seeds the total tocopherols were found out to be substantially higher in the kernels ($P<0.05$) in all the sunflower hybrids. The concentrations of tocopherols in sunflower seeds and kernels ranged from 200.56 to 220.04 $\mu\text{g/g}$ and from 255.52 to 268.49 $\mu\text{g/g}$ respectively, where α -tocopherol had been found to be most abundant amongst the sample. Accordingly, it was deduced that sunflower kernels had a higher DPPH scavenging activity, and a higher nutritive value than sunflower seeds.

The effect of powdered safflower seed and sunflower seeds on total cholesterol level in the rats that were fed on high fat and high cholesterol diets was studied by Moon *et al.* (2001). A high cholesterol diet (1%, weight/weight) or diet high in cholesterol augmented with the powder of sunflower seeds (5% weight/weight; SSP) or extract of sunflower seeds and ethanol (0.15% weight/weight; SSE) or water extract with safflower seeds (0.5% weight/weight; SSW) were administered to male rats for a time period of about 5 weeks. It was found that all the compositions of the safflower seed considerably reduced the concentration of total cholesterol; on contrary the SSE and SSW supplementations that lowered the plasma triglyceride concentration. In the SSW group the plasma hepatic cholesterol contents were found to be considerably lesser with regard to the control group, on contrary in both the cases the triglyceride hepatic content had been found to be markedly lesser. It was also analysed that, the hepatic HMG-CoA functions had been considerably raised in the two groups SSE and SSW groups in comparison to the remaining both groups. As per the results it becomes indicative that the SSE and SSW administration had proven to

be potent in improving the risk of atherosclerosis in the rats which were administered with diets high in cholesterol.

Flaxseeds, wheat germ, sunflower seeds, buckwheat along with twenty eight plant products were selected and their total phenolic content and antioxidant activities were analysed by Velioglu *et al.* (1998). Folin-Ciocalteu method was used to determine the phenolic content. Phenolic content was determined to be varying from 168 to 10549 mg/100 g of dry product. β -carotene bleaching method had been utilised to evaluate the antioxidant activity of the methanolic extract being expressed as AOX (log A_{470}/min), A4 (percent inhibition relative control), ORR (Oxidation Rate Ratio), and ACC (Antioxidant Activity Coefficient) varied from 0.05, 53.6, 0.009, and 51.7 to 0.26, 99.1, 0.46, and 969.3, respectively. Amongst the antioxidant activities and the total phenolic content the correlation coefficient came out to be statistically significant.

The outcome of intake of different types of fat on cholesterol levels in the blood serum was investigated by Bronte *et al.* (1956). 2 European men with high serum cholesterol initially along with 1 Cape Coloured man and 5 Bantu men with low serum cholesterol level initially were given diets added with several fats and oils. The diet of the 2 Europeans comprised of 50 g of fat daily mainly from the animal origin whereas the diet of the 6 Non Europeans comprised of a low fat content. The serum cholesterol and serum lipoprotein were recorded along with the cholesterol content of the faeces. It was found out that beef muscle, beef drippings, butter, and hydrogenated groundnut oil caused a raise in the serum cholesterol levels.

A cross over double-blind study was directed by Barham *et al.* (1998) to analyse the effects of sunflower seeds and whole flax seeds on the lipid profile of the post-menopausal women as a part of their diet. 38 postmenopausal women were selected with mild, moderate or severe hypercholesterolemia. They were randomly assigned the 6-week period with two regimens: flaxseeds or sunflower seeds. Either treatment provided to the subjects comprised of 38 g in breads and muffins. After six weeks of supplementation a two week washout phase was conducted. Post the washout phase the subjects were switched. The Blood samples were collected at the start and then 6, 8 and 14th week of the study. For both the treatments significant reductions ($p < 0.01$) in total cholesterol were recorded (6.9 and 5.5% for flaxseed

and sunflower seed, respectively). The cholesterol lowering effects of the sunflower and flax seeds were attributed to the presence of α -linolenic acid or the linoleic acids, the non-protein constituents in the seeds along with total and soluble fiber.

2.2 Effect of food processing (roasting) on the quality attribute of sunflower seeds

Roasting is a common cooking method that incorporates dry heat method, usually practised at 140-400°C. This method of cooking prepares food with radiating heat and also by convecting heat through forced air (Singh *et al.* 2016). This is basically a short time and a high temperature procedure (Mayer *et al.* 1985). Roasting results into drying that further leads to a reduction in the moisture content. The moisture diffusion occurring at a high temperature causes puffing and hence a crisp texture. With the decrease in the moisture content the shelf life of food, seeds or grains increases as the water activity is also decreased. The colour, flavour and odour evolved during roasting in the food product give a characteristic appearance and taste to it (Sharma *et al.* 2011).

One appliance that is nowadays found in majority of houses is the Microwave oven. The microwaves are used widely by masses these days for reheating as well as cooking certain foods. The principle used by microwaves is heating through interaction of electric component of the electromagnetic field with the polar molecules. As and by the polar molecules strive to position in the oscillating field the heat is generated (Burfoot *et al.* 1990). The microwave provides many advantages for industrial as well as home cooking, thawing, baking, pasteurization, sterilization, tempering and blanching (Decareau *et al.* 1985). Microwave energy works instantaneously with penetration and heating of food (Mudgett *et al.* 1989; Watanabe *et al.* 1998).

The roasting of sunflower seeds was performed in a study by Fozia *et al.* (2005). 10 g of sunflower seeds were placed uniformly in pyrex-petri dishes. They utilised a consumer-model microwave oven for the experiment. The roasting was conducted for 5, 10 and 15 min at 2450 MHz frequency (oven adept of generating 500 W and medium power setting). Once the roasting was completed the sunflower seeds were kept at an ambient temperature to cool, post to which they were intensively mixed before the crushing and oil extraction. In another study, Farooq *et al.* (2005) studied the impact on the sunflower seed composition of microwave heating. They also explored the oxidative stability changes, Fatty Acid distribution and

tocopherol content of the sunflower oil. Two varieties KL-39 and FH 330 of sunflower seeds were taken and extracted with n-hexane. In their experiment, a significant difference ($P<0.05$) in the oil content of seeds was observed. However, content of protein and fiber depicted no change in the oilseed residue. Although, a significant ($P<0.05$) decrease in the tocopherol amount was found but still around 76-81% of α -tocopherol was detected in it even after 15 min of roasting. Microwave heating in regard to the FA composition lead to decrease in linoleic acid 17-19% and an increase in oleic acid 16-42%, while the content of palmitic and stearic acid remained unaffected.

In an experiment Yoshida *et al.* (1999) analysed the oxidative stability and the tocopherol content in the oils prepared from soybean conducted roasting of whole soybeans. 12.0 cm diameter pyrex-petri dishes were taken. The beans were placed uniformly in a single layer. Next they covered the petri dishes, and placed them in the microwave oven on its glass rotating plate (model R-5550; Sharp, Osaka, Japan). In turntable mode, beans were roasted for six, eight, twelve and 20 minutes respectively. To prepare a full fat soy flour without any burnt odour roasting for about 6-10 min was found to be favourable. In another study– to analyse the functional and antioxidant properties of chick pea (*Cicer arietinum*), post to its exposure to the microwave roasting (Jogihali *et al.* 2017) performed the roasting of chickpeas seeds. The seeds were soaked in water for 45 minutes at room temperature (Ratio being; water: seeds =2:1). Post to this they were air-dried in open for 10 minutes. The treated chickpeas at different powers (450, 600 and 900W) for 5, 10 and 15 minutes were then roasted in microwave oven. Post to this the roasted and non-roasted chickpeas were converted to a flour using hammer mill.

2.3 Antioxidant potential of sunflower seeds

Antioxidants are considered to be additives in the food industry as they play a major role in reducing oxidation of food components, specifically that of lipids. The process of oxidation results in deterioration of the quality of food as well as its shelf life (St Angelo *et al.* 1996). The antioxidants are treated to be of a high importance even in the living organisms since they interfere and hence prevent the formation of excessive free radical in cells. These free radicals if present in excess may cause degradation and deterioration of the biologically important molecules and thus result in progression of various diseases. It is the oxidative processes that results in onset of various infectious diseases, diabetes, cancer, rheumatoid

diseases, arthritis, eye issues, respiratory diseases, atherosclerosis etc. (Temple *et al.* 2000). Among various plant products, the eminent sunflower seeds have been found out to possess a high antioxidant potential. In comparison to other vegetables oils, sunflower oil was found out to be rich in α -tocopherol (Schmidt *et al.* 2005).

Antioxidant activity and the phenolic compound profiles of six fractions (I-VI) of sunflower seed extract was analysed by Magdalena *et al.* (2012). The HPLC-MS (ESI) analysis method was applied for the qualitative and quantitative analysis of the fractions for its phenolic compounds profiles. In terms of their ability to scavenge DPPH and ABTS and also in terms of their ability to reduce Fe^{3+} ferricyanide complex to the ferrous form which was expressed as TEAC, EC50, and the reducing power values the antioxidant activity of the fraction were studied respectively. Their experiment showed a pragmatic result as in a positive correlation was obtained between the antioxidant activity and the phenolic content of the individual fraction.

The antioxidant activities and total phenolics of 28 plant products, including sunflower seeds, flaxseeds, wheat germ, buckwheat and several fruits, vegetables and other medicinal plants were determined by Velioglu *et al.* (1998). The total phenolic content, determined according to the Folin-Ciocalteu method varied from 169 to 10548 mg/100g of dry product. Antioxidant activity of methanolic extract evaluated according to β -carotene bleaching method expressed as AOX ($\Delta \log A_{470}/\text{min}$), AA (percent inhibition relative to control), ORR (Oxidation Rate Ratio) and AAC (Antioxidant Activity Coefficient) ranged from 0.05, 53.7, 0.009 and 51.7 to 0.26, 99.1, 0.46 and 969.3 respectively. The correlation coefficient between total phenolics and antioxidative activities was statistically significant. In a similar study, Bolivar *et al.* (2009) aimed to check the antioxidant activity (TAC) at different germination states (dormant, imbibed and 7d sprouts) for 13 edible seeds. Selected seeds included mungbean, alfalfa, fava, fenugreek, mustard, wheat, broccoli, sunflower, soybean, radish, kale, lentil and onion. Sunflower seed sprouts had higher TAC on a DB ($40202 \mu\text{g Trolox g}^{-1}$) compared to other seeds.

In another study conducted by Paulina *et al.* (2013) aimed at investigating the effect of germination on the phenolic acids and flavonoids profile, as well as antioxidant activity (AA), in selected edible seeds of mung beans, radish, broccoli and sunflower. Germination increased the total phenolic (TP) and flavonoid (TF) levels, as well as the AA of the seeds,

and influenced the profile of free and bound phenolic compounds. Among the samples, mung bean was characterised by lowest levels of TP and TF, as well as AA, evaluated using ABTS, DPPH and FRAP assays. Sunflower and radish sprouts were the most rich in phenolic compounds.

Phenolic compound profiles and antioxidant activity of six fractions (I–VI) acquired from the extract of sunflower seeds was analyzed by Karamac *et al.* (2012). For the quantitative and qualitative estimation of phenolic content of fractions the HPLC-MS (ESI) analysis method was put to use. The evaluation of the antioxidant activity of the fractions was done in reference to their potential to scavenge ABTS and DPPH along with their ability to reduce ferricyanide complex to ferrous form. It was expressed as EC50, TEAC respectively. Good correlation was shown amongst the antioxidant activity as well as the phenolic content of the fractions.

2.4 Relationship between diet and diseases

It has been recorded during the last few years that a bad diet is directly proportional to the progression of certain metabolic disorders and chronic diseases as like cardiovascular disease, cancer, cataract, diabetes mellitus, hypertension, obesity etc. (Willet *et al.* 1998). The records depict that a daily diet regime that consists of ample vegetables, fruits, plant foods, legumes with non-processed foods in it decreases the development of such chronic diseases considerably. It was forwarded by Jacobs *et al.* (1998) in their study that vegetables, fruits and minimally processed foods form protective foods that make a shield against the progression of the various chronic diseases.

In another study Scoztek *et al.* (2013) reviewed and identified the major contributing factors of diabetes, cardiovascular disease (CVD), chronic diseases of respiratory system, malignant cancer to be unhealthy nutritional practices and adverse lifestyle. In accordance to the WHO guidelines, it was forwarded that a healthy lifestyle would require replacing saturated fatty acids (SFA) with polyunsaturated fatty acids (PUFA) along with elimination of the trans-fatty acids from diet and reducing the consumption of simple carbohydrates. Present study reviewed the current evidences and the most appropriate type of dietary fat for preventing arteriosclerosis was discussed. Increased intake of PUFA in the diet in both America and Northern Europe resulted in n-6 PUFAs being dominant in diets in comparison

to n-3 PUFAs. The resultant non-proportion led to increase in mortality due to CVD in these countries. It was analysed that in contrast to the above, the conventional Mediterranean diet that yielded a PUFA n-6/n-3 ratio of 2:1 proved to be more beneficial. Also it was added by the recent studies that the idea of replacing the SFAs with carbohydrates could not reduce the risk or arteriosclerosis. Also, substituting carbohydrates with MUFA gave ambiguous findings but only the PUFAs and that too n-3 was found to reduce the risk of IHD (Ischemic Heart Disease). Till now the debate about n6 and n3 goes on. However, its noteworthy that adopting a Mediterranean diet pattern might help reduce the risk of IHD.

In a study conducted by Nicolosi RJ *et al.* (2004), the diets which were higher in PUFA i.e. polyunsaturated and their relation with cholesterol in the blood serum levels was evaluated. In contrast, meal plans with elevated quantities of MUFA and saturated fats did not lead to any such decrease. The given study had been conducted to analyse the impact of meal plans with high- or mid-linoleic oil in comparison to the high-linoleic containing sunflower oil on oxidation of LDL which would lead to progression of cardiac diseases on earlier stages in the hamsters with a raised serum cholesterol levels. The hamsters were given a high cholesterol diet consisting of 10% sunflower oil (mid-oleic), or sunflower oil (high linoleic) (wt/wt), olive oil (high oleic) in addition to cholesterol 0.4% (wt/wt) for a period of 10 weeks. After completion of 10 weeks, only the animals that had been fed with first group showed considerable decrease in the serum levels of LDL (a decrease of about 17%) in comparison to the second group. Hamsters fed upon third group showed considerably raised levels of serum triglycerides (an increase of about 41%) in comparison to the fourth group. Amount of the serum LDL in the animals administered with the fourth group were significantly higher (+77%) in comparison to hamsters given either the olive oil (high-oleic) or sunflower oil (high linoleic). As per the LDL oxidation parameter measurements the animals fed on the third and second group had notably an extended lag phase (ranging from an increase of 66% to 145%). With regard to the sunflower oil (high linoleic), the ester of the aortic cholesterol ester had been found to be decreased by 13% and 34% in the sunflower oil (mid oleic) and Olive oil group (high oleic).

The LDL atherogenicity was studied by Juan *et al.* (1996). For the study about 18 subjects were selected as volunteers. The subjects were given a diet with 31% of its calories coming from the sunflower oil for 3 weeks which was then changed to a diet in which 30.5% of calories were obtained from the olive oil for an additional 3 weeks. The LDL after SFO

(Sunflower oil) displayed the ratio of fatty acids as (18:2 + 18:3 + 20:4) to (16:0 + 16:1 + 18:0 + 18:1) of 1.06 ± 0.11 compared to 0.73 ± 0.06 after the OO (Olive Oil) period. The LDL levels were found to be significantly lower after SFO than as compared to after OO. Against the expectation, the LDL oxidation catalysed by copper was significantly less than period of SFO intake in comparison to the period of OO intake. The result obtained could also be contributed to the larger size of the SFO-LDL. Thus, it was found that the³ LDL properties: oxidizability, circulation, along with the intima proteoglycans affinity, alteration in the atherogenesis, was found to be directed in the favourable condition with intake of natural antioxidants and linoleic acid in the diet.

Functional foods and its properties were studied by Claire *et al.* (2002). They forwarded it to could be certain group of foods that are whole, enriched, enhanced or fortified. The functional foods have been known to consist of certain medicinal value in addition to its usual nutritional profile or they are considered to boast certain health properties apart from their nutritional abundance (e.g., vitamins and minerals), when they are consumed in adequate quantities as part of their diet on a regular basis. Correlating the intake of certain foods considered to be functional foods with health benefits should be established on some scientific evidences. The study was conducted on the normal people taken as subjects. Although many claimed to be functional foods with sufficient amount of data for back-up but still all foods available in market and claiming to be functional foods are not. The given study on the basis of certain proofs on their role categorized a number of foods that were considered to be functional. These foods have become mightily explored & broadly popular field of nutritional research these days. Nevertheless, special consideration should be given to the fact that the functional foods should not considered as a magic wand against improper lifestyle and habits.

In another study conducted by Rui *et al.* (2005) the intake of nuts on a regular basis was found to be directly lower the risk of diabetes type 2 and cardiovascular disease. The authors studied in the Multi-ethnic study of atherosclerosis the relation between the consumption of nuts and seeds along with C-reactive protein, interleukin-6, and fibrinogen. A cross-sectional study was done and it incorporated around 6080 participants from US with their age ranging from included 6,080 US participants aged 45–84 years with ample background study of their diet and biomarkers. The consumption of nuts and seeds was categorised as 5 or more times per week, 1-4 times per week, less than once a week, rare or

never. After certain adjustment in age, gender, income, education, race/ethnicity, physical activity, drinking, smoking, use of dietary supplements mean biomarkers were as follows interleukin-6—1.25, 1.24, 1.21, C-reactive protein—1.98, 1.97, 1.80, and 1.72 mg/litre and fibrinogen—343, 338, 338, and 331 mg/dl ($p < 0.01$). Further changes in hypertension, lipid levels, diabetes and medication use furnished identical results. Secondary changes in the BMI mildly enhanced intensity of the union delivering statistical significance at borderline. Frequent consumption of seeds and nuts was found to be linked with lower inflammatory marker levels.

It was investigated by Kathleen *et al.* (2007) that diet influenced the prevailing risk factors for cardiovascular diseases (CVDs). In lieu of the study, intake cholesterol rich foods along with the total dietary fat, particularly Trans-fats and saturated fats was recommended in moderation. Dietary fats were allowed mainly from plant sources and fatty fish, providing polyunsaturated (including omega-3) and monounsaturated fatty acids. Whole grains, legumes, vegetables, fruits, and other fiber-rich sources, were used as the carbohydrate source rather than sugars. Although vitamins such as E, C, and some B vitamins have been correlated with decrease in CVD risk, data supports foods rich in these nutrients than the use of supplements. Dietary minerals such as calcium, potassium and magnesium have been found to be favourable for heart health, on contrary the risk of hypertension decreases with the reduction corresponding with reduced risk of CVD. In contrast, the data present has been quite powerful to advocate the association of healthy body weight management and cardiovascular health. In general, diets based mainly of plant origin and less processed foods, along with an active lifestyle were found to be helpful in heart health. A similar study conducted by Levya *et al.* (2010) demonstrated the utilisation of nutritional interventions to prevent the advent of cardiovascular diseases (CVD). One such nutritional strategy was increased usage of omega (ω)-3 fatty acids to produce considerable cardio vascular benefits. Amongst the rich sources of ω -3 fatty acids marine food products are one. Apart from marine products flaxseed is also one plant based ω -3 fatty acid source. As per the results acquired from various epidemiological investigations, experimental studies and clinical trials consumption of ALA (alpha-linolenic acid) has been found to be beneficial in CVD.

The effects of essential fatty acids on health and in chronic diseases was analysed by Artemis *et al.* (1999). It was forwarded that the diets of human beings evolved have ever since consisted of around similar quantities of n-3 and n-6 essential fatty acids, but in the last century there has been a relative increase in the intake of n-6 fatty acids. In the western diets

nowadays the proportion of n-6 to n-3 fatty acids instead of the prescribed 1-2:1 ranges from $\approx 20-30:1$. The high intake of n-6, as per the study's lead to a shift in the anatomical condition to prothrombotic which is designated by an increase in vasospasm, blood viscosity, decrease in bleeding time and vasoconstriction. The omega 3 Fatty acids on the other hand possess antithrombotic, anti-inflammatory, antiarrhythmic, vasodilatory and hypolipidemic properties. The n-3 fatty acids is known to prevent HTN, type 2 diabetes, coronary heart disease along with rheumatoid arthritis, renal disease, Crohn's Disease, Ulcerative Colitis in certain patients.

2.5 Product Development

2.5.1 Cookies

From a very long time cookies as snack foods have played a vital role in life of human as antiquity and are very much relished by large section of society. The percentages of its ingredients might differ but in the end the final product is always expected to be same- sweet, crunchy and nutty. Cookies have a lower moisture content and hence they are protected from microbial spoilage and provides longer shelf life. The incorporation of sunflower seeds flour may be a very good option for its easy administration due to its wide acceptability by masses

In a study conducted by Pasha *et al.* (2011) mung beans were used to develop high protein cookies (100:0, 95:5, 90:10, 85:15, 80:20, 75:25). It was found that there was an increase in the ash, crude fiber and the protein content as the percentage of mung beans was increased. Also, it was found that the thickness of the cookies along with the above parameters was increased. In a similar study Mishra *et al.* (2012) blended soybean flour and maize flour to develop the cookies in the ratio of 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100. It was found that the cookies with highest percentage of soy flour were high in crude fiber, ash, protein and fat in comparison to the cookies containing high maize flour content which showed a high carbohydrate content. The cookies containing ninety percentile of maize flour and ten percentile of soy flour attained highest in sensory quality attributes.

In a similar study conducted by Aziah *et al.* (2012) legume flour was incorporated to develop cookies. Three formulations had been developed (1) control with 100% wheat flour (2) wheat flour 50% + moong bean 35% + corn starch 15% (3) wheat flour 50% + chick pea

35% + corn flour 15%. Significant difference ($p < 0.05$) were obtained in total carbohydrates, crude fiber, protein and ash content in the cookies. It was deduced that the sensory attributes of cookies incorporated with legumes were better than the control. Another study conducted by Chilungo (2013) used Cassava flour and Pigeon Pea flour in different percentage. The flours were blended in the ratio wheat flour: Cassava flour: Pigeon pea flour (90:5:5, 80:10:10, 70:15:15). 100% wheat flour cookie was used as the control. An increase in the protein as well as the fiber content was observed when the content of pigeon pea flour and cassava were raised. Cookies with the highest content of cassava and pigeon pea flour had highest cookie weight, diameter and spread ratio whereas the control ranked lowest in all.

2.5.2 Biscuits

Hesham *et al.* (2007) developed biscuits incorporated with germinated and non-germinated legume seed flour or mushrooms. Germinated and non-germinate legume flour along with mushroom flour was blended with wheat flour in the percentile of 5, 10 and 15 respectively. As per the results dough developing time and water absorption capacity increased whereas the dough stability and tolerance index decreased in the flour blend of 10 and 15 percentile. In a similar study, Masur *et al.* (2008) developed high protein biscuits supplemented with Bengal gram. Wheat biscuits with added Bengal gram flour with 10, 15, 20, 25 percent level along with modifications in water, fat and baking powder to improve the nutritional and textural quality of biscuits were made. The diameter (cm) and height (cm) were found to be constant with 15% incorporation of Bengal gram flour. It was found that supplementation of Bengal gram flour in about 15-20% level improved the dough texture, sensory parameters and protein quality.

A study performed by Banurekha and Mahendran (2009) developed biscuits incorporated with soybean flour as a protein supplemented cereal snack food. In the percentage of 5,10,15,20 and 25 the soybean flour and wheat flour was thoroughly blended. It was found that protein, fat and calorie of wheat-soybean biscuits increased as the per cent of soybean flour increases. But there was a decrease in the moisture and ash content of the biscuits as the soybean flour per cent was increased. Similarly, Abu-Salem and Abou- Arab (2011) prepared biscuits supplemented with Bambara groundnut. Wheat flour and the Bambara groundnut flour were blended thoroughly at a percentile 5, 10, 15, 20, 25, and 30 per cent. The biscuits made from 100 per cent were used as control. It was found that the

mean quality score of the decrease as groundnut flour increases. Thickness and diameter increases as groundnut flour increases.

2.5.3 Physicochemical Composition of Biscuits and Cookies

High protein biscuits were developed from Bengal gram in a study conducted by Masur *et al.* (2008). Wheat biscuits with Bengal flour tried at 10, 15, 20, and 25 per cent levels along with modifications in water, fat and baking powder were made to improve the nutritional and textural quality of biscuits. It was reported that the height of the biscuits remained constant with increasing levels of Bengal gram flour up to 20 per cent and also the diameter remains constant (58.5) at different levels up to 15 per cent of Bengal gram flour. But the spread factor and spread ratio decreased with increasing ratio of Bengal gram flour. Another study was conducted on similar lines where Mishra *et al.* (2012) prepared cookies from blended flour of soybean and maize flour in different proportion (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100) and the cookies were assessed for the physical and chemical attributes. It was seen that the cookies' weight reduced gradually from 16.3 to 4.9 with the rising fraction of soy flour and the spread ratio reduced significantly from 7.1 to 6.7 with increase in the concentration of maize flour. The protein content of the cookies was also found to reduce from 39.3 to 9.9 % with the increase in maize flour proportion. The fat, crude fiber and ash content reduced from 27 to 16 per cent, 4.2 per cent to 2.1 per cent and 7.08 to 4.58 per cent respectively.

The result of utilisation of protein pea isolate (decorticated) on basal baking attributes of the biscuits made from wheat was studied by Hassan *et al.* (2009). Decorticated pigeon pea isolate (DPPI) was incorporated with wheat flour at protein levels of 15, 20 and 25 per cent respectively. There was a decrease in the gluten quantity and increase in water absorption, dough development time and dough stability. Also an increase in the protein and the ash content is found along with decrease in the carbohydrate and the calorific value. Another study conducted by Kohajdova *et al.* (2013) studied the suitability of pea flour in cracker biscuits production. Refined wheat flour are substituted with different levels of pea flour (0, 10, 20 and 30%). The evaluation on rheological properties and physical characteristics found that addition of pea flour result in increased absorption of water and dough development time whereas dough stability was decreased. It was also found that there was a decrease in the

volume index, width and spread ratio and an increase in thickness. It was observed that pea flour had considerably higher protein (21.46%) and ash (3.11%) content than wheat flour.

2.5.4 Phytonutrient Composition of Biscuits and Cookies

The antioxidant activity (AOA) of frequently consumed pulses, cereals, legumes and millets in India was studied by Sreeramulu *et al.* (2009). AOA assessed by DPPH radical scavenging assay, ferric reducing antioxidant powder (FRAP) assay. It was observed that the finger millet and rajmah has highest FRAP ranged from 16.21 to 47.71 moles/g and DPPH scavenging activity 1.73 and 1.07. Finger millet and black gram dhal has the highest TPC ranged from 373 to 418 mg/100gm, respectively.

The nutrient composition of cereal (wheat) bambara and groundnut based cookies was analysed Maduke *et al.* (2013). Wheat flour and Bambara groundnut flour were used in ratio 70:30 to provide 10 per cent protein. It was observed that higher Zinc and iron (5.51 and 12.82 mg/100mg) were found in Bambara groundnut-wheat cookies as compared to the wheat cookies. Another study performed by Zhang *et al.* (2014) studied the selected dietary polyphenols in a cookie model for its antioxidant and anti-glycation activity. Five dietary polyphenols named epicatechin, naringenin, chologenic acid, quercetin and rosmarinic acid were selected for the cookie fortification. The increase in the antioxidant capacity was not as per the expectations since the antioxidant capacity was considerably decreased by thermal degradation during the baking process.

2.5.5 Organoleptic Evaluation

Hemenda and Mohamad (2010) prepared a cake fortified with 5 per cent and 10 per cent of chick pea and soybean. Sensory evaluation was performed using a 5-point semi structure scale method in terms of appearance, colour, cell uniformity, firmness, odour, taste, and overall acceptability. It was found that addition of soy flour blend at 5 per cent and 10 per cent levels to wheat flour had no adverse impact on sensory attributes of the end product. In another study, Gratin *et al.* (2010) developed school children snacks based on baked fermented legumes and cereals. Cakes were prepared by substituting 20 per cent of the refined wheat flour and kidney bean flour, brownies with 30 per cent of pigeon pea flour and cookies with 30 per cent of black eyed pea flour using fermented and non-fermented

legumes. The sensory evaluation of the products using the hedonic scale of 7 points observed that the product was higher than 5 in the attribute, taste, colour and overall acceptability.

Similar study was conducted by Howard *et al.* (2011) where they analysed pasta supplemented with the peanut flour for its formulation optimisation as well as the ingredient functionality and formulation optimization. Peanut flour substituted with durum wheat flour at a level of 30 per cent, 40 per cent, and 50 per cent. And also carrageenan were added at a level of 2.4 per cent, 2.65 per cent and 2.9 per cent and the drying temperature (60, 70 and 88°C) were used respectively on final pasta product. The sensory evaluation were done where the values of colour lightness varied from 42.43 to 64.01, decreasing (becoming darker) with an increase in drying temperature along with increase in the peanut flour level. The content of moisture varied from 56.24 per cent to 68.37 per cent and the values reduced as the drying temperature increased. The pasta was found to be light in colour, softer in texture and higher in moisture when dried at 60°C with 30 % of peanut flour in it in comparison to the other relative varieties with higher percentile of peanut flour and dried at a higher temperature.

CHAPTER-3

HYPOTHESIS

Sunflower seeds have been mainly used to extract the oil which is majorly used for cooking or other culinary purpose in various parts of the world besides its use in cosmetic industries. The seeds of sunflower are well known for its physico-chemical, phytochemical potential as required by the human body like presence of Vitamin E, B complex Vitamins, essential fatty acids, poly-phenols etc. in it. However, still lacking its identity in the pharmaceutical industries. Therefore, the present study is aimed to fulfil this gap by using sunflower seeds as a pharmaceutical weapon for curing various deranged metabolic parameters including serum cholesterol levels, blood lipid levels, Fasting blood glucose levels, SGOT, SGPT levels; and its further utilization in food product development for its efficient delivery in term of health and nutraceutical foods. Moreover preventing various metabolic disorders with nutritional intervention is therapeutic strategy that is widely being adopted.

CHAPTER-4
OBJECTIVES

1. To evaluate the tocopherol and phenolic content of sunflower seeds.
2. To study the effect of sunflower seeds on serum lipid levels, FBS in patients with diabetes type-2 and SGOT, SGPT levels in patients with fatty liver grade 1, with and without medication.
3. To compare the sunflower seeds with routine medicines of the relative metabolic disorders,
4. To prepare and evaluate the sunflower seeds' enriched food product.

The present investigation entitled “**Effect of Sunflower seeds on Hypercholesterolemia, Fatty liver and fasting blood glucose in Diabetes Mellitus type 2 patients**” was conducted under the Department of Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Phagwara, Punjab. The material used, detail of the experiments and techniques employed in the investigation have been furnished in this chapter under the following headings.

5.1 Analysis of tocopherol and phenolic content of sunflower seeds

5.1.1 Raw Material

The sunflower seeds’ samples were procured from the local market at Amritsar, Punjab, India. Seed weight varied from 120–200 mg. All seed were sealed up in polythene bags and stored in airtight container until needed.

5.1.2 Sample Preparation

The sunflower seeds were cleaned manually in order to abolish damaged, broken or cracked grains along with the foreign materials if any. The cleaned seeds were then kept in sealed aluminium pouches stored till further analysis and utilisation.

5.1.3 Roasting

The sunflower seeds were positioned on the turntable plate of the oven (Model: Samsung, CE104VD, 230 V-50 Hz, 2450 MHz, 100-900 W-6 Levels) after being placed in a single uniform layer in the 12 cm diameter Pyrex petri dishes (Yoshida *et al.* 2001). The contents of the dishes were then roasted at 150°C for 5 min. Once the roasting was done the seeds were kept to cool at room temperature (Patricia *et al.* 2014).

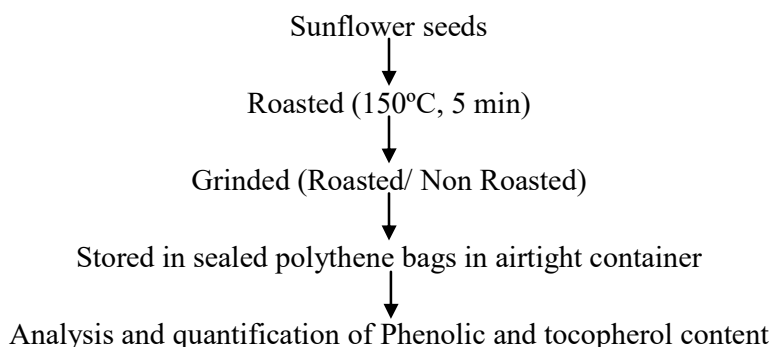


Fig 5.1 Flowchart for preparation and analysis of the sunflower seeds

5.2 Analysis of non-roasted and roasted sunflower seeds

5.2.1 Free Radical Antioxidant Scavenging Capacity:

The DPPH assay method works on the principle of the reduction of DPPH. DPPH, the free radical delivers the maximal absorption at 517 nm (purple colour) with one odd electron. When the antioxidant molecule reacts with DPPH, the stable free radical, pairing occurs in the presence of a Hydrogen donor and it's reduced to DPPHH. Resultant to this the absorbance is decreased DPPH radical to the DPPH-H, which leads to the decolourization (yellow colour). The decolourization is directly proportional to the reducing capacity.

The sunflower seeds have been reported to possess antioxidant properties. So in the present study sunflower seeds, both Roasted and Non Roasted have been evaluated for their possible potential to produce antioxidant action by the DPPH scavenging method. The extract of sunflower seeds was composed by dispersing 0.15 gm of powdered sunflower seeds in 10ml of 70% (v/v) acetone for the conduction of DPPH test. The solution was centrifuged at 20,000 g for 20 minutes after shaking for 30 min continuously at room temperature. Then the extract's aliquot (50µl) was taken and was made to blend with the acetate buffer (100 mM, pH 5.5, 0.5 ml) and ethanol DPPH solution (0.5 mM, 0.25 ml). After keeping the blend in dark for about thirty minutes, at 517 nm its absorbance was measured with absolute ethanol taken as a blank. Results obtained have been displayed as an IC₅₀ value. It shows the total quantity of sample (in mg) that provided 50% inhibition of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (Zilic *et al.* 2010).

The DPPH scavenging effect percentage was calculated by the given equation:

$$\text{DPPH scavenging effect (\%)} \text{ or percent inhibition} = \frac{A_0 - A_1}{A_0 \times 100}$$

Where A_0 was the absorbance of the control and A_1 was the absorbance of the sample.

5.2.2 Total Phenolic Content

Total phenolic content was estimated with Singleton and Rossi method (1965). Similar extract that had been used in the test of DPPH was used. 20% of 1.25 ml sodium carbonate, Folin reagent 0.25 ml, and 0.4 ml deionized water were taken and then the extract about 0.1 ml was blended with these solutions. At 750 nm the absorbance was measured after the solution was kept at room temperature for about 40 minutes. Gallic acid equivalents (eq.) were put to use in order to analyze the total phenolic content with respect to the gallic acid the calibration curve. The solutions obtained were manifested as milligrams of gallic acid per gram of dry matter (d.m.).

5.3 Effect of sunflower seeds on the biochemical parameters of sample patients (n=300)

5.3.1 Design of the Study

The present study conducted was a randomised, case controlled and a prospective study. Methodological aspects in the study have been discussed as under:

- Sample selection
- Data collection
- Pre supplementation data collection (Dietary survey, Anthropometric measurement, Biochemical testing)
- Supplementation of Sunflower seeds
- Post supplementation data collection (Biochemical testing, Dietary survey)
- Statistical analysis

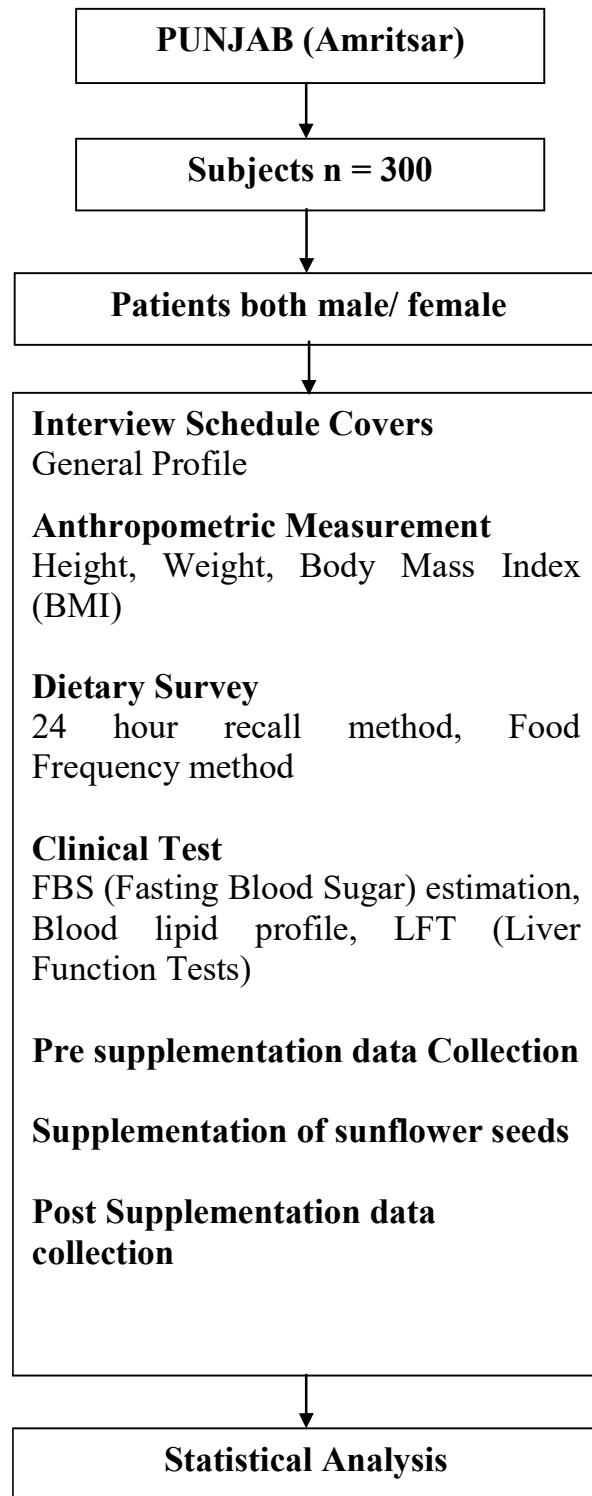


Fig 5.2 Flowchart elucidating design of the study

5.3.2 Sample Selection:

The entire sample comprising of 300 patients was selected from Punjab state (from the cities Amritsar, Batala, Jalandhar) amongst people of age ranging from 45-55 years (including both males and females) with raised blood glucose levels, deranged lipid profile or liver function tests. The subjects included in the study were selected from OPD/IPD (Out-Patients Department/ In-Patients Department) of the hospital.

5.3.3 Data Collection:

A schedule for an interview was prepared in order to collect the required information with the help of self-prepared questionnaire so that detailed information could be obtained. The interview schedule (questionnaire) consisted of both closed and open ended questions (Ivan *et al.* 2000). It was designed in such a manner so as to obtain the information related to:

- General Information
- Dietary Pattern
- History of Diabetes or any other medical problem
- Undergone any major or minor surgery

Pretesting of Interview Schedule - Before conducting the actual survey the interview schedule was tested on a few patients having same characteristic to find out the general level of understanding of some basic terms, process and questions and also to find out the need of modification in questions, if any (Richard *et al.* 1998).

5.3.4 Pre Supplementation Data Collection:

5.3.4.1 Dietary Survey:

Diet survey was carried by 24 hour recall method. The subjects were asked to provide estimates of the amount of meal they had taken during past 1 day or 24 hours. In the given method subject was asked to name the food eaten with approximate amounts during the previous day at each meal and between meals. Quantities were stated in household units such as a glass of milk etc. by providing the subjects the measuring cups or other devices to aid in recalling (Glady *et al.* 1982).

5.3.4.2 Food Frequency Method:

In this method the subjects were asked about the number of times certain foods or combination of food was consumed per day/per week/per month or any other period of time. The food frequency list is inclusive of a large number of food groups so as to get a clue to the nutritive adequacy (Walter *et al.* 1984).

5.3.4.3 Anthropometric Measurements:

The Anthropometric measurements taken were:-

- (i) Height
- (ii) Weight
- (iii) BMI (Body Mass Index)

(i) Measurement of Height:

Height is a linear measurement that reflects skeletal growth. It is a measure of chronic malnutrition or under nutrition and should be measured as accurate as possible. It is made up of the sum of component, legs, pelvis, spine and skull (Jatinder *et al.* 2004).

The equipment used for taking height of the subject was a non-stretch tape which was fixed on a flat wall.

(ii) Measurement of Weight:

Recording of weight is the most widely used measurement both for assessing under nutrition as well as for over nutrition. Weight of an individual reflects the more recent nutrition. It is a measurement of body mass. A portable platform weight beam balance was used to assess weight of the subject as it is sturdy, easily transportable and accurate to within limits required (Nisa *et al.* 2010).

Table: 5.1 Ideal Weight for Height Chart

<u>Height (Meters & Feet)</u>	<u>Males Weight (Kgs)</u>	<u>Females Weight (Kgs)</u>
(1.524 m)5'-0"	50.6 – 54.7	50.7 – 54.3
(1.549 m)5'-1"	51.8 -55.5	51.6 – 55.2
(1.575 m)5'-2"	56.5-60.4	53.0 – 56.6
(1.598 m)5'-3"	57.7 – 61.8	54.3 – 58.0
(1.625 m)5'-4"	58.8 – 63.6	56.2 – 59.8
(1.651 m)5'-5"	60.9 – 65.4	57.5 – 61.1
(1.676 m)5'-6"	62.3 – 66.8	58.8 – 63.4
(1.701 m)5'-7"	64.1 – 68.6	60.7 – 65.2
(1.727 m)5'-8"	65.7 – 70.9	62.1 – 66.6
(1.752 m)5'-9"	67.7 – 72.8	64.1 – 68.4
(1.778 m)5'-10"	69.3 – 74.5	65.7 – 70.2
(1.803 m)5'-11"	71.3 – 76.3	67.0 – 71.6
(1.828 m)6'-0"	73.1 – 78.6	68.4 – 73.8
(1.854 m)6'-1"	73.4 – 80.8	73.2 – 80.6
(1.879 m)6'-2"	77.7 – 83.6	77.5 – 83.4
(1.905 m)6'-3"	79.9 – 85.8	79.7 – 85.8

Source: National Centre for Health Statistics in collaboration with the National Centre for Chronic Disease Prevention and Health Promotion. Ideal weight for height chart for adults (2000).

(iii) BMI:

BMI (Body Mass Index) is the estimation of mass of the human body with respect to an individual's weight and height. It is the body fat's measure on the basis of weight and height of adults. It is also called the Quetelet index

BMI is measured with the help of a simple formula which includes a person's weight in Kg that is Kilograms to the person's height taken in meters (m).

$$\text{BMI} = \frac{\text{Weight in Kg}}{\text{Height in meter square}}$$

5.3.4.4 Biochemical Testing of the parameters

Biochemical testing helps in determining different parameters, and also identifying the main biological chemical compounds, by using molecular and biochemical tools. It helps to measure the amount of a substance in the body through blood or urine analysis.

(i) Fasting Blood Glucose

A sample of the blood was obtained when the person was in a fasting state and the amount of sugar was assessed. Blood sugar was measured with the help of an apparatus known as glucometer with the help of which fasting as well as random level of blood sugar were determined. First of all, a sharp edge blood lancet was used to the prick over the tip of the finger. A drop of blood from the prick area was taken and put over the specific mark, then the strip was inserted into the glucometer and count-down of time was started. When it reached zero the final value of blood sugar level appeared on screen of glucometer and noted (Kaul *et al.* 2013).

(ii) Estimation of Glycosylated Haemoglobin (HbA1c)

Hemoglobin A1c (HbA1c) test was done in order to evaluate the control of blood glucose in patients suffering from diabetes (usually type-2). The serum glucose testing on a daily basis gives a view of present control of blood sugar whereas the HbA1c gives a view of blood sugar control of the patient in the past 120 days. Because of the reason that glucose molecule stays attached to hemoglobin molecule for whole life of the red blood cell (about 120 days). This test is done to analyze the blood glucose level on an average in the patient for not just one day but also for previous 2-3 months (Kaul *et al.* 2013).

The test was performed with the help of the HPLC equipment Bio-Rad D-10, as per the DCCT referral source (i.e. Diabetes Control and Complications) in the latest issues. It is well recorded by the NGSP i.e. the National Glycohemoglobin Standardization Program. Before performing automated analysis, the sample haemosylate was prepared manually. As per the method used for testing the tetra decyltrimethyl ammonium bromide were mixed with the obtained samples consisting of the haemolysing reagent for several mins (1000µl haemolysing reagent + 10µl whole blood). The value of the Glycosylated haemoglobin was estimated and determined with the help of DDS kit (Diasis Diagnostic Systems) as per the

given instructions on its kit. A DDS calibrator was used for its calibration. The total haemoglobin that was required Glycosylated Haemoglobin's measurement was estimated in a different column of the same equipment in the DDS Kit (Fatih *et al.* 2010).

(iii) Estimation of Lipid Profile

A set of blood tests that help in identifying the range of lipid content in the blood as like cholesterol, triglycerides etc. is known as the Lipid profile. The results of this test help to identify approximate risks for disease if any as like coronary artery disease, atherosclerosis etc. also it helps to determine certain genetic diseases. The lipid profile includes complete cholesterol, triglycerides, LDL, HDL. Here, LDL is commonly known as the bad cholesterol whereas HDL is known as the good cholesterol (Sidhu *et al.* 2012).

The lipid profile and its relative tests were estimated by enzymatic calorimetric method especially the total cholesterol (Allain *et al.* 1974). The triglycerides were estimated by Van Denmark and Jacobs enzymatic method. The High Density and Low Density Lipoprotein were analysed with the help of Gordon and Gordon method 1977 and Friedewald formula 1972. All the above mentioned tests were estimated in the blood serum. All parameters were determined in the blood of the patients and controls bringing into use special kits of the reagent which are commercially available.

(iv) Estimation of Liver Function Tests (LFT)

The LFTs are groups of laboratory assays of blood in biochemistry which have been designed to analyse the status of an individual's liver. The parameters measured in this include albumin, bilirubin (indirect, direct), globulin, SGOT (AST) and SGPT (ALT) the Liver transaminases. The LFTs prove to be a helpful screening tool in detecting hepatic dysfunction in a patient if any (Sultana *et al.* 2004).

For enzymes- SGOT/AST (Serum Glutamate Oxaloacetic Transaminase/ Aspartate Aminotransferase), SGPT/ALT (Serum Glutamic Pyruvate Transaminase/ Alanine Aminotransferase) the procedure approved worldwide is used with p- nitrophenol phosphate taking part as a substrate, in an environment of basic pH. Un-haemolysed fresh blood was used as the sample for the evaluation (Thapa *et al.* 2007).

5.3.5 Grouping and Supplementation

The roasted sunflower seeds were advised to be added to hot or cold beverages or cereals (2gm).

(i) Control Group: It comprised of patients with high serum lipid levels, high blood glucose levels or high LFT levels than normal with minor medications for the same like lovastatin, glycomet, avas etc. along with the specific diet modifications. The groupings were as follows:

Group 1- Deranged lipid levels on specific medications and diet modifications.

Group2- Raised blood glucose levels on medications and diet modifications

Group3-Increased LFT levels on medications and diet modifications

Group4- Deranged lipid levels + increased blood glucose + deranged LFT levels with the medications and the diet modifications

Group5- Patients with high serum lipid levels or high blood glucose levels or high LFTs and only on diet modifications but not on any medications.

(ii) Case Group: - comprised of as follows:

Group1 (a) - Deranged lipid levels receiving 2 g of sunflower seeds in addition to the medications and the dietary modifications

Group2 (a) –Raised blood glucose levels receiving 2 g of sunflower seeds in addition to the medications and the dietary modifications

Group3 (a) - Increased LFT levels receiving 2 g of sunflower seeds in addition to the medications and the dietary modifications

Group4 (a) - Deranged lipid levels + increased blood glucose + deranged LFT levels receiving 2 gram of sunflower seeds in addition to the medications and the dietary modifications

Group5 (a) –Patients receiving 2gram of sunflower seeds including patient with high serum lipid levels or high blood glucose levels or high LFTs but not on any medications.

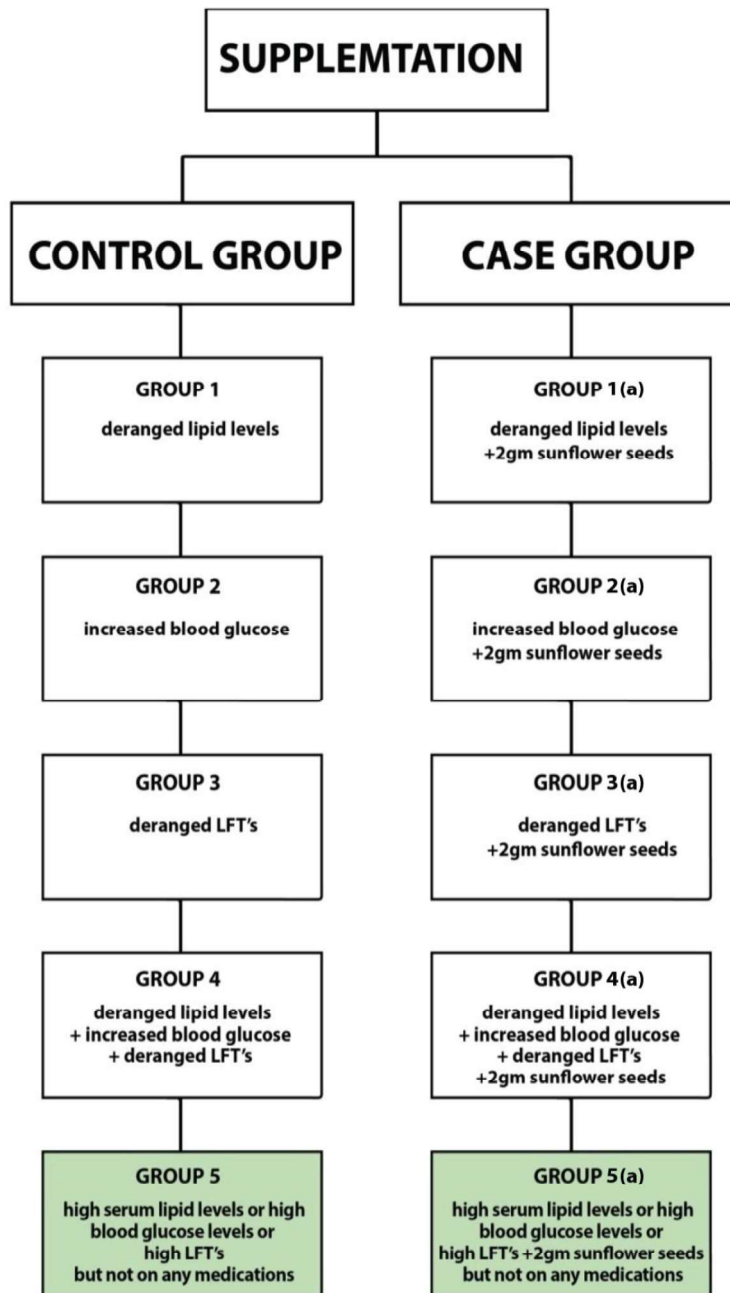


Fig 5.3 Flowchart showing the classification of the Case and the Control Group

5.4 To prepare and evaluate the sunflower seeds' enriched food product

Product formulation process is a systematic set of activities targeted at development of an acceptable product (Earle *et al.* 2007). Keeping in mind the nutritional attributes of sunflower seeds, different flour blends were used to design and develop healthy sunflower based cookies made from these blends. The result obtained was put to use for the nutritional therapeutic purpose.

Cookies are very common and well acceptable in all the countries. The percentile as discussed might differ but the final product is always expected to be same sweet, crunchy and nutty. Cookies are named variedly in different areas in accordance to the place of origin. From a very long time cookies have been served as or with a dessert and even nowadays they are a very common snack consumed at various times of the day with tea/coffee and even used as a gift item.

5.4.1 Procurement of raw material

To prepare the cookies, the materials needed were procured from the local market: sunflower seeds, wheat flour, sodium bicarbonate and white butter.

5.4.2 Flour Preparation

The seeds were first graded, then sorted and finally cleaned. The seeds then were soaked for 24 hrs in water. Post to soaking the seeds were washed thoroughly and then oven dried for 24 hrs at 60°C or till the moisture content came to around 11.4%. Seeds once dried were ground with the help of a grinding machine, the ground seeds were then sieved through a 1-mm sieve. The ground and sieved seeds were then stored in airtight containers or sealed packets until further analysis at room temperature. (Morton, 1987)

5.4.3 Experimental Plan

The experimental plan is given in **Table 5.2** and **Table 5.3** shows the different composition of flour. In **Table 5.4** the different ingredients used in making the cookies were given in gm and **Fig 5.4** shows flowchart of the preparation of cookies.

Table 5.2 Experimental plan for product formulation

S.No	Parameter	Level	Description
1.	Product	1	Cookies
2.	Ingredient	5	sunflower seeds, white butter, wheat flour, salt and sodium bicarbonate
3.	Samples	4	A ₁ , A ₂ , A ₃ and A ₄
4.	Analysis	4	Physical analysis, Sensory analysis, Functional Analysis, Physicochemical Analysis

Table 5.3 Composition of various flour blends

S.No	Flour Blend	Wheat Flour (WF), %	Sunflower Seeds Flour (SSF), %
1.	A ₁	100	0
2.	A ₂	80	20
3.	A ₃	70	30
4.	A ₄	60	40

Table 5.4 Ingredients for cookies' preparation

S.No	Ingredients	A ₁	A ₂	A ₃	A ₄
1.	WWF(gm)	250	200	175	150
2.	SSF (gm)	0	50	75	100
3.	White Butter (gm)	125	125	125	125
4.	Sodium Bicarbonate [Baking powder] (gm)	3.5	3.5	3.5	3.5

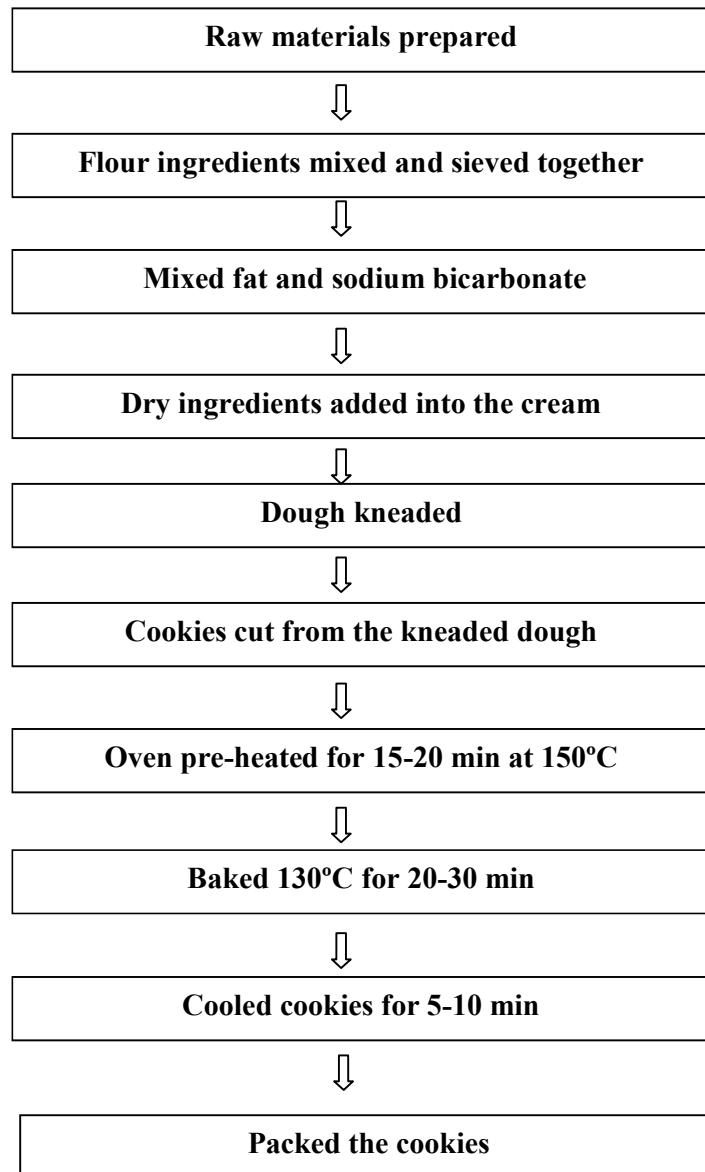


Fig 5.4 Flowchart of cookies' preparation

5.4.4 Evaluation of functional properties of the sunflower enriched flour

5.4.4.1 Bulk Density

A known amount of sample was weighed into 50 ml graduated measuring cylinder. The sample was packed by gently tapping the cylinder on the bench top 10 times from a height of 5 cm. The volume of the sample was recorded.

Bulk Density (g/ml or g/cm³) = Weight of sample/Volume of sample after tapping.

5.4.4.2 Water Absorption Capacity

15 ml of distilled water was added in a centrifuge tube of 25 ml with 1 gm of sample in it. The centrifuge tube was agitated for two minutes on a vortex mixer. It was then centrifuged for 20 minutes at 4000 rpm. The supernatant was decanted and discarded. The adhering water drops were removed and the tube was weighed again.

WAC % = (Weight Tube + Sediment – Weight of empty tube)/ Weight of Sample × 100

5.4.4.3 Oil Absorption Capacity

10 ml of oil was added in a centrifuge tube of 25 ml with 1 gm sample in it. The centrifuge tube was agitated for 2 minutes on a vortex mixer. It was then allowed to stand for 30 minutes at room temperature.

To 1 g of the sample, 10 ml of oil was added in a 25 ml centrifuge tube and agitated on a vortex mixer for 2 minutes. It was allowed to stand at room temperature for 30 minutes. The mixture was then centrifuged for 30 minutes at 500 µg in a high speed micro centrifuge. The supernatant was then decanted and discarded. The adhering oil drops were removed and the tube was reweighed again.

OAC % = (Weight tube + sediment – weight of empty tube)/ weight × 100

5.4.4.4 Swelling Power

The swelling power (SP) was measured at 70°C and 80°C independently for each flour sample. 0.1 g of sample was taken and heated for 15 minutes at 70°C and 80°C in a water bath with intermittent shaking. A high speed micro centrifuge was used to centrifuge the sample. The supernatant was decanted into a test tube and the sediment was weighed. The decanted supernatant was also collected, dried and weighed.

$$\text{SP \%} = (\text{dry matter weight} / \text{sediment weight}) \times 100$$

5.4.5 Physical evaluation of sunflower seeds enriched cookies

5.4.5.1 Weight

A digital top loading balance was used to check the weight of cookies which consists of different units of weight as like gram, milligram etc.

5.4.5.2 Diameter and Height

A Vernier calliper was used to measure the cookie diameter and height.

5.4.5.3 Spread ratio

Spread ratio is calculated as diameter/height

5.4.6 Physicochemical composition of sunflower seeds enriched cookies

5.4.6.1 Protein Content

The protein content had been determined by Lowry's method. Different dilutions of BSA solutions were prepared by mixing stock BSA solution (50 mg/ 50 ml) and water in standard flask. Extraction of sample was carried out with buffers used for enzyme assay. 0.5 gm of sample was weighed and ground in a pestle mortar with 5ml of buffer. The mixture was centrifuged and supernatant was collected. 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard was taken into series of test tubes. 0.1 ml of sample was taken in other test tube. 5 ml of alkaline copper solution was added in each test tube including blank (1ml distilled water). Mixture was mixed properly and was allowed to stand for 10 minute. 0.5 ml of Folin-Ciocalteu reagent was added to each test tube and kept in dark for 30 minutes. Readings

were taken at 660 nm. Standard graph was plotted and amount of protein was calculated as mg/g or 100gm of sample (Lowry *et al.* 1951).

5.4.6.2 Fat Content

Soxhlet extraction method was used to determine the fat content using petroleum ether (40-600°C) as the reagent. Dried samples (2gm) were extracted with petroleum ether in Soxhlet extraction apparatus for 6-8 hours in pre weighed round bottom flask. The extract containing fat and petroleum ether was evaporated over boiling water bath and dried in an oven at low temperature and weighed. The difference in the weight of the round bottom flask represented the ether extract (fat content) present in the sample (AOAC, 2000)

Weight of sample = W (g)

Weight of empty round bottom flask = W₁ (g)

Weight of empty round bottom flask + Fat content = W₂ (g)

$$\text{Fat Content \%} = \frac{\text{Amount of Ether extract}}{\text{Weight of Sample (g)}} \times 100$$

$$\text{Fat content \%} = \frac{W_2 - W_1}{W} \times 100$$

5.4.6.3 Total Carbohydrate Content

Total carbohydrates were calculated by the given formula (Rangana, 1986)

Total CHO% = 100- (Moisture + Crude Ash + Crude Protein + Crude Fat + Crude Fiber)

5.4.6.4 Crude Fiber Content (AOAC, 2000)

In order to analyse the crude fiber content moisture and fat free sample (2g) were mixed with 200 ml of 1.25 percent H₂SO₄ by gentle boiling for half an hour. The contents were filtered and the residue was washed many times with distilled hot water till it all the acid washes off. Acid free residue was then transferred to the same flask to which 200ml of 1.25 per cent of NaOH was added. The contents were mixed again for half an hour, filtered it and residue was again washed with hot distilled water till it became alkali free. The residue was dried overnight at 100°C and weighed and then placed in muffle furnace at 600°C (±50°C) for

4 hours. The loss in weight after ignition of the sample represented the fiber in the sample (AOAC, 2000). The per cent crude fiber was calculated as follows:

$$\text{Weight of sample} = W \text{ (g)}$$

$$\text{Weight of empty crucible} = W_1 \text{ (g)}$$

$$\text{Weight of empty crucible + sample before ignition} = W_2 \text{ (g)}$$

$$\text{Weight of empty crucible + sample after ignition} = W_3 \text{ (g)}$$

$$\text{Fiber content \%} = \frac{(W_2 - W_1) - (W_3 - W_1)}{W} \times 100$$

5.4.6.5 Moisture Content (AOAC, 2000)

Moisture Content in the edible immature seeds of pulses was determined by following the oven drying method. 5g of sample was taken in a previously weighed, dried aluminium cups. These cups are kept in a hot air oven at 60°C (±5°C) for 8 hours. The aluminium cups were taken out from the oven and kept in the desiccator for cooling for 30 minutes in order to attain a constant weight. After cooling, the samples were weighed with aluminium cups. The loss in the weight represented the moisture content of the sample.

$$\text{Weight of empty aluminium cup} = W_1 \text{ (g)}$$

$$\text{Weight of sample} = W_2 \text{ (g)}$$

$$\text{Weight of aluminium cup + sample before drying} = X \text{ (g)}$$

$$\text{Weight of aluminium cup + sample after drying} = Y \text{ (g)}$$

$$\text{Moisture content \%} = \frac{\text{Loss in weight (g)}}{\text{Weight of sample (g)}} \times 100 = \frac{X - Y \text{ (g)}}{X \text{ (g)}} \times 100$$

5.4.6.5 Ash Content

The weighed amount of sample (1g) was taken and put in previously dried and weighed silica crucibles. Samples were first incinerated over an electric hot plate followed by ashing in muffle furnace at a temperature of 550°C (±25°C) for 6 hours (until pale white residue was

obtained). These ashed samples were taken out from the muffle furnace and kept in desiccator for 2 hours for cooling (AOAC, 2000). After cooling samples were weighed again and per cent ash content was calculated as follows:

$$\text{Weight of empty crucible} = W \text{ (g)}$$

$$\text{Weight of crucible + sample before ashing} = W_1 \text{ (g)}$$

$$\text{Weight of crucible + sample after ashing} = W_2 \text{ (g)}$$

$$\text{Ash content \%} = \frac{\text{Weight after ashing (g)}}{\text{Weight of sample}} \times 100$$

$$\text{Ash content \%} = \frac{(W_2 - W_1)}{(W_1 - W)} \times 100$$

5.4.7 Total Phenolic Content

Similar to section 5.2.2; 0.2 g of finely ground sample was weighed and taken in a beaker and 10 ml of 70 per cent acetone was added. The beaker was placed in a water bath (adjusted at 37°C for 2 hours). Frequent shaking was given for better extraction. After expiry of this period, extract was centrifuged for 20 minutes at 3000 rpm. The supernatant was collected in a test tube and was further used for the estimation of total and simple phenols. 0.1 ml of aliquot extract as obtained above was taken and volume was made 1ml with distilled water. 2.5 ml of 20 per cent sodium carbonate solution was added followed by 0.5 ml Folin-Ciocalteu reagent. Contents were left for 40 minutes for colour development (purplish blue). Absorbance was read at 725 nm after 40 minutes against a suitable blank and calculations were done for total phenols using standard curve which was prepared using gallic acid (0.1 mg/ml) (Makkar *et al.* 1997).

5.4.8 Antioxidant activity by DPPH Assay (Brand-William *et al.* 1997)

Similar to section 5.2.1; the antioxidant properties were evaluated using the DPPH radical scavenging method. Ascorbic acid was used as the natural antioxidant for the antioxidant activity comparison. Each sample's antioxidant activity was expressed as IC 50, and was calculated in accordance to the standard protocol from the graph after plotting inhibition percentage against extract concentration DPPH assay. 1.5 ml of 0.1 mM DPPH

solution was mixed with 1.5 ml of various concentrations (10-500 µg/ml) of extract. The experiment was replicated in three independent assays. Ascorbic acid was used as positive controls.

The DPPH scavenging effect percentage was calculated by the given equation:

$$\text{DPPH scavenging effect (\%)} \text{ or percent inhibition} = \frac{A_0 - A_1}{A_0 \times 100}$$

Where A_0 was the absorbance of the control and A_1 was the absorbance of the sample.

All test were run in triplicates (n=3) and average values were calculated.

5.4.9 Organoleptic evaluation:

Nine point Hedonic scale method as given by Amerine *et al.* (1965) was followed for conducting the sensory evaluation of sunflower seeds incorporated cookies. The panel of 10 judges comprising of faculty members and post-graduate students of the Department of Food Technology and Nutrition, Lovely Professional University were selected with care to evaluate the cookies for sensory parameters such as colour, crispiness, taste, mouthfeel and overall acceptability. Efforts were made to keep the same panel for sensory evaluation throughout the entire period of study. The samples were presented to judges and plain water was given to them to rinse their mouth in between the evaluation of samples. No discussion during evaluation was allowed.

CHAPTER-8

SUMMARY AND CONCLUSION

The present study on “**Effect of Sunflower seeds on Hypercholesterolemia, Fatty liver and fasting blood glucose in Diabetes Mellitus type 2 patients**” was undertaken to explore the possibility of control of various deranged parameters in certain metabolic disorders like dyslipidaemia, Fatty Live (grade 1), Diabetes Type 2. As per the study, the sunflower seeds were first roasted and then about 2gm of the sunflower seeds were given to the sample patients for a period of six months.

A comparative analysis of phenolic, tocopherol content and antioxidant activity was done for both roasted and non-roasted sunflower seeds to find out the effect of roasting on the sunflower seeds. The sunflower seeds both roasted and non-roasted showed a good antioxidant potential. A significant difference ($p < 0.05$) was observed amongst the antioxidant potential of roasted and non-roasted seeds. Although less but insignificant difference ($p < 0.05$) was observed amongst the total phenolic content of roasted and non-roasted seeds.

The incorporation of sunflower seeds in the diet for six months in the sample patients showed visible and significant reductions ($p < 0.05$) in various relative parameters. The reductions were in agreement with the statistical analysis. In this study, it has been found that the supplementation of 2 gm in the diets of sedentary men and women (age ranging from 45-55 yrs.) can lower concentrations of serum total cholesterol, triglycerides, LDL, FBS, SGOT, SGPT not only in patients who are on medications but also in ones who have not yet been advised any medication for the deranged levels respectively. Apart from these findings the HDL also known as the good cholesterol showed a good increase. It has been suggested that the consumption of up to 2 g sunflower seeds in roasted form has been considered safe. Also, in the given study it has been analysed that roasting does not hamper much of the seed's antioxidant potential and capacity as much difference was not countered amongst the roasted and non-roasted seeds as far as the antioxidant capacity, phenolic content, tocopherol content, protein, fat content etc. are concerned.

Product formulation and development was also performed considering the nutritional attributes of sunflower seeds. Different flour blends were used to design and develop healthy sunflower based cookies. The result obtained was put to use for the

nutritional therapeutic purpose. The cookies made with different sunflower seeds enriched flour blends were T₁ - 100% wheat flour, T₂ - 80% wheat flour and 20% sunflower seed flour, T₃ - 70% wheat flour and 30% sunflower seed flour and T₄ - 60% wheat flour with 40% sunflower seed flour.

It was observed in functional analysis of the flour blends, T₄ (60% wheat flour with 40% sunflower seed flour) was found to have maximum water absorption capacity, bulk density and swelling power. The flour blend T₂ with 20% sunflower seed flour was found to be highest in oil absorption capacity. The results of the proximate composition analysis revealed that protein along with crude fiber and fat was also highest in T₄ with 13.95 per cent, 2.58 and 4.1 per cent respectively. The total phenolic content was found to be maximum in A₄ 179.06 mg/100 g. The antioxidant activity was also found to be highest in T₄.

In the physical analysis of the cookies made from the various flour blends, T₄ was observed to have maximum diameter whereas T₂ was found to have maximum height and T₁ was found to have maximum weight and spread ratio. The proximate analysis of the cookies showed that T₄ had maximum protein (12.95 per cent), fat (27.1 per cent) and crude fiber (2.45 per cent) whereas T₁ had maximum carbohydrate content (75.23 per cent). In antioxidant activity analysis maximum content was found in T₄. With the addition of sunflower flour the highest improvement was found in T₄.

The physicochemical and sensory evaluation of cookies, revealed that up to 20% substitution of wheat flour with sunflower seeds flour (T₂) produced acceptable cookies similar to the control (100% wheat flour) cookies. It showed the maximum score in colour (8.5), flavour (8.1), texture (8.1) and overall acceptability (8.3), taste (8.2). Hence, T₂ i.e. 20% sunflower seed flour was found to be most accepted in this study.

The second product formulation – the capsules also proved to be effective as about 42% of the sample patients opted to take the capsular form of the powdered seeds finding it easier to consume whereas the remaining 58% opted for the seeds in the natural form.

Thus in a nutshell, it can be concluded that sunflower seeds can be used as an adjuvant and an inordinate remedy to render control over the deranged biochemical parameters like cholesterol, triglycerides, LDL, FBS, SGOT and SGPT along with a good increase in HDL (the good cholesterol). In addition it was found that roasting did not hamper

much of its nutritional composition (as mentioned in **table 6.4**) hence it can be incorporated in light cooking and roasting recipes as well. Further, cookies enriched with sunflower seeds were developed for its efficient delivery and administration. Moreover preventing various metabolic disorders with nutritional intervention is therapeutic strategy that is widely being adopted.

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INDEX

CHAPTERS	TOPICS	PAGE NO.
1.	Introduction	3-6
2.	Review of literature	7-21
3.	Hypothesis	22
4.	Research Objectives	23
5.	Materials and Research Methodology	24-43
6.	Result and Discussion	44-78
7.	Conclusion	79-81
8.	References	82-89

Appendix I

GENERAL INFORMATION QUESTIONNAIRE

Date:

Q: 1 Name:

Q: 2 Sex:

Male Female

Q: 3 Age (Tick as appropriate):

Under 45 yrs 46–55 yrs 56–65 yrs Over 65 yrs

Q: 4 Height:Ft.....inches

Q: 5 Weight:Kg

Q: 6 Occupation:

Q: 7 Annual Income:

Q: 8 Does anyone from your immediate family member or other relative been diagnosed with any health issue (Tick the appropriate)

: grandparent, aunt, uncle or first cousin (but no own parent, brother, sister or child)

: parent, brother, sister or own child

Q: 9 Are you suffering from any below mentioned ailment?

a) Heart problem b) Hypertension c) High Cholesterol d) Diabetes e) Fatty liver

f) Any other.....

Q: 11 When did you last visited your physician?

.....

Q: 22 How many times in a month you eat outside?

- Once a month
- Twice a month
- Three to four times or more
- Never

Q: 24 How often do you get your blood parameters checked?

- Never
- Yearly
- After 6 months
- Quarterly

Q: 26 How active are you in terms of physical activity resultant of housework, garden work, other daily activity?

- Very inactive
- Inactive
- A little activity
- A moderate amount of activity
- Active

Q: 27 How active are you in terms of physical activity resultant from cycling, walking, going to gym, running?

- I never exercise
- A couple times a month
- 1 or 2 times a week
- 3 to 4 times a week
- 5 to 6 times a week
- Once a day
- More than once a day

Q: 29 Have you ever had an admission to hospital?

YES

NO

Q: 31 Do you have allergies with any food substance?

YES

NO

If yes, please specify.....

Q: 31 Undergone any surgery in past?

YES

NO

If yes, please specify.....

Appendix II

DIETARY RECALL

1. What is first thing in the morning you take after getting up?

2. In Breakfast what do you take usually and at what time?

3. Between Breakfast and Lunch anything to eat?

4. Lunch at what time and what do you take ususally?

5. Tea or coffee in evening if any?

6. Dinner at what time and what do you have?

7. Anything you take before sleeping?

8. How many cups of tea or coffee in a day do you take?

- One Cup
- Two-three cups
- Four-five cups
- More than five cups
- None

9. How many glasses of water in a aday?

- Two-four glasses
- Four to six glasses
- Six to eight glasses
- Eight to ten glasses
- More than then glasses

10. How much sugar you take in your tea/coffee or milk?

- None
- Half teaspoon or less
- One teaspoon
- Two teaspoon
- More than two teaspoon

Appendix III

QUESTIONNAIRE POST CAPSULAR SUPPLEMENTATION OF SUNFLOWER SEEDS

Date:

Q1 Name:

Q2 Sex:

Male Female

Q3 Age (Tick as appropriate):

Under 45 yrs 46–55 yrs 55–64 yrs over 64 yrs

Q4 Did you take the sunflower seeds as advised to you?

Yes No taken sometimes missed sometimes

Q5 Did you take the capsules which consisted the powdered sunflower seeds?

a) Yes b) No

Q6 Taking sunflower seeds was easier than taking the capsules. Please tick:

- a) Strongly disagree
- b) Disagree
- c) Neutral
- d) Agree
- e) Strongly Agree

Q7 Taking capsules was easier than taking the sunflower seeds. Please tick:

- a) Strongly disagree
- b) Disagree
- c) Neutral
- d) Agree
- e) Strongly Agree

Q8 Did consumption of the sunflower seeds prove to be beneficial for you?

- a) Yes
- b) No
- c) Can't Say

Appendix IV

EVALUATION PERFORMA FOR HEDONIC RATING TEST OF THE FOOD PRODUCTS

Name of the Product:

Name of the Evaluator:

Dated:

Sample Code	Color	Flavor	Taste	Texture	Overall Acceptability	Remarks (if any)

Signature _____

Hedonic Scale

Expression	Points to be assigned
Dislike extremely	1
Disliked very much	2
Disliked moderately	3
Disliked slightly	4
Neither liked nor disliked	5
Liked slightly	6
Liked moderately	7
Liked very much	8
Liked extremely	9

Appendix V

REFERENCE VALUES FOR ALL THE REQUIRED PARAMETER

Parameter	Satisfactory	Satisfactory
Cholesterol (mg/dl)	<200	>200
Triglycerides (mg/dl)	<150	>150
LDL (mg/dl)	<140	>140
HDL (mg/dl)	<40	>40
FBS (mg/dl)	70-100	>100
SGOT (U/L)	5-40	>40
SGPT (U/L)	7-56	>56

**Appendix VI
Master-sheet**

Group: I Control	Visit 1			Visit 2			Visit 3			Visit 4			Visit 5			Visit 6											
	Ht	Wt	BMI	Chol	Tgl	LDL	HDL	Chol	Tgl	LDL	HDL	Chol	Tgl	LDL	HDL	Chol	Tgl	LDL	HDL								
Patient's Name																											
1. Lakhwinder Kaur (45yrs)	154	67	30.4	207	168	127	59	192	155	125	55	188	142	128	51	180	140	121	50	175	137	119	48	177	138	123	49
2. Surjit Singh (51yrs)	168	81	28.9	196	178	92	34	185	163	94	37	180	155	97	38	183	147	95	40	172	140	100	40	170	136	99	41
3. Shamsher Singh (53yrs)	177	89	28.4	219	163	91	37	203	152	95	38	190	139	100	39	183	136	98	39	185	138	97	40	178	135	101	40
4. Sanjit Singh (46yrs)	162	85	32.6	231	190	83	41	212	177	86	40	198	154	90	41	190	146	92	41	192	141	90	42	183	135	95	42
5. Mohinder Kaur (53yrs)	150	71	32.2	193	271	89	38	183	252	90	38	180	214	89	39	185	185	92	40	181	162	85	40	183	149	87	41
6. Naander Kaur (52 yrs)	165	68	25.1	246	318	164	34	220	260	152	37	201	219	136	38	194	181	130	40	187	165	132	41	189	142	135	41
7. Ranjit Singh (45yrs)	181	89	27.8	220	271	135	33	202	248	122	38	190	222	120	38	192	181	124	40	186	166	118	40	188	152	121	41
8. Rajni Sehgal (48yrs)	155	67	30.4	249	190	178	42	223	174	162	41	198	161	153	41	190	155	145	41	193	142	146	43	189	140	145	43
9. Anju Kumari (50yrs)	152	70	31.8	243	187	178	37	218	167	155	38	200	159	143	38	192	142	135	40	185	140	132	40	177	143	137	41
10. Sarabjeet Singh (46 yrs)	178	86	26.1	191	236	111	46	186	209	100	45	183	191	97	42	185	175	99	43	181	163	101	43	179	152	103	42
11. Amrith Khosla (54yrs)	169	91	31.1	167	232	98	43	165	217	97	43	166	195	96	42	162	179	100	42	163	161	99	42	169	149	101	42
12. Surinder Kaur (56yrs)	151	82	32.5	178	223	169	33	170	205	154	37	174	188	140	39	175	172	138	40	177	158	135	40	174	145	137	41
13. Jhirmal Singh (52yrs)	170	85	30.5	255	189	154	37	226	176	148	38	203	162	131	39	191	150	133	39	185	143	135	40	188	139	127	41
14. Jaspal Singh (51 yrs)	178	84	27.9	227	210	168	42	204	189	151	41	191	165	140	41	195	151	136	42	190	144	132	42	193	141	135	42
15. Mukhtar Singh (47yrs)	172	93	32.6	235	198	154	39	210	177	142	39	192	162	135	40	188	150	131	41	185	145	130	41	181	138	128	42
16. Kuldeep Kaur (45 yrs)	167	72	26.6	202	236	190	40	191	212	168	41	187	189	157	41	185	166	142	41	183	151	135	42	185	146	138	42
17. Sharampal Kaur (52 yrs)	160	98	39.2	198	219	182	36	189	196	170	38	185	171	155	39	180	166	142	40	182	152	140	41	187	143	141	4

Patient's Name	Ht	Wt	BMI	Visit 1			Visit 2			Visit 3			Visit 4			Visit 5			Visit 6								
				Chol	Tgl	LDL	HDL	Chol	Tgl	LDL	HDL	Chol	Tgl	LDL	HDL	Chol	Tgl	LDL	HDL	Chol	Tgl	LDL	HDL				
18. Parchan Singh (55yrs)	177	89	28.7	260	192	168	42	233	173	155	42	199	155	149	42	191	143	143	43	188	138	141	43	192	135	133	43
19. Pooran Singh (53yrs)	175	90	30.2	231	175	150	32	202	160	141	35	195	148	135	36	197	145	128	38	193	136	131	39	190	137	128	40
20. Surinder Singh (47yrs)	181	89	27.8	206	208	132	41	191	189	130	41	188	171	122	41	183	158	118	42	186	142	123	42	185	135	120	42
21. Sarita Sharma (49yrs)	151	68	30.9	210	204	134	45	192	186	130	43	186	171	128	43	188	155	125	43	184	143	122	42	185	140	123	42
22. Sawinder Singh (54 yrs)	172	78	26.8	390	182	324	45	316	165	276	44	275	152	235	44	231	147	187	45	217	141	162	43	202	143	151	43
23. Jeet Kaur (55 yrs)	155	70	29.1	238	542	117	28	211	410	120	33	195	325	119	35	182	261	123	37	185	189	124	39	180	155	121	40
24. Dharam Singh (49yrs)	168	83	28.6	237	468	151	21	219	349	143	36	202	301	137	37	197	276	135	38	185	211	138	40	188	168	132	41
25. Sukhwinder Kaur (45yrs)	157	64	26.6	242	274	170	34	213	239	155	36	196	201	142	37	189	180	139	39	191	162	140	40	182	145	135	42
26. Harchand Singh (51yrs)	179	85	26.5	265	219	132	35	234	200	130	36	210	182	135	38	191	168	129	39	175	155	130	40	181	141	133	41
27. Harjit Kaur (53yrs)	162	83	31.9	233	298	152	37	219	252	145	38	198	203	141	38	187	172	138	39	172	166	139	40	166	152	137	41
28. Seema Arora (45 yrs)	157	61	25.4	248	184	173	36	208	161	160	37	189	155	158	38	176	143	151	38	179	140	150	39	174	141	146	41
29. Ramesh Kumar (52yrs)	165	78	28.8	298	213	156	27	252	199	149	30	221	172	147	34	209	165	142	36	195	151	138	39	192	142	139	40
30. Nirmala Devi (55 yrs)	152	73	31.7	313	245	186	37	271	198	178	39	245	181	165	40	228	164	159	40	210	148	151	41	195	145	150	42

Group I (Case)		Visit1		Visit2		Visit3		Visit4		Visit5		Visit6													
S.No	Patient's Name	Ht	Wt	BMI	Chol	Tgl	LDL	HDL	Chol	Tgl	LDL	HDL	Chol	Tgl	LDL	HDL									
1	Dilawar Singh (52yrs)	171	86	30.7	284	245	172	36	267	220	168	37	249	197	160	37	228	169	155	152	39	197	146	148	44
2	Narinder Singh (47yrs)	168	81	32.4	213	288	139	30	202	265	135	34	199	230	133	35	195	201	177	134	38	185	153	130	39
3	Sohan Singh (52yrs)	173	89	31.7	253	176	131	33	237	165	130	35	222	162	126	36	219	157	150	126	39	189	144	130	43
4	Surjit Kaur (53 yrs)	150	64	29	321	218	143	29	286	189	137	32	265	177	135	33	242	162	156	138	38	201	139	137	45
5	Sushila Devi (55yrs)	153	71	32.27	298	189	161	34	265	171	155	36	241	165	150	37	229	152	144	142	39	183	136	147	40
6	Krishna (51yrs)	145	75	39.4	212	256	143	28	202	221	140	31	197	191	138	32	192	176	160	136	38	186	146	133	46
7	Jaasvir Singh (46yrs)	177	79	28.2	242	162	166	51	236	155	160	47	222	148	156	45	209	142	145	150	46	193	140	146	47
8	Saroop Chand (50yrs)	170	75	26.8	222	168	153	37	210	165	155	38	202	160	147	38	199	158	152	144	40	186	146	143	41
9	Hajit Kaur (44yrs)	155	75	34.1	216	276	161	38	208	243	158	39	203	212	156	39	200	189	161	150	40	191	151	147	43
10	Harwant Singh (56yrs)	165	79	31.6	290	95	121	51	267	101	122	49	241	105	123	47	222	106	103	120	45	188	109	121	46
11	Suresh Gupta (49yrs)	178	87	31.1	253	236	185	33	241	210	171	35	235	186	166	36	221	169	162	157	38	195	152	147	40
12	Pratap Singh (49yrs)	172	79	28.2	234	414	131	38	222	352	133	38	216	302	135	38	208	264	213	135	40	185	165	134	43
13	Swaran Singh (50yrs)	178	85	30.3	212	490	167	30	204	411	162	33	196	354	154	34	192	303	261	145	38	190	170	140	40
14	Roop Kumar (53yrs)	165	86	34.4	289	231	174	35	265	200	161	36	241	176	162	37	225	165	158	150	40	197	149	143	45
15	Neeta Kaushik (52yrs)	156	62	28.2	252	187	152	38	231	180	145	39	219	172	147	39	205	166	154	141	40	190	143	139	43
16	Sarabjeet khullar (51yrs)	165	72	28.8	276	190	159	33	251	181	151	35	224	166	146	37	213	153	148	143	40	189	145	141	44
17	Naresh Kumari (46yrs)	150	55	25	211	245	144	27	205	223	140	30	199	204	138	34	196	187	164	139	37	190	150	142	41
18	Mahesh Vij (46yrs)	170	82	29.2	284	245	168	29	298	186	159	33	265	163	155	36	243	155	149	148	39	198	141	145	42
19	Sukhwinder Kaur (53yrs)	168	88	30.2	321	201	135	33	266	223	137	35	258	197	138	36	241	174	156	131	39	203	148	134	40

20	Nirmala Deei (52 yrs)	152	88	27.8	285	254	179	39	226	161	165	39	220	156	161	40	213	152	158	40	206	147	154	41	183	144	151	42
					Visit1				Visit2				Visit3				Visit4			Visit5				Visit6				
S.No	Patient's Name	Ht.	Wt.	BMI	Chol	Tgl	LDL	HDL	Chol	Tgl	LDL	HDL	Chol	Tgl	LDL	HDL	Chol	Tgl	LDL	HDL	Chol	Tgl	LDL	HDL	Chol	Tgl	LDL	HDL
21	Javed Iqbal (46 yrs)	165	70	30.9	234	166	189	36	210	223	168	37	205	198	156	37	198	179	155	38	195	162	152	38	192	151	148	39
22	Dilbagh Singh (49 yrs)	170	64	26.8	226	256	143	32	218	287	140	35	213	243	141	36	207	211	145	38	202	186	140	39	195	158	142	39
23	Gurpreet Singh (44 yrs)	170	82	29.1	222	311	164	28	261	182	160	33	245	179	157	35	223	164	150	36	211	152	148	38	189	143	146	38
24	Satwant Kaur (52 yrs)	177	75	28.6	273	196	195	36	252	149	181	38	241	147	172	38	226	144	158	39	213	146	152	39	199	142	148	40
25	Sardool Singh (55yrs)	152	75	26.6	269	154	186	34	244	155	169	36	229	150	161	37	221	147	159	39	215	142	155	40	202	140	153	41
26	Punjab Kaur (46 yrs)	181	96	26.5	251	168	148	29	240	174	145	33	233	167	143	36	219	159	146	37	210	150	140	39	197	139	138	41
27	Sarup Singh (53 yrs)	165	66	31.9	247	187	181	43	211	282	177	43	205	254	171	42	199	211	158	43	195	186	146	44	183	158	141	44
28	Rachpal Singh (52 yrs)	171	85	25.4	217	333	177	35	222	156	165	37	214	151	153	37	202	149	148	39	197	144	145	39	195	142	138	40
29	Darshan Singh(47 yrs)	178	75	28.8	238	162	132	30	211	199	130	32	210	178	127	35	202	165	120	37	198	152	119	37	190	148	121	38
30	Ramesh Handa (50 yrs)	180	86	31.7	244	221	187	32	289	243	176	35	265	210	161	37	231	187	156	38	222	162	149	40	201	151	143	41

Group 2 (Control)		Visit1		Visit2		Visit3		Visit4		Visit5		Visit6		
S.No	Patient's Name	Ht	Wt	BMI	FBS	HDL	FBS	HDL	FBS	HDL	FBS	HDL	FBS	HDL
1	Sarla devi (52 yrs)	151	67	30.4	212	38	186	39	163	39	140	40	129	41

2	Narinder Singh (47 yrs)	180	89	27.8	186	40	169	40	152	41	143	41	131	42	122	41
3	Suraj Parkash (46 yrs)	172	86	30.7	192	39	179	40	166	40	158	40	141	40	127	40
4	Manohar Lal (50 yrs)	168	77	27.5	146	29	138	33	130	36	126	37	120	38.5	116	40
5	Rajwant Kaur (53 yrs)	158	65	27	178	33	165	35	152	36	137	37	120	39	117	39
6	Davinder Singh (45 yrs)	170	93	33.2	166	36	153	37	140	37	132	37.5	126	39	120	39
7	Kiran Kumari (49 yrs)	150	55	25	189	35	170	36	163	36.5	158	37	141	38	124	40
8	Mohan Lal (52 yrs)	165	72	28.8	149	40	140	40	133	40	128	39	121	40	118	40.5
9	Rajinder Krishan (48 yrs)	166	83	30.7	234	38	198	39	176	39	162	39	148	40	123	40
10	Laehi Kaur (48 yrs)	155	71	29.5	276	34	224	36	189	37	163	38	141	38	133	39
11	Parveen Sharma (55 yrs)	172	88	31.4	162	39	158	39	151	39	144	40	136	40	123	41
12	Parkash Kaur (50 yrs)	162	67	25.7	187	40	169	40	156	40	143	40.5	138	41	129	41
13	Asha (51 yrs)	152	70	31.8	203	32	188	35	171	37	158	37	143	38	122	39
14	Sarabjeet Singh (46 yrs)	181	83	25.9	142	37	138	38	131	38	125	39	118	39	110	40
15	Ravinder Singh (49 yrs)	177	79	25.2	156	29	148	33	140	35	133	36	129	36	120	38
16	Maminder Kaur (53 yrs)	159	66	26.4	162	33	155	35	148	36	136	36	122	39	112	39
17	Krishan Kapoor (55 yrs)	164	70	26.9	193	39	176	39	159	40	141	40	128	40	119	41
18	Sudha Mehta (48 yrs)	155	59	24.5	141	40	136	40	131	40	124	41	119	41	110	42
19	Rashmi Khanna (46 yrs)	152	61	27.7	167	26	159	29	145	34	138	36	129	36	121	38
20	Shiv Kumar (54 yrs)	178	77	24.8	210	34	189	36	168	38	151	39	136	39	125	41
S.No	Patient's Name	Ht	Wt	BMI	Visit1 FBS	HDL	Visit2 FBS	HDL	Visit3 FBS	HDL	Visit4 FBS	HDL	Visit5 FBS	HDL	Visit6 FBS	Visit HDL
21	Davinder Kaur (51 yrs)	150	82	37.2	188	38	171	38	158	39	145	39	131	40	123	41

22	Punjab Singh (45 yrs)	175	89	29.6	142	31	137	34	131	36	125	37	116	38	108	39
23	Atma Ram (49 yrs)	160	68	27.2	159	40	143	40	138	40	125	41	119	41	112	41
24	Renu Singh (55 yrs)	165	67	24.8	173	39	161	39	148	40	135	40	127	40	119	41
25	Rajesh Arora (53 yrs)	172	77	26.5	163	35	151	36	143	36	129	37	121	38	113	39
26	Mamir Kaur (48 yrs)	158	64	26.6	169	38	156	38	141	38	135	39	122	40	118	40
27	Seema Sharma (45 yrs)	160	57	22.8	222	40	191	40	177	40	153	40.5	135	41	129	41
28	Baljeet Singh (54 yrs)	161	68	27.2	195	36	177	37	163	38	148	38	132	39	126	39
29	Balbir Malhotra (50 yrs)	170	71	25.3	156	39	145	39	138	39	129	122	118	40	110	40
30	Barinder Kaur (46 yrs)	175	93	31	171	29	155	33	142	35	128	36	117	38	109	38

Group 2 (Case)																
S.No	Patient's Name	Ht	Wt	BMI	Visit1 FBS	HDL	Visit2 FBS	HDL	Visit3 FBS	HDL	Visit4 FBS	HDL	Visit5 FBS	HDL	Visit6 FBS	HDL
1	Sawinder Kaur (50yrs)	152	80	36.3	134	38	121	41	111	40	107	42	104	43	103	44
2	Darshan Kaur (50yrs)	155	63	28.6	211	33	165	36	140	38	129	36	121	37	118	40
3	Narinder Singh (52yrs)	180	83	25.9	197	37	162	35	123	40	119	40	116	41	112	43
4	Harjit Kaur (55yrs)	153	65	29.54	182	32	159	28	138	39	122	38	111	39	107	42
5	Santa Singh (56yrs)	167	72	28.8	203	31	159	40	138	36	129	38	121	39	114	41
6	Rattan Lal (46yrs)	171	90	32.14	225	21	177	40	149	32	133	35	124	37	117	39.5
7	Sanjit Singh (47yrs)	168	73	29.2	186	39	151	40	126	41	116	41	114	40	109	44
8	Rajwant Kaur (46 yrs)	165	68	27.2	160	36	139	42	106	40	114	41	110	40	105	42
9	Jeevan Lata (52yrs)	156	60	27.3	178	40	154	41	131	41	120	41	109	42	102	46
10	Sanjit Singh (51yrs)	180	90	28.12	220	38	179	38	151	41	133	41	121	42	112	47
11	Kanta Devi (46yrs)	154	62	28.2	157	39	137	38	120	40	105	40	106	41	103	43
12	Vijay Kumar (53yrs)	170	101	36.07	188	36	162	40	141	39.5	120	40	108	41	104	45
13	Om Prakash (55yrs)	166	86	34.4	196	37	161	39	148	40	131	40	120	40	119	44
14	Sukhdeep Singh (45yrs)	180	110	34.37	166	38	145	46	122	41	111	41	109	42	104	48
15	Prabhjeet Singh (50yrs)	171	70	25	146	34	123	39	117	40	112	40	106	40	101	43
16	Rupinder Kaur (46yrs)	162	70	28	197	42	161	34	143	45	127	45	114	45	104	46
17	Suvridha Chadda (48yrs)	158	63	28.6	148	38	124	40	112	40	108	40	102	40	103	42.5

18	Krishan Bhalia (52yrs)	171	79	28.2	129	29	120	40	116	38	117	39	112	41	107	43
19	Mangal Singh (48yrs)	184	86	26.8	169	38	145	39	122	40	119	41	114	42	110	46
S.No	Patient's Name	Ht	Wt	BMI	Visit1 FBS	HDL	Visit2 FBS	HDL	Visit3 FBS	HDL	Visit4 FBS	HDL	Visit5 FBS	HDL	Visit6 FBS	HDL
20	Sanjeev Mahajan (48yrs)	170	77	27.5	149	38	120	39	116	39	115	39	114	41	109	42
21	Sarabjeet Kaur (50yrs)	185	83	25.9	258	38	214	39	179	39	155	40	114	40	110	44
22	Pripal Singh	152	50	22.7	194	40	172	40	156	40	139	41	131	40	116	45
23	Kuljeet Kaur(55yrs)	171	85	30.3	160	45	142	47	130	49	126	50	122	41	108	50
24	Nirmaljeet Kaur (46 yrs)	158	69	31.3	184	35	160	40	141	40	137	40	116	51	115	45
25	Kanta Devi (47 yrs)	160	72	28.8	237	37	201	42	172	41.5	149	42	125	40.5	114	46
26	Jaswant Singh(51 yrs)	153	80	36.3	157	35	139	37	128	38	120	38.5	123	42	112	42
27	Rashpal Singh (45 yrs)	168	80	28.5	204	39	173	40	151	40	134	40	116	40	115	46
28	Satwant Kaur (49 yrs)	173	89	30.6	185	36	165	38	141	39	129	40	122	41	102	42
29	Poonam Kumari (53 yrs)	156	67	30.4	173	38	155	39	143	39	135	39	111	40	116	45
30	Mangal Singh (48yrs)	152	71	32.2	192	32	169	37	154	41	141	41	123	41	113	44

Group 3 (Control)																							
S.No	Patient's Name	Ht	Wt	BMI	Visit1 SGPT	Visit2 SGPT	Visit3 SGPT	Visit4 SGPT	Visit5 SGPT	Visit6 SGPT	Visit1 SGOT	Visit2 SGOT	Visit3 SGOT	Visit4 SGOT	Visit5 SGOT	Visit6 SGOT	Visit1 SGPT	Visit2 SGPT	Visit3 SGPT	Visit4 SGPT	Visit5 SGPT	Visit6 SGPT	
1	Manvinder Singh (46yrs)	171	84	30	58	84	55	75	69	48	60	44	57	42	53								
2	Sarabjeet Kaur (52yrs)	155	68	28.3	65	101	61	88	73	48	68	45	61	41	55								
3	Sheela Devi (50yrs)	151	57	25.9	48	76	45	69	62	38	59	38	55	37	50								
4	Maksudan Singh (48yrs)	181	89	27.8	89	156	78	122	98	62	71	53	60	45	57								
5	Surinder Kumar (51yrs)	177	91	29.3	103	185	86	149	121	52	100	43	82	41	65								
6	Raman Kumar (55yrs)	167	88	32.5	71	153	67	120	99	50	75	45	63	42	58								
7	Lovejeet Kaur (49yrs)	157	60	25	83	171	72	143	111	52	79	44	65	40	57								
8	Narinder Chawla (53yrs)	171	78	27.8	94	189	76	151	125	53	91	46	74	43	60								
9	Parvinder Kaur (45yrs)	161	71	28.4	55	89	51	75	67	43	60	41	56	38	51								
10	Sewa Singh (48yrs)	174	76	25.3	68	130	65	100	91	42	77	40	65	39	59								
11	Mukhtar Singh (56yrs)	179	87	28.6	74	141	65	119	101	48	88	45	76	44	65								
12	Raunaki Ram (54yrs)	165	83	30.7	111	180	89	155	122	58	94	48	71	45	62								
13	Mohan Lal (52yrs)	170	82	29.2	85	158	69	121	98	55	76	51	68	47	55								
14	Paramjit Kaur (49yrs)	153	60	26.8	70	161	65	129	97	52	83	49	72	45	64								
15	Shashi Bala (45yrs)	157	55	22.9	93	167	75	134	103	55	87	50	71	47	60								
16	Mithun Das (46yrs)	172	76	27.1	64	123	60	95	81	52	70	47	63	43	56								
17	Rita Khanna (52yrs)	155	82	35.6	51	87	48	80	73	42	65	39	61	38	54								
18	Parminder Singh (55yrs)	169	78	27.8	46	78	45	71	66	41	60	38	54	38	50								
19	Jatinder Kaur (47yrs)	150	67	30.4	95	153	82	122	98	55	71	50	64	48	58								

S.No	Patient's Name	Ht	Wt	BMI	Visit1		Visit2		Visit3		Visit4		Visit5		Visit6	
					SGOT	SGPT	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT
20	Surat Singh (51 yrs)	182	92	28.7	82	145	71	115	63	91	54	73	51	62	47	54
21	Harinder Kaur (45yrs)	158	66	26.4	79	135	65	111	61	95	56	81	52	70	48	59
14	Partap Singh (49yrs)	176	81	27.1	61	123	57	101	52	87	50	73	47	64	42	58
22	Hardev Singh (50yrs)	172	86	30.7	50	86	49	73	47	65	44	61	42	58	41	52
23	Ranjit Chawla (55yrs)	168	79	28.2	105	182	87	149	68	113	58	89	51	71	48	60
24	Harish Arora (56yrs)	167	82	30.3	72	134	65	105	61	89	59	73	53	65	49	54
25	Sarita Rani (51yrs)	151	67	30.4	89	156	80	81	69	73	62	65	56	60	50	58
26	Gul Ahmed (46yrs)	180	78	24.3	93	167	81	129	67	97	59	71	51	64	48	56
27	Asha Kumari (48yrs)	161	72	28.8	100	172	86	134	73	100	65	78	55	67	49	59
28	Surjit Khureana (54yrs)	160	75	30	64	129	58	97	52	84	49	71	45	64	44	55
29	Manjit Singh (45yrs)	175	83	28.6	69	121	60	99	53	87	48	80	46	71	43	62
30	Manvinder Singh (46yrs)	171	84	30	58	84	55	75	50	69	48	60	44	57	42	53

S.No	Group 3 (Case)		Ht	Wt	BMI	Visit1		Visit2		Visit3		Visit4		Visit5		Visit6	
	Patient's Name					SGPT	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT	SGOT
1	Mohinder Kaur (48yrs)	23 Sep	160	75	30	117	127	93	101	75	86	56	72	38	56	33	51
2	Surej Maimi (55yrs)		177	81	28.9	88	124	72	112	66	103	58	89	47	71	40	54
3	Babli (54yrs)		160	62	24.8	86	98	75	91	60	83	48	71	41	58	37	50
4	Ahmed Singh (53yrs)		180	81	25.3	90	111	82	96	68	84	55	68	42	51	39	44
5	Prakash Kaur (48yrs)		153	69	31.4	97	126	89	112	78	100	65	87	52	62	46	45
6	Simarjeet Singh (44yrs)		182	96	30	85	130	79	116	71	98	56	76	48	59	41	48
7	Gulshan Kaur (48yrs)		145	57	30	98	121	84	103	71	91	58	77	51	61	44	47
8	Amarjeet Singh (51yrs)		178	84	30	80	112	65	98	52	83	48	68	41	55	38	49
9	Boota Singh (53yrs)		170	76	27.1	69	97	59	81	46	64	41	52	37	49	33	47
10	Amar Singh (51 yrs)		170	85	30.3	108	132	91	117	79	102	64	86	55	69	43	51
11	Punjab Singh (51yrs)		173	83	29.6	110	132	91	119	82	101	68	83	53	67	42	52
12	Surjit Kaur (47yrs)		154	61	27.7	61	88	55	72	47	59	41	51	36	48	34	46
13	Neelam Saini (48yrs)		152	58	26.3	75	101	69	88	58	73	51	58	43	45	38	47
14	Varsha (55 yrs)		149	56	29.5	103	129	88	106	71	83	58	69	42	52	39	45
15	Dhanwant Singh (47yrs)		171	85	30.3	66	92	58	79	51	63	45	51	41	48	35	45
16	S.M.Verma (50yrs)		167	90	36	59	85	50	71	43	59	39	52	35	45	34	42
17	Ramesh Kumar (55yrs)		178	109	35.1	118	178	103	132	87	101	68	83	54	65	41	52
18	Harjot Singh (51yrs)		168	85	30.3	127	189	100	156	82	118	71	89	59	61	42	49
19	Harjinder Kaur (48yrs)		160	72	28.8	79	152	65	123	59	105	52	87	41	60	38	52

S.No	Patient's Name	Ht	Wt	BMI	Visit1		Visit2		Visit3		Visit4		Visit5		Visit6	
					SGOT	SGPT	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT
20	Rabinder Singh (45yrs)	160	76	30.4	88	175	76	143	61	117	56	94	42	76	35	54
21	Pawan Singh (46yrs)	171	78	27.8	94	189	79	155	65	121	53	100	46	79	41	56
14	Rajwant Kaur (52yrs)	155	61	25.4	72	158	61	122	53	98	43	81	38	63	36	51
22	Gurbhlej Singh (54yrs)	181	86	26.8	69	137	57	104	52	83	47	65	43	54	39	48
23	Kanta devi (50yrs)	150	68	30.9	78	181	65	149	53	123	46	91	40	72	38	56
24	Amrit Singh (47yrs)	178	89	27.8	105	169	88	130	67	101	51	84	45	63	40	51
25	Gursharan Kaur (52yrs)	160	78	31.2	118	194	85	156	61	117	48	81	42	65	38	52
26	Irfaan Ahmed (44yrs)	179	81	26.1	93	165	74	129	59	97	48	72	41	55	39	48
27	Naresh Kumar (49yrs)	174	91	30.3	102	188	86	149	61	110	49	77	42	59	38	51
28	Amrik Kaur (53yrs)	152	59	25.6	81	154	69	110	53	91	41	69	39	53	37	48
29	Rajeev Kundra (56yrs)	178	80	25.8	76	163	63	121	55	93	41	67	38	57	35	51
30	Mohinder Kaur (48yrs)	160	75	30	117	127	93	101	75	86	56	72	38	56	33	51

Group 4 (Control)		Visit1		Visit2		Visit3		Visit4		Visit5		Visit6		Visit7		Visit8		Visit9		Visit10											
Patient's Name	Ht	Wt	BMI	Chol	Tgl	LDL	HDL	FBS	OT	PT	Chol	Tgl	LDL	HDL	FBS	OT	PT	Chol	Tgl	LDL	HDL	FBS	OT	PT							
1.Kamal Kumar (48yrs)	177	100	35.7	194	172	72	37	123	82	124	185	161	77	38	118	66	101	181	154	81	40	111	60	86	185	146	78	42	108	55	72
2.Kulwinder Singh (45yrs)	165	78	31.2	244	180	81	38	140	69	105	220	166	79	39	123	63	96	203	150	78	40	110	55	89	199	148	85	41	103	51	81
3.Nirmal Singh (50yrs)	171	93	33.2	179	220	70	41	191	90	131	170	203	76	42	160	83	112	172	186	79	42	143	81	99	175	171	82	44	132	69	81
4.Balwinder Singh (49yrs)	177	89	31.7	208	191	96	37	201	75	121	197	178	100	39	162	63	99	190	161	93	40	134	58	90	188	154	95	41	121	51	79
5.Mangwinder (46yrs)	168	72	28.8	197	231	81	39	183	59	98	183	211	92	40	152	47	86	179	187	95	40	130	50	79	181	162	93	41	121	48	71
6.Harinder Kaur (53yrs)	155	68	30.9	215	217	150	30	108	77	111	191	182	138	34	102	69	104	190	171	126	37	97	62	91	193	162	129	39	95	55	76
7.Neelam Kumari (56yrs)	152	61	27.7	258	232	192	41	136	66	134	232	208	178	40	124	56	109	211	181	160	40	113	55	93	203	170	152	41	106	51	81
8.Harjinder Singh (48yrs)	181	96	30	250	213	180	43	112	79	129	222	189	162	43	110	77	112	216	178	155	44	106	67	100	199	162	151	44	102	60	89
9.Bachan Singh (46yrs)	165	68	27.2	129	110	93	33	116	70	86	133	105	94	35	110	61	74	135	111	95	35	106	56	71	133	106	97	36	105	50	63
10.Sadhvi Singh (49yrs)	171	78	27.8	201	228	183	41	152	83	145	190	205	166	41	131	70	121	178	189	151	41	119	62	103	185	171	145	42	111	55	91
11.Lalita Sharma (45yrs)	152	81	36.8	231	187	121	35	185	98	122	212	169	119	38	166	83	100	192	153	120	39	141	71	86	195	150	122	40	130	60	72
12.Jaswinder Kaur (51yrs)	160	61	24.4	214	187	142	37	128	101	147	194	162	135	39	114	90	121	190	155	128	40	110	74	102	188	151	129	41	106	63	88
13.Amanpal Singh (53yrs)	165	78	31.2	197	204	180	33	129	81	141	188	189	162	38	121	71	119	185	172	157	39	118	60	98	189	164	146	40	114	52	82
14.Balkar Singh (48yrs)	177	79	28.2	222	179	150	41	157	72	133	213	160	133	40	138	61	108	207	156	136	41	121	52	93	198	150	138	41	115	44	80
15.Asha Khanna (50yrs)	160	84	33.6	245	170	108	45	133	82	111	225	159	110	43	125	70	98	211	152	114	43	116	62	91	197	146	115	43	111	48	78
16.Kashmir Singh (46yrs)	177	84	35	198	217	158	42	135	80	132	180	198	142	43	123	74	112	183	191	140	43	119	68	101	186	182	138	42	112	54	88
17.Manjeet Singh (49yrs)	168	91	36.4	255	189	166	32	180	70	109	228	171	157	38	164	63	82	212	156	151	38	151	55	71	205	144	147	39	138	47	60
18.Kashmir Kaur (55yrs)	162	74	29.6	231	201	156	39	151	58	94	209	188	140	39	124	56	87	197	165	138	40	118	50	72	195	159	135	41	111	46	65
19.Balwant Singh (51 yrs)	167	81	32.4	227	96	179	42	175	47	61	212	100	161	55	155	40	55	202	103	155	51	139	38	51	198	99	148	48	123	35	47

181	87	27.1	173	338	92	36	114	60	95	175	301	100	38	110	51	82	172	276	102	39	108	46	70	103	241	170	39	105	42	61
20.Gurwinder Singh (46yrs)																														
157	68	30.9	164	186	166	34	160	77	106	165	169	165	35	146	63	91	168	155	164	35	132	58	79	159	150	164	37	120	50	65
21.Daljit Kaur (54yrs)																														
182	88	27.5	319	369	220	38	171	48	81	275	310	197	39	159	46	72	248	269	162	39	136	45	64	158	234	221	40	122	43	55
22.Amanpreet Singh (52yrs)																														
178	89	31.7	213	129	162	27	266	93	114	206	133	157	31	188	74	90	202	130	155	34	162	58	79	134	150	197	37	148	42	68
23.Kuldeep Singh (47yrs)																														
171	78	27.8	141	308	59	39	123	38	78	145	271	76	40	120	37	64	149	253	79	40	117	38	61	75	222	147	41	109	35	58
24.Sanjeev Kumar (56yrs)																														
181	90	28.4	177	222	116	27	200	45	67	178	200	120	30	178	44	62	180	187	121	34	152	43	58	126	169	182	36	139	40	53
25.Uttam Singh (54yrs)																														
165	71	28.4	236	109	145	44	172	89	128	219	111	140	45	159	71	100	211	114	138	44	145	62	86	139	115	203	43	130	54	63
26.Vijay Kaur(51yrs)																														
170	77	27.5	181	239	122	27	327	74	130	189	202	125	31	285	61	109	186	181	127	35	237	58	92	130	168	183	37	198	51	79
27.Gurjeet Singh (45yrs)																														
151	64	29	233	185	113	32	208	46	89	218	168	121	35	181	41	80	212	151	122	37	165	38	65	119	149	206	38	141	36	52
28.Paramjeet Kaur (48yrs)																														
165	79	35.9	198	204	154	39	253	78	124	195	190	150	40	201	59	101	197	186	145	40	180	51	85	147	165	194	41	158	42	72
29.Pawan Bhanot (55yrs)																														
176	80	28.5	233	198	132	40	153	88	137	210	182	135	40	145	68	110	202	168	140	41	131	61	95	138	157	196	41	128	52	80
30.Savrajdeep Singh (49yrs)																														

Group 4 (Control) Continued						Visit5										Visit6									
S.No	Patient's Name	Ht	Wt	BMI	Chol	Tgl	LDL	HDL	FBS	OT	PT	Chol	Tgl	LDL	HDL	FBS	OT	PT							
1	Kamal Kumar (48yrs)	165	89	35.6	180	140	80	42	102	44	59	178	141	80	44	103	42	50							

2	Kulwinder Singh (45yrs)	162	68	27.2	191	145	87	41	99	48	69	187	147	88	42	100	44	57
3	Nirmal Singh (50yrs)	178	81	28.9	176	155	83	44	119	56	64	173	150	80	45	107	49	49
4	Balwinder Singh (49yrs)	178	81	28.9	193	147	94	42	116	45	67	189	145	90	42	108	39	58
5	Mangwinder Singh (46yrs)	171	83	33.2	177	157	90	41	111	45	63	180	150	88	42	103	41	54
6	Harinder Kaur (53yrs)	170	78	27.8	188	155	132	40	98	52	65	184	148	133	40	94	48	57
7	Neelam Kumari (56yrs)	170	88	31.4	198	166	147	41	101	48	74	195	154	146	42	99	45	65
8	Harjinder Singh (48yrs)	177	70	25	194	150	147	44	103	54	76	190	146	148	45	99	51	62
9	Bachan Singh (46yrs)	168	79	31.6	131	108	100	42	101	39	60	136	103	101	39	102	35	53
10	Sadhu Singh (49yrs)	156	69	31.4	182	156	142	42	105	48	76	186	150	143	43	103	42	61
11	Lalita Sharma (45yrs)	154	72	32.7	189	148	120	40	119	55	60	186	145	117	41	111	39	54
12	Jaswinder Kaur (51yrs)	173	78	27.8	192	147	130	41	105	48	75	189	143	133	42	101	42	63
13	Amanpal Singh (53yrs)	168	76	30.4	185	158	142	41	108	46	69	182	153	145	41	102	42	58
14	Balkar Singh (48yrs)	188	98	30.6	195	149	135	42	107	40	71	196	145	134	42	101	38	60
15	Asha Khanna (50yrs)	160	65	26	195	145	112	44	109	41	65	198	140	118	43	99	38	51
16	Kashmir Singh (46yrs)	150	59	26.8	182	169	132	43	110	52	75	184	160	130	43	105	45	64
17	Manjeet Singh (49yrs)	168	86	34.4	197	148	145	40	121	42	52	192	145	148	41	110	39	46
18	Kashmir Kaur (55yrs)	180	89	27.6	194	156	137	41	106	44	58	190	152	134	42	101	41	51
19	Balwant Singh (51 yrs)	155	95	43.2	196	96	145	47	111	36	40	195	100	143	47	108	33	42
20	Gurwinder Singh (46yrs)	172	80	28.5	178	209	98	40	102	40	52	176	174	99	41	98	35	48
					Visits							Visit						
21	Daljit Kaur (54yrs)	152	69	31.4	169	147	156	38	112	48	57	167	145	151	38	107	45	50
22	Amanpreet Singh (52yrs)	160	58	23.2	213	201	149	41	110	39	48	205	169	145	42	106	33	45

23	Kuldeep Singh (47yrs)	178	78	27.8	195	132	149	38	130	37	55	196	135	143	39	125	35	49
24	Sanjeev Kumar (56yrs)	180	90	28.1	143	187	79	41	104	35	50	145	163	81	42	100	36	48
25	Uttam Singh (54yrs)	160	75	30	183	155	124	38	122	36	49	179	152	125	39	111	34	44
26	Vijay Kaur(51yrs)	173	75	26.7	198	112	142	43	133	42	55	195	110	140	42	137	40	46
27	Gurjeet Singh (45yrs)	168	92	36.8	188	151	129	39	161	45	65	182	143	133	40	145	42	58
28	Paramjeet Kaur (48yrs)	177	86	27.7	195	145	123	39	127	34	49	193	147	120	41	116	35	44
29	Pawan Bhanot (55yrs)	160	67	26.8	193	154	143	41	131	35	66	195	149	142	42	120	33	52
30	Savrajdeep Singh (49yrs)	153	71	32.2	191	150	137	42	115	47	71	193	148	133	42	104	42	58

Group 4 (Case)		Visit1			Visit2			Visit3			Visit4																					
S.No	Patient's Name	Ht	Wt	BMI	Chol	Tgl	LDL	HDL	FBS	OT	PT	Chol	Tgl	LDL	HDL	FBS	OT	PT	Chol	Tgl	LDL	HDL	FBS	OT	PT							
1	Mohinder Singh (51yrs)	165	89	35.6	194	172	72	37	123	82	124	185	161	77	38	118	66	101	181	154	81	40	111	60	86	185	146	78	42	108	55	72
2	Harjindee Kaur (49yrs)	162	68	27.2	244	180	81	38	140	69	105	220	166	79	39	123	63	96	203	150	78	40	110	55	89	199	148	85	41	103	51	81
3	Baldev Singh (56yrs)	178	81	28.9	179	220	70	41	191	90	131	170	203	76	42	160	83	112	172	186	79	42	143	81	99	175	171	82	44	132	69	81
4	Hadial Singh (48yrs)	178	81	28.9	208	191	96	37	201	75	121	197	178	100	39	162	63	99	190	161	93	40	134	58	90	188	154	95	41	121	51	79
5	Ramnak Singh (54yrs)	171	83	33.2	197	231	81	39	183	59	98	183	211	92	40	152	47	86	179	187	95	40	130	50	79	181	162	93	41	121	48	71
6	Sarabjeet Singh (48yrs)	170	78	27.8	215	217	150	30	108	77	111	191	182	138	34	102	69	104	190	171	126	37	97	62	91	193	162	129	39	95	55	76
7	Varinder (48yrs)	170	88	31.4	238	232	192	41	136	66	134	232	208	178	40	124	56	109	211	181	160	40	113	55	93	203	170	152	41	106	51	81
8	Ankush Sharma (45yrs)	177	70	25	250	213	180	43	112	79	129	222	189	162	43	110	77	112	216	178	155	44	106	67	100	199	162	151	44	102	60	89
9	Sukhdeep Singh (50yrs)	168	79	31.6	129	110	93	33	116	70	86	133	105	94	35	110	61	74	135	111	95	35	106	56	71	133	106	97	36	105	50	63
10	Davinder Kaur (47yrs)	156	69	31.4	201	228	183	41	152	83	145	190	205	166	41	131	70	121	178	189	151	41	119	62	103	185	171	145	42	111	55	91
11	Kulwinder Kaur (55yrs)	154	72	32.7	231	187	121	35	185	98	122	212	169	119	38	166	83	100	192	153	120	39	141	71	86	195	150	122	40	130	60	72
12	Surinder Singh(45yrs)	173	78	27.8	214	187	142	37	128	101	147	194	162	135	39	114	90	121	190	155	128	40	110	74	102	188	151	129	41	106	63	88
13	Sewa Singh (55yrs)	168	76	30.4	197	204	180	33	129	81	141	188	189	162	38	121	71	119	185	172	157	39	118	60	98	189	164	146	40	114	52	82
14	Anil Kumar (49yrs)	188	98	30.6	222	179	150	41	157	72	133	213	160	133	40	138	61	108	207	156	136	41	121	52	93	198	150	138	41	115	44	80
15	Mohinder Kaur (50yrs)	160	65	26	245	170	108	45	133	82	111	225	159	110	43	125	70	98	211	152	114	43	116	62	91	197	146	115	43	111	48	78
16	Amba Devi (52yrs)	150	59	26.8	198	217	158	42	135	80	132	180	198	142	43	123	74	112	183	191	140	43	119	68	101	186	182	138	42	112	54	88
17	Punjab Chand (51yrs)	168	86	34.4	255	189	166	32	180	70	109	228	171	157	38	164	63	82	212	156	151	38	151	55	71	205	144	147	39	138	47	60
18	Jugraj Singh (48yrs)	180	89	27.6	231	201	156	39	151	58	94	209	188	140	39	124	56	87	197	165	138	40	118	50	72	195	159	135	41	111	46	65
19	Santosh Rani (48yrs)	155	95	43.2	227	96	179	42	175	47	61	212	100	161	55	155	40	55	202	103	155	51	139	38	51	198	99	148	48	123	35	47
20	Vanceet Vij (56yrs)	172	80	28.5	173	338	92	36	114	60	95	175	301	100	38	110	51	82	172	276	102	39	108	46	70	170	241	103	39	105	42	61

Group 4 (Case Continued)																					
S.No	Patient's Name	Ht	Wt	BMI	Visit5		Visit6		Tgl	LDL	HDL	FBS	OT	PT	Tgl	LDL	HDL	FBS	OT	PT	
1	Mohinder Singh (51yrs)	165	89	35.6	180	140	80	42	102	44	59	178	141	80	44	103	42	50			

2	Harjindee Kaur (49yrs)	162	68	27.2	191	145	87	41	99	48	69	187	147	88	42	100	44	57
3	Baldev Singh (56yrs)	178	81	28.9	176	155	83	44	119	56	64	173	150	80	45	107	49	49
4	Hadial Singh (48yrs)	178	81	28.9	193	147	94	42	116	45	67	189	145	90	42	108	39	58
5	Raunak Singh (54yrs)	171	83	33.2	177	157	90	41	111	45	63	180	150	88	42	103	41	54
6	Sarabjeet Singh (48yrs)	170	78	27.8	188	155	132	40	98	52	65	184	148	133	40	94	48	57
7	Vaerinder (48yrs)	170	88	31.4	198	166	147	41	101	48	74	195	154	146	42	99	45	65
8	Ankush Sharma (45yrs)	177	70	25	194	150	147	44	103	54	76	190	146	148	45	99	51	62
9	Sukhdeep Singh (50yrs)	168	79	31.6	131	108	100	42	101	39	60	136	103	101	39	102	35	53
10	Davinder Kaur (47yrs)	156	69	31.4	182	156	142	42	105	48	76	186	150	143	43	103	42	61
11	Kulwinder Kaur (55yrs)	154	72	32.7	189	148	120	40	119	55	60	186	145	117	41	111	39	54
12	Surinder Singh(45yrs)	173	78	27.8	192	147	130	41	105	48	75	189	143	133	42	101	42	63
13	Sewa Singh (55yrs)	168	76	30.4	185	158	142	41	108	46	69	182	153	145	41	102	42	58
14	Anil Kumar (49yrs)	188	98	30.6	195	149	135	42	107	40	71	196	145	134	42	101	38	60
15	Mohinder Kaur (50yrs)	160	65	26	195	145	112	44	109	41	65	198	140	118	43	99	38	51
16	Amba Devi (52yrs)	150	59	26.8	182	169	132	43	110	52	75	184	160	130	43	105	45	64
17	Punjab Chand (51yrs)	168	86	34.4	197	148	145	40	121	42	52	192	145	148	41	110	39	46
18	Jugraj singh (48yrs)	180	89	27.6	194	156	137	41	106	44	58	190	152	134	42	101	41	51
19	Santosh Rani (48yrs)	155	95	43.2	196	96	145	47	111	36	40	195	100	143	47	108	33	42
20	Vaneet Vij (56yrs)	172	80	28.5	178	209	98	40	102	40	52	176	174	99	41	98	35	48
21	Kusum chopra (56yrs)	152	69	31.4	169	147	156	38	112	48	57	167	145	151	38	107	45	50
22	Sawinder Kaur (50 yrs)	160	58	23.2	213	201	149	41	110	39	48	205	169	145	42	106	33	45
23	Sarabjeet Singh (46yrs)	178	78	27.8	195	132	149	38	130	37	55	196	135	143	39	125	35	49

24	Munish Talwar (54 yrs)	180	90	28.1	143	187	79	41	104	35	50	145	163	81	42	100	36	48
25	Surender Singh (51 yrs)	160	75	30	183	155	124	38	122	36	49	179	152	125	39	111	34	44
26	Deepak (45 yrs)	173	75	26.7	198	112	142	43	133	42	55	195	110	140	42	137	40	46
27	Kewal Krishan (54 yrs)	168	92	36.8	188	151	129	39	161	45	65	182	143	133	40	145	42	58
28	Annik Singh (52 yrs)	177	86	27.7	195	145	123	39	127	34	49	193	147	120	41	116	35	44
29	Navdeep kaur (49 yrs)	160	67	26.8	193	154	143	41	131	35	66	195	149	142	42	120	33	52
30	Sarup Rani (55 yrs)	153	71	32.2	191	150	137	42	115	47	71	193	148	133	42	104	42	58

Group5 (Control)		Visit1		Visit2		Visit3		Visit4		Visit5															
S.No	Patient's Name	Ht	Wt	BMI	Chol	Tgl	LDL	HDL	FBS	OT	PT	Chol	Tgl	LDL	HDL	FBS	OT	PT	Chol	Tgl	LDL	HDL	FBS	OT	PT
1	Kashmir Kaur (45yrs)	150	62	24.8	183	191	82	39	-	-	-	170	185	88	39	-	-	-	165	172	92	40	-	-	-
2	Parinder Singh (49yrs)	172	91	32.5	210	132	124	42	-	-	-	202	133	119	49	-	-	-	198	127	111	42	-	-	-
3	Kanwal Bajraj (46yrs)	180	88	27.5	240	158	163	35	-	-	-	221	152	155	35	-	-	-	215	147	144	35	-	-	-
4	Amarjeet Singh (53yrs)	176	80	28.5	122	185	89	36	-	-	-	128	172	95	36	-	-	-	130	162	98	36	-	-	-
5	Ashok Kumar (48yrs)	170	67	23.9	219	71	144	35	-	-	-	213	80	140	35	-	-	-	202	89	131	35	-	-	-
6	Madhu Tuli (44yrs)	152	60	27.3	226	166	164	36	-	-	-	215	158	159	38	-	-	-	211	155	152	39	-	-	-
7	Amar Kaur (55yrs)	152	64	29	207	141	72	43	-	-	-	200	138	80	41	-	-	-	198	135	88	42	-	-	-
8	Ashu Verma (46yrs)	161	62	24.8	206	121	128	41	-	-	-	202	122	130	38	-	-	-	199	126	133	38	-	-	-
9	Kiran Jolly (52 yrs)	151	67	30.4	204	115	124	39	-	-	-	200	121	129	39	-	-	-	195	125	122	39	-	-	-
10	Bhushan Kumar (50 yrs)	165	75	31.6	144	179	119	36	-	-	-	139	171	120	36	-	-	-	131	165	122	36	-	-	-
11	Neelam (46yrs)	158	69	31.3	-	-	-	-	111	-	-	-	-	-	-	-	-	-	-	-	-	-	105	-	-
12	Bharat Lal (56yrs)	173	70	25	-	-	-	-	115	-	-	-	-	-	-	-	-	-	-	-	-	-	108	-	-
13	Rajinder Singh (53yrs)	168	68	27.2	-	-	-	-	106	-	-	-	-	-	-	-	-	-	-	-	-	-	102	-	-
14	Kaushalya Devi (53yrs)	155	68	31	-	-	-	-	109	-	-	-	-	-	-	-	-	-	-	-	-	-	104	-	-
15	Raunak Singh (50yrs)	178	80	28.6	-	-	-	-	104	-	-	-	-	-	-	-	-	-	-	-	-	-	100	-	-
16	Kashmir Singh (55yrs)	180	88	27.5	-	-	-	-	102	-	-	-	-	-	-	-	-	-	-	-	-	-	100	-	-
17	A.P. Chatha (50yrs)	169	83	33.2	-	-	-	-	110	-	-	-	-	-	-	-	-	-	-	-	-	-	103	-	-
18	Nathaniai Singh (48 yrs)	178	85	30.3	-	-	-	-	112	-	-	-	-	-	-	-	-	-	-	-	-	-	107	-	-
19	Vinod Kumar (51yrs)	170	72	25.7	-	-	-	-	107	-	-	-	-	-	-	-	-	-	-	-	-	-	99	-	-

Group 5 (Control) Continued				Visit5				Visit6										
S.No	Patient's Name	Ht	Wt	BMI	Chol	Tgl	LDL	HDL	FBS	OT	PT	Chol	Tgl	LDL	HDL	FBS	OT	PT
1	Kashmir Kaur (45yrs)	150	62	24.8	160	160	96	40	-	-	-	177	156	92	41	-	-	-

2	Parminder Singh (49yrs)	172	91	32.5	195	132	118	43	-	-	-	193	135	116	42	-	-
3	Kanwal Balmaj (46yrs)	180	88	27.5	206	142	143	36	-	-	-	202	143	142	36	-	-
4	Amarjeet Singh (53yrs)	176	80	28.5	128	153	100	37	-	-	-	131	151	105	37	-	-
5	Ashok Kumar (48yrs)	170	67	23.9	192	90	138	36	-	-	-	193	98	139	36	-	-
6	Madhu Tuli (44yrs)	152	60	27.3	198	149	145	41	-	-	-	197	146	143	41	-	-
7	Amar Kaur (55yrs)	152	64	29	193	132	97	43	-	-	-	196	130	98	43	-	-
8	Ashu Verma (46yrs)	161	62	24.8	190	123	138	38.5	-	-	-	191	128	136	39	-	-
9	Kiran Jolly (52 yrs)	151	67	30.4	192	127	129	41	-	-	-	187	125	132	40	-	-
10	Bhushan Kumar (50 yrs)	165	75	31.6	137	158	121	37	-	-	-	138	152	120	37	-	-
11	Neelam (46yrs)	158	69	31.3	-	-	-	-	101	-	-	-	-	-	-	36	-
12	Bharat Lal (56yrs)	173	70	25	-	-	-	-	102	-	-	-	-	-	-	38	-
13	Rajinder Singh (53yrs)	168	68	27.2	-	-	-	-	99	-	-	-	-	-	-	34	-
14	Kaushalya Devi (53yrs)	155	68	31	-	-	-	-	100	-	-	-	-	-	-	37	-
15	Raunak Singh (50yrs)	178	80	28.6	-	-	-	-	100	-	-	-	-	-	-	39	-
16	Kashmir Singh (55yrs)	180	88	27.5	-	-	-	-	98	-	-	-	-	-	-	37	-
17	A.P. Chatha (50yrs)	169	83	33.2	-	-	-	-	99	-	-	-	-	-	-	34	-
18	Nathaniai Singh (48 yrs)	178	85	30.3	-	-	-	-	102	-	-	-	-	-	-	36	-
19	Vinod Kumar (51yrs)	170	72	25.7	-	-	-	-	100	-	-	-	-	-	-	36	-
20	Robin Sharma (46 yrs)	152	88	27.8	-	-	-	-	106	-	-	-	-	-	-	40	-
21	Ashish Parkash (45 yrs)	170	77	27.5	-	-	-	-	-	55	72	-	-	-	-	-	68
22	Kuldeep Singh (50yrs)	181	95	29.6	-	-	-	-	-	45	60	-	-	-	-	-	54
23	Rajwant Singh (56yrs)	175	82	27.3	-	-	-	-	-	47	71	-	-	-	-	-	64

24	Ravinder Singh (52yrs)	172	82	28.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	41	58
25	Gurvinder Singh (50yrs)	177	90	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	38	65
26	Rajwinder Singh (52yrs)	170	83	29.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	46	67
27	Chanan Singh (48yrs)	180	87	27.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	49	58
28	Sarabjeet Kaur (45yrs)	164	81	31.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	45	50
29	Anil Vohra (49yrs)	170	79	28.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	39	53
30	Ashish Parkash (45 yrs)	170	77	27.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	51	68

Group 5 (Case Continued)																					
S.No	Patient's Name	Ht	Wt	BMI	Visit5		Tgl	LDL	HDL	FBS	OT	PT	Visit6		Tgl	LDL	HDL	FBS	OT	PT	
1	Deepak Sharma (46 yrs)	167	74	167	194	147	133	38	-	-	-	-	194	140	131	39	-	-	-	-	

2	Munish Gupta (48yrs)	167	70	167	209	121	148	36	-	-	-	200	115	145	38	-	-	-
3	Ishwar Singh (53 yrs)	180	83	180	199	145	120	37	-	-	-	195	140	122	39	-	-	-
4	Makhan Singh (47yrs)	175	76	175	194	165	128	37	-	-	-	192	160	129	38	-	-	-
5	Resham Singh (52yrs)	175	88	175	168	149	152	38	-	-	-	173	141	149	40	-	-	-
6	Veena Kasra (54yrs)	150	67	150	202	137	125	41	-	-	-	198	133	128	42.5	-	-	-
7	Joginder Kumar (50yrs)	168	81	168	200	148	110	38	-	-	-	197	143	111	39	-	-	-
8	Niranjan Singh (51yrs)	178	84	178	180	156	137	39	-	-	-	177	151	135	40	-	-	-
9	Simmrjeet Kaur (47yrs)	152	86	152	154	158	143	38	-	-	-	158	153	144	38	-	-	-
10	Deljit Kaur (45yrs)	158	65	158	204	124	93	39	-	-	-	200	120	97	40	-	-	-
11	Paramjeet Kaur (47yrs)	155	78	155	173	157	139	40	-	-	-	171	150	135	41	-	-	-
12	Palwinder Kaur (52yrs)	154	68	30.9	-	-	-	-	104	-	-	-	-	-	-	101	-	-
13	Kulwant Kaur (54yrs)	160	71	28.4	-	-	-	-	105	-	-	-	-	-	-	102	-	-
14	Madhu Sharma (46yrs)	157	68	28.3	-	-	-	-	111	-	-	-	-	-	-	108	-	-
15	Yadwinder Singh (51yrs)	172	77	26.5	-	-	-	-	109	-	-	-	-	-	-	107	-	-
16	Manjeet Kaur (46yrs)	160	68	27.2	-	-	-	-	108	-	-	-	-	-	-	105	-	-
17	Kavita Sharma (48yrs)	156	60	25	-	-	-	-	107	-	-	-	-	-	-	105	-	-
18	J.S. Chaudhary (48yrs)	176	77	25.6	-	-	-	-	101	-	-	-	-	-	-	102	-	-
19	Raman Grover (49yrs)	171	80	28.5	-	-	-	-	106	-	-	-	-	-	-	103	-	-
20	Amarjeet Singh (53yrs)	165	76	28.1	-	-	-	-	100	-	-	-	-	-	-	98	-	-
21	Asha Khosla (55yrs)	155	68	28.3	-	-	-	-	106	-	-	-	-	-	-	102	-	-
22	Raunak Singh (50yrs)	178	80	28.6	-	-	-	-	-	50	62	-	-	-	-	-	46	55
23	Balwinder Kaur (52yrs)	152	68	30.9	-	-	-	-	-	53	65	-	-	-	-	-	48	59
24	Nathaniai Singh (48 yrs)	178	85	30.3	-	-	-	-	-	56	63	-	-	-	-	-	50	56

25	Ramesh (44yrs)	173	96	34.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	42	51
26	Anju Bala (45 yrs)	150	65	29.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37	52
27	Gurinder Singh (53 yrs)	168	65	26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	46	58
28	Sajjan Singh (52 yrs)	169	72	28.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	39	58
29	Radhia Rami (47 yrs)	156	63	28.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37	50
30	Jatinder Kaur (50yrs)	152	63	28.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	39	53

