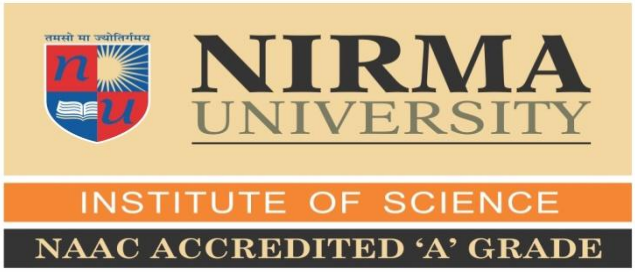
Prevalence of Diabetes in Bharuch & Narmada District and Effect of Insulin on ErbB 3 receptor in Cerebral Cortex &Corpus Striatum of Diabetic Rats

A Dissertation thesis submitted to Nirma University in partial fulfillment of requirement for degree of

MASTER OF SCIENCE

IN

BIOTECHNOLOGY/ MICROBIOLOGY



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Acknowledgement

Firstly, *I thank Almighty for giving me the strength to work even in unfavorable conditions without losing faith in myself.*

Most important I would like to thank my mother who has been a source of encouragement and inspiration to me throughout my life and also for the myriad of ways in which she actively supported me in my determination to find and realize my potential, and to make this contribution to our world.

I would like to thank all the people who contributed in some way to the work described in this thesis. First and foremost, I thank my guide, Dr. Amee K. Nair, for accepting me into her group. I appreciate all her contributions of time, ideas, and funding to make my M.Sc experience productive and stimulating. Secondly, I would like to thank Ms. Madhavi Joshi for teaching me, both consciously and unconsciously, how good experimental project is done. The joy and enthusiasm she has for her research was contagious and motivational for me. Also, I would like to thank Mr. Manoj Patidar for his great support in my tough times during last session of MSc. He encouraged and motivated us and showed us a right path at every stage of MSc.

It's my great honour to thank **Prof. Sarat Dalai** Director (I/c), Institute of Science, Nirma University for his valuable advice, motivation and critical suggestions and for providing me with all the facilities in the institute for the successful completion of this project.

I express my gratitude to all the faculty members of the Institute of Science, **Dr. Shalini Rajkumar, Dr. Sriram Seshadri, Dr.**

Nasreen Munshi, Dr. Mili Das, Dr. Vijay Kothari, Dr. Sonal Bakshi, for their unconditional support and valuable time.

I thank Mr. Hasit Trivedi, Mr. Sachin Prajapati, Mr. Rajendra patel, Ms. Shewtal Shukla,Ms. Shweta Patel for providing me with all kinds of possible help.

Last but not least, i am thankful to my friends who supported me throughout this project. Especially, Nisarg Gohil, Monica Khapra, Viraj Mangrola, Dhavan Sharma, Milan Patel, Khyati Patel, Shrusti Dave.

Dedicated

TO

GOD...

&

My Parents and My

Family

1. Introduction

1.1 Diabetes Mellitus

Diabetes mellitus was considered as a disease of minor significance to world health till now. But it is taking place as one of the major threats to mankind in the 21st century. The last two decades have seen a significant increase in the number of diabetic patients worldwide (Zimmet et al., 2001).

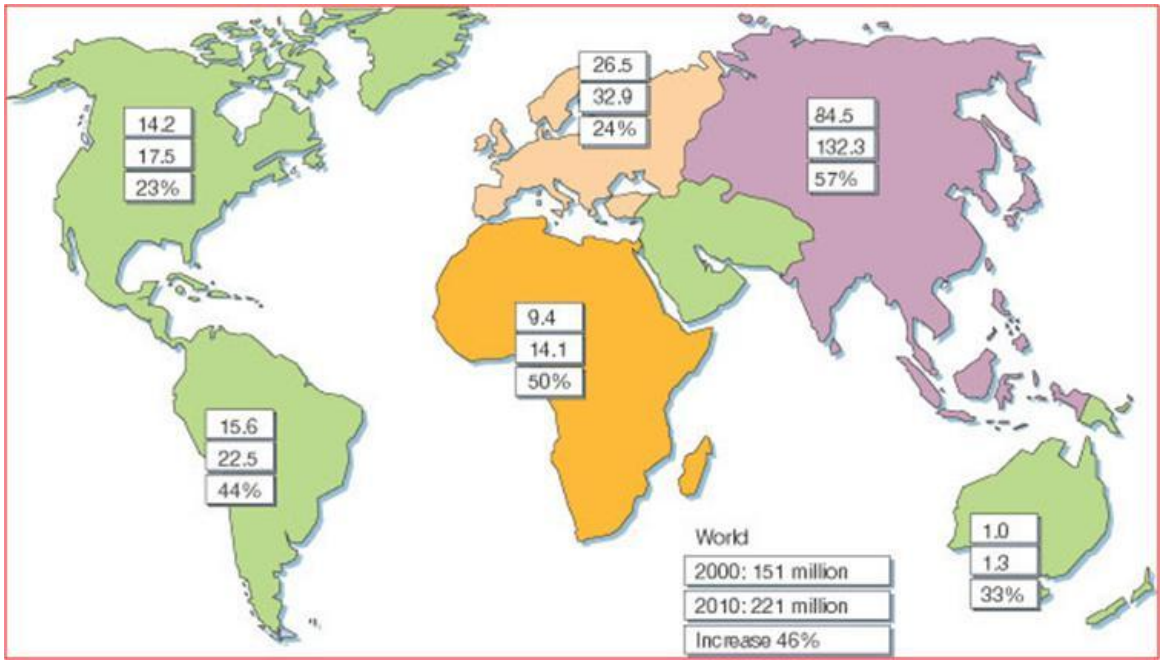


Figure 1: Numbers of diabetic patients (in millions) for the year 2000 & 2010 (top values and middle values, respectively) and the percentage increase (Adapted from Amos et al., 2010).

Diabetes is an intricate group of diseases with a variety of causes. People suffering from diabetes have high level of glucose in the blood, also called high blood sugar or hyperglycemia (American Diabetes Association., 2009). Also, it is a diverse group of disorders that have in common abnormal tolerance of glucose. Type I diabetes or Insulin dependent diabetes mellitus is characterized by complete dependency exogenous insulin. Type II diabetes or non-insulin-dependent diabetes mellitus is characterized by hyperglycaemia but no tendency to ketoacidosis except under extraordinary stress. People with type II diabetes may take treatment of insulin to control hyperglycemia, but it is not exactly required (Molitch, 1990). According to the Diabetes Atlas 2006, India is having

highest number of diabetic patients in the whole world: 40.9 million in 2007 and expected to increase to 69.9 million by 2025. (Pradeepa et al., 2011)

1.2 Types of Diabetes

The global increment in diabetes is due to population growth, ageing, rising trends towards an unhygienic diet, obesity and sedentary lifestyles (Ahmed et al., 2010).

1.2.1 Type I diabetes

Type I diabetes has a strong genetic component, inherited mainly through the HLA complex, but the factors that stimulates the onset of clinical disease remain largely unknown. Complications of type I diabetes consist of microvascular and macrovascular disease, which account for the major mortality and morbidity associated with type I diabetes.

1.2.1.1 Prevalence

Type I diabetes is present in 5–10 percent of all diabetics, but is increasing in teenage groups (Maiese et al., 2007). The prevalence of type I diabetes advances with age and the overall occurence of disease may be increasing (Lueder and Silverstein, 2005).

1.2.1.2 Etiology

Genetic predisposition, autoimmunity and viral infection are the leading etiological factors implicated in the pathogenesis of type I diabetes mellitus (Muthukrishnan et al., 2007). Type I diabetes, which may develop at any age is a chronic autoimmune disorder, characterized by irreversible autoimmune destruction of the insulin secreting β-cells of the islets in the pancreas. Glycogenolysis and Gluconeogenesis produces more amount of glucose and decreased cellular uptake of glucose from the circulation. Life-long treatment is required in Type I diabetes with regular insulin for survival (Mehra et al., 2007).

1.2.2 Type 1.5 diabetes

Latent autoimmune diabetes in adults or type 1.5 diabetes is considered to be an autoimmune disease mediated by T-cell. However, identification of patients is based commonly on autoantibody (Ab) detection. Type 1.5 diabetes is considered as an autoimmune disorder, mediated by autoreactive T-cells. T-cells reacting to various islet

proteins in peripheral blood have been demonstrated in patients with type 1 diabetes. (Goel et al., 2007).

1.2.2.1 Prevalence

Diagnosis of type 1.5 diabetes is usually after age of 35 years and there is no immediate need for insulin (Goel et al., 2007).Type 1.5 diabetics are phenotypically indistinguishable to type II diabetic patients but they are also positive for the autoantibody commonly observed in type I diabetes. Approximately 10% to 30% of adults with type II diabetes test positive for autoantibodies, determined by the age and ethnicity of the study group (Stenstrom et al., 2005). This group of phenotypic adult type II diabetic patients (approximately 10%) are said to have latent autoimmune diabetes in adults (LADA) or type 1.5 diabetes or type 1 diabetes (Palmer et al., 2005).

1.2.2.2 Etiology

Patients with type 1.5 diabetes have an autoimmune activity similar to that found with type I diabetes. Though patients with type 1.5 diabetes have genes such as HLA DR2, DQB1*0602, which protects any individual from developing diabetes , the beta-cells become so inflamed by continuous environmental insults that they start autoimmune destruction within the beta-cells. This destruction of beta-cells by autoimmunity in type 1.5 diabetics leads to insulin dependency more rapidly than in type II diabetes, but the more attenuated genetic and immune factors associated with type 1.5 diabetes as compared with type I diabetes lead to an older age at onset and a slower progression to insulin dependency (Unger, 2008).

1.2.3 Type II diabetes

Type II DM or non-insulin dependent DM is the most common form of DM characterized by hyperglycaemia, resistance to insulin, and deficiency of relative insulin. Type II DM results from interaction between genetic, environmental and behavioral risk factors. Type II DM is due primarily to lifestyle factors and genetics. A number of lifestyle factors plays a crucial role in development of type II DM. These are physical inactivity, sedentary lifestyle, smoking and regular alcohol consumption. Obesity has been found to contribute to approximately 55% of cases of type II DM. The high rate of childhood

obesity between the 1960s and 2000s is believed to have led to the increase in type II DM in children and adolescents (Olokoba et al., 2012).

1.2.3.1 Prevalence

Type II diabetes is the most common form of the disease, accounting for about 90 to 95 % of all diagnosed cases of diabetes. In type II diabetes, the body does not produce sufficient amount of insulin or the cells ignore the insulin (Badyal and Kaur, 2008). The rise in the prevalence of type II diabetes has been seen in the middle-aged and elderly, there is now strong evidence of a rise among younger adults (Alberti et al., 2004).

1.2.3.2 Etiology

Type II diabetes occurs usually in individuals over 40 years of age and dramatically increases as a result of changes in human behaviour and increased body mass index (Elmer et al., 2004). The increasing proportion of the ageing, consumption of calorie rich diet, increasing obesity and sedentary lifestyle has led to a tremendous increase in the number of diabetic patients in the whole world (El-Shenawy and Abdel-Nabi, 2006). This form of diabetes encompasses individuals who have insulin resistance and usually have deficiency of relative insulin. At least initially and often throughout their lifetime, these individuals do not require insulin treatment to survive. Most patients with this form of diabetes are have obesity, which causes some degree of insulin resistance. This form of diabetes frequently goes undiagnosed for many years (American Diabetes Association, 2010). Both genetic and intrauterine environmental factors mediates familial predisposition to type II diabetes. (Seshiah et al., 2008).

1.3 Geographic Profile of Gujarat

Most of the land of Gujarat is dry and arid in nature on account of nearness to Thar Desert. It consists of four broad zones viz., (i) the Upland zone (hilly area), (ii) the Pediment Zone with a thin sediment cover, (iii) the Basinal Zone and (iv) Coastal Zone .The Hilly Zone comprises the highlands of Aravallis & Vindhyans, which practically forms a water divide for river flowing into the Arabian Sea & Rann of Kutch. The Pediment Zone is the prominent feature bordering the hilly areas. The Basinal Zone is

mainly a featureless plain area. It includes the Aeolian landforms of north Gujarat and small hilly areas in the north. The coastal zone includes broad estuarine inlets, mudflats, mangrove swamps, salt marshes and other associated features of the Narmada, Tapi, Mahi, Sabarmati rivers. The Saurashtra peninsula forms watershed of almost all the radially flowing rivers, falls into Arabian Sea. The highlands of Kathiawar have resulted from the denudation of the basaltic plateau. The Chotila hill forms the highest topographic feature in north (340 m AMSL). More rugged topography occupied part of Sabarkantha, Panchmahals, Baroda, Surat and Bharuch districts, include Vanmala, Rajpipla, Satpura, Ratanmal and Pavagadh hills.

Table 1: Agro climatic features of South Gujarat

Sub Region	Rainfall(in mm)	Climate	Soil	Crop	Sub Region
South Gujarat	974	Semi-arid to dry sub-humid	Deep black, coastal alluvium	Jowar, Arhar, cotton, wheat	South Gujarat
South Gujarat (heavy rainfall)	1793	Semi-arid to dry sub-humid	Deep black, coastal alluvium	Rice, Ragi, sugarcane, Jowar	South Gujarat (heavy rainfall)

Risk factors affecting diabetes are:

Body Mass Index (BMI): BMI is short for Body Mass Index. This uses a formula which indicates the ratio between weight and height of a person.

1. **BMI = weight (kg) / height (m) / height (m)**

Table 2: Interpretation of BMI result

BMI	Classifications (by the WHO)
Less than 18.5	Underweight
18.5 or more and less than 25	Normal
25 or more and less than 30	Overweight
30 or more	Obese

Body Fat: Body fat serves a role in energy storage and internal organ protection. We have two types of fats in our system: 1) essential fat which is deposited in small amounts to protect the body and 2) adipose tissue (stored fat) protection to internal organs, covers the nerves, and is the largest reserve of stored energy. Too much body fat is unhealthy; having too little fat can be just as unhealthy. In men and women, Body fat is distributed differently so, classifying the body fat percentage is different between the males and females.

2. Body fat percentage refers to the amount of body fat mass in regards to the total body Weight expressed as a percentage.

Body fat percentage (%) = {Body fat mass (kg) / Body weight (kg)} × 100

Table 3: Interpretation of Body Fat percentage result

Body Fat Percentage (MALE)	Classification	Body Fat Percentage (FEMALE)
>25%	Very High	>35%
20 - 25%	High	30 – 35%
10 - 20%	Normal	20 - 30%
<10%	Low	< 20%

3. **Visceral Fat:** Visceral fat is found in the abdomen and nearer vital organs. High visceral fat is closely linked to increased levels of fat in blood, which may lead to high

cholesterol, heart disease and type II diabetes. In order to prevent this problem, it is necessary to try to reduce the amount of visceral fat levels to an acceptable level.

Table 4: Interpretation of Visceral Fat result

Visceral Fat Level	Level Classification
0.5 - 9.5	0 (Normal)
10.0 - 14.5	+ (High)
15.0 - 30.0	++ (Very High)

1.4 Diabetic Neuropathy

Diabetic neuropathy (DN) is a common disorder in which peripheral nerve dysfunction is a main symptom in a patient with diabetes mellitus (DM). There is 4.3% prevalence of DM in India as compared to western countries (1%–2%). Probably Indians are more prone for insulin resistance and cardiovascular mortality. (Bansal et al., 2006). DN is a chronic, disabling condition that affects approximately 60 to 70 % of diabetic individuals. Next to cardiovascular complications associated with diabetes, 75% of diabetic patients suffer from diabetic neuropathy (Bansal et al., 2006).

Degeneration of axons due to demyelination is a clear hallmark of diabetic neuropathy due increased level of glucose in neurons (Selvarajah et al., 2011). Hyperglycaemia causes an explosive increase in neuronal glucose levels. If this is persistent then intracellular glucose metabolism leads to myelin sheath damage. This is a serious consequence of long-term diabetes, is referred as diabetic neuropathy (Tomlinson and Gardner; 2008).

In neuropathy, fine nerve ending is damaged in the peripheral body including the damage of myelin sheath causing intense pain, decreased motility and remains the leading cause of non-accidental limb amputation. Traditionally, diabetes has been handled as a peripheral metabolic disease. But most of the non-invasive brain imaging techniques have demonstrated structural and functional abnormalities related to diabetes (Zsombok et al., 2009). Studies from Electron Microscopy have revealed the diabetes induced degeneration in axons of CNS and Oligodendrocytes(Myelin producing cells in CNS) (Juan et al., 2009).

1.4.1 Increased Glucose in PNS causes myelin damage in CNS

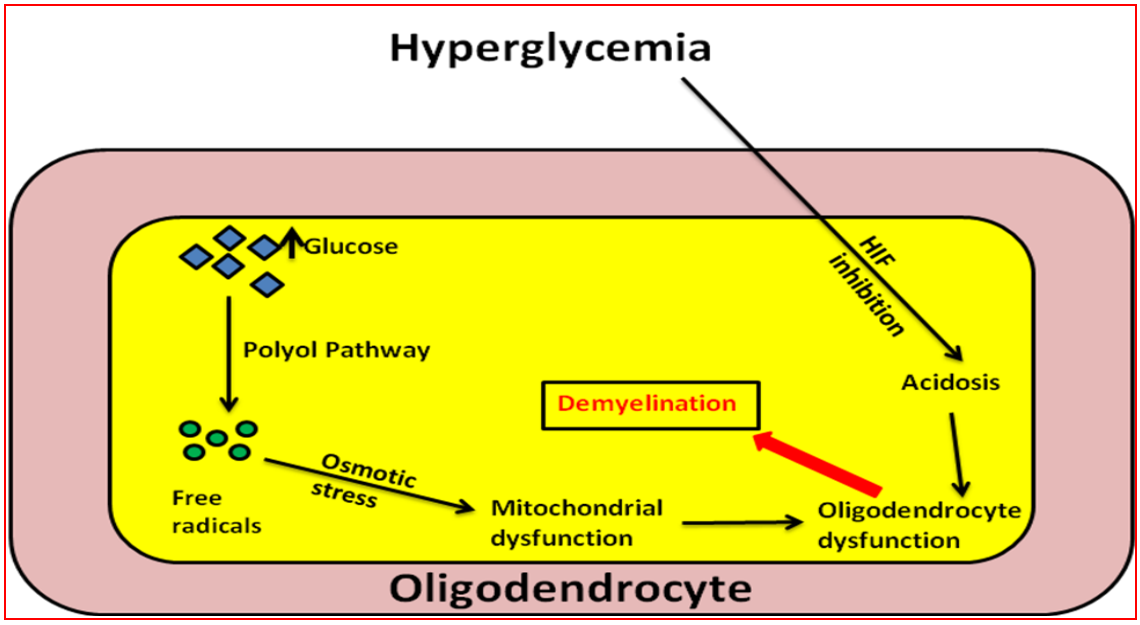


Figure 2: Increased glucose causes demyelination in CNS: Hyperglycemia activates polyol pathway which leads to oligodendrocyte dysfunction and ultimately causes demyelination.

Certain metabolites which are produced due to excess glucose metabolism in periphery body has been well established to affects CNS. Raised intracellular glucose drives the polyol pathway to form sorbitol - as an end product that causes tissue swelling through increased osmotic effect (Burg, 1988). When sorbitol is formed as an end product of polyol pathway, causes tissue swelling through increased osmotic effect because sorbitol is non charged intra cellular osmolyte (Burg, 1988). The increased consumption of NADPH therein compromises recycling of glutathione disulphide (GSSG) to glutathione (GSH) (Tomlinson and Gardiner; 2008) along with formation of advanced glycated end products (James et al., 2008) does not allow a favourable cellular environment for the process of remyelination to be initiated in damaged oligodendrocytes. Compromised anti oxidant system is accounted, which is decreased activity of superoxide dismutase and catalase, enzymes that provide antioxidant defense to brain (Tomlinson et al., 2007). During hyperglycemia, decreased level of anti-oxidant enzymes and increased DNA damage has been reported. During severe energy deprivation during diabetes, down regulation of mitochondrial free radicals scavenger system is observed which leads to generation of high level of reactive oxygen species (ROS) which in turn activate the processes leading to DNA damage (Singh et al., 2001). These reports support the fact that oxidative stress is the biochemical trigger for myelin damage during CNS diabetic

neuropathy. Furthermore, additional consequence of hyperglycaemia is non-enzymatic glycation of proteins, which form a stable advanced glycation end-product (AGE) which have capacity to induce cellular responses through RAGE receptors (receptors for AGEs) (Singh et al., 2001;Jakus and Rietbrock, 2004). RAGE expression on oligodendrocytes during experimental diabetes induces abnormal RAGE signalling with further cellular dysfunction (Toth et al., 2006) and oligodendrocyte damage.

CNS is one of the representative end-organ targets of metabolic insult related to diabetes mellitus. Brain remains the final target by hyperglycemia leading to neuronal activation, functional change in synapse and morphology of oligodendrocytes (Lyoo et al., 2013). Thus, altered glucose homeostasis and impaired insulin signaling is reported during diabetes leads to myelin damage in CNS (Wrighten et al., 2009).

Moreover, hyperglycemia is also causes cellular acidosis. This hypothesis of "acidotoxicity" states that anaerobic metabolism of conversion of glucose to lactic acid under conditions of lack of oxygen leads to intracellular and extracellular acidosis. Indeed, cellular pH after ischemia is a straight function of levels of lactic acid in the brain. The pannecrosis in the brain is envisioned by acidity, i.e., cellular damage not only to neurons but also to glia and vascular elements (Huang et al., 2012) leading to oligodendrocyte dysfunction.

1.4.2 Role of ErbB signaling in myelination

ErbB signaling is not necessary for the initiation of CNS myelination, but that Erbb3 is required for late events in oligodendrocyte maturation and myelination; i.e., oligodendrocyte Erbb3 and Erbb4 loss does not produce myelin defects but loss of oligodendrocyte ErbB3 causes hypomyelination in the corpus callosum and optic nerve.(Makinodan et. al 2012).

ErbB3: ErbB3 signals play significant roles in oligodendrocyte myelination and salutatory conduction of nerve impulses. (Nawa H. et.al.2013).

1.5 OBJECTIVES

Objective 1: To Study Prevalence of Diabetes in Bharuch and Narmada District

1. To obtain the risk profile of Diabetes Mellitus patients from district of South Gujarat so that prevention or decrease the burden of Diabetes Mellitus in Gujarat can be possible.
2. To determine prevalence of Diabetes and its Socio-demographic profile.
3. To examine the strength and effect of family history of Diabetes in first degree relatives on the prevalence of self-reported, physician-diagnosed Diabetes among participants.
4. To estimate Diabetes management, Diabetes control and late complication status among patients afflicted with Diabetes

Objective 2: Effect of Hyperglycemia and Insulin treatment on ErbB Receptors Mediated Remyelination of Diabetic Rats

- To investigate the effect of progressive diabetes and therapeutic effect of insulin on ErbB receptors in Cerebral Cortex and Corpus striatum.

1.6 Expected Outcome:

Proposed demographic survey study of diabetes will help to identify prevalence of diabetic neuropathy in West Gujarat which will be helpful to medical practitioners for therapeutic management and control of diabetes. Generation of specific data regarding how therapeutic role of insulin affects ErbB mediated myelination in Cerebral Cortex and Corpus Striatum of streptozotocin induced diabetic rats.

2. Materials and Methods

2.1 Demographic Studies

A survey study was conducted in two districts of South Gujarat namely Bharuch and Narmada to describe the effect of occupation, dietary pattern, inheritance and migration on diabetes management, diabetes control and late complication status among people who are affected from diabetes. The survey was conducted unbiasedly. People were interrogated with the help of a questionnaire and the whole data were recorded in case forms. The data obtained was analysed for over a period of one month. 350 people from Bharuch and 400 people from Narmada were considered as the size of the sample population. The sample size of the population is calculated by the formula given below:-

$$Sample\ Size = \frac{\frac{z^2 \times p(1-p)}{e^2}}{1+(\frac{z^2 \times p(1-p)}{e^2 N})}$$

Where,

Population size = N

Margin of error = e (e is percentage , put in decimal form)

Z score = Z

Desired confidence level	Z - score
80%	1.28
85%	1.44
90%	1.65
95%	1.96
99%	2.58

People were individually examined by Karada Scan HBF-375 (Omron) Body Composition Monitor to measure the different parameters which are likely to affect diabetes. These parameters are Weight, BMI, Body Fat, and Visceral Fat.

Random Blood Sugar (RBS) of people was scrutinized with the help of Glucometer (CareSensN). Finger of an individual was pricked by lancet and glucometer inserted with blood glucose test strip (CareSensN) was placed on the drop of blood. The blood automatically draws within the test strip and hence the reading of RBS is displayed on the screen of glucometer.

Thereafter, People were physically assessed for hypertension. A blood pressure monitor (Omron) is used to check the blood pressure of an individual. People were asked to sit on a chair in a relaxed position for getting exact results of blood pressure.

And lastly, People were interrogated with the help of a questionnaire for complications like Neuropathy, Retinopathy, Nephropathy, Cardiomyopathy, and Hypoglycaemia.

2.2 Instruments and Chemicals

Blood pressure monitor (Omron) & Body composition monitor (Omron) were procured from Chamunda Surgical, Ahmedabad. Glucometer (I-Sens) and Caresens - N Blood Gluco strips were procured from Simandhar Healthcare, Ahmedabad.

Streptozotocin (Qualigens fine chemicals, Mumbai), Actrapid human insulin (Novo Nordisk, Denmark), sucrose (Sisco Research Lab, Mumbai), KH_2PO_4, (Sisco Research Lab, Mumbai), α keto glutarate (Sisco Research Lab, Mumbai), Lactate dehydrogenase (Sisco Research Lab, Mumbai), sodium hydroxide (NaOH; Sisco Research Lab, Mumbai), Tri reagent (Sigma Aldrich), DEPC water (Himedia laboratories pvt. Ltd.), chloroform (Sisco research Lab, Mumbai), cDNA synthesis kit (Fermentas, USA), PCR Mastermix (Fermentas, USA), primer ErbB-3 and ErbB-4 Receptors (), Agarose (Sisco Research Lab, Mumbai), ethidium bromide (Central Drug House, Mumbai), DNA ladder (Fermentas, USA). All biochemicals used in the present study were of analytical grade.

2.3 Model Induction

2.3.1 Induction of diabetes

Animals were allocated into following groups:-

1) Control [C]
2) Diabetic [D]
3) Diabetic + Insulin treated [D + I]

Each and every group consisted of 3 animals. A single intrafemoral dose (50mg/kg body weight) of Streptozotocin prepared in citrate buffer, pH 4.5 was used to induce diabetes (Arison et al., 1967; Hohenegger and Rudas et al., 1971). Estimation of blood glucose was done by blood glucose test strip (CareSensN) with the help of glucometer (CareSensN). Daily 2 doses (1-1.5 IU/Kg body weight) of regular human insulin (Actrapid) was delivered to [D + I] group for 6 weeks. (Flanagan et al., 2003).

Consumption of food, water and change in body weight were recorded throughout the experiments. Daily, Food and water uptake by the animals were regularly monitored and recorded during the active phase (12 hrs) and inactive phase (12 hrs) of the experimentation period.

2.4 Animals

Adult male Wistar rats of 100-150 gms body weight were purchased from Haffkine Biopharmaceuticals Ltd., Mumbai and used for all experiments. They were housed in separate cages under 12-h light and 12-h dark periods and were maintained on standard food pellets and water at 25±3°C. Animal care and procedures were done according to the Institutional and National Institute of Health Guide lines.

2.5 Glucose estimation and Body Weight measurement

Daily body weight was recorded during the inactive phase throughout the experiment. Glucose estimation was carried out by Caresens Glucometer (Amrit AG Health & Lifecare, Ludhiana, India).

2.6 Tissue preparation

Rats were sacrificed on the 6 weeks of model induction by cervical dislocation. The cerebral cortex and corpus striatum were dissect out and frozen in ice according to the procedure of (Iversen & Glowinski, 1966). The tissues were stored at -80^{o}C until assay.

2.7 Gene Expression

2.7.1 Total RNA Isolation

Total RNA was isolated using Tri-reagent (Sigma, #T9424) from the cerebral cortex and corpus striatum of the control and experimental group of rats' brain. RNA purity was checked at 260/280 nm in Agilent Technologies Ltd. UV–Visible spectrophotometer. Isolated RNA was observed on an agarose gel using agarose gel electrophoresis.

2.7.2 cDNA synthesis

cDNA was made from the total RNA by using cDNA synthesis kit (Thermo Scientific #K1612).

2.7.3 PCR analysis

ErbB 3 specific primers were designed by using IDT, Primer quest and primer 3.

Table 5: ErbB 3 specific primers

ErbB 3 Primers	Sequence (5'-3')	Tm (^{o}C)	% GC
Forward	GTGCTGTGCTTCCTTCTC	60	55.6
Reverse	GTACTGGTTGTCTGCATCTC	60	50

Table 6: PCR profile for amplification of ErbB 3

Steps	Temperature	Time
Initial Denaturation	94^{o} C	3 minute
Number of cycles		1
Denaturation	94^{o} C	30 seconds
Annealing	53^{o} C	45 seconds
Amplification	72^{o} C	45 seconds
Number of cycles		35
Final extension	72^{o} C	10 Minutes

3.RESULTS AND DISCUSSION

3.1 Demographic Studies

3.1.1 Prevalence of Diabetes in Bharuch

In demographic survey study of Bharuch, 28.28% of people have found to be diabetic. A total of 450 individuals falling in four age groups viz. 15-24, 25-34, 35-44, 45-54, 55-64, 65-74 and >75. The maximum diabetic patients (31.31 %) are found in age group of 55-64. The present study shows highest no. of females (38.98%) are found to be diabetic in age group of 55-64 whereas highest no. of males (25%) are found to be diabetic in age group of 45-54.

Table 7: Prevalence of Diabetes among the population of Bharuch District

Population	Male N (%)	Female N (%)	Total N (%)
Diabetic	40, (28.57%)	59, (28.09%)	99, (28.28%)
Non-Diabetic	100, (71.42%)	151, (71.90%)	251, (71.71%)

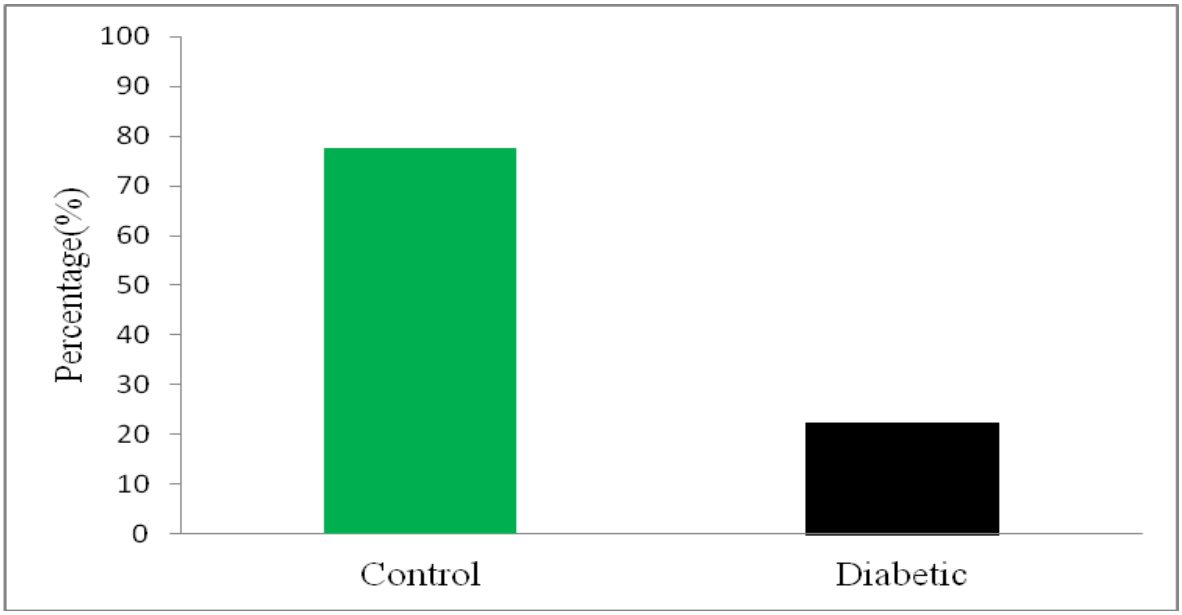


Figure 3. Prevalence of diabetes in total sample of studied population in Bharuch District

Table 8: Age group & gender wise distribution of Diabetics among the population of Bharuch District

Age Groups (Years)	Male N (%)	Female N (%)	Total N (%)
15 to 24	1, (2.5%)	0, (0%)	1, (1.01%)
25 to 34	6, (15%)	4, (6.77%)	10, (10.1%)
35 to 44	7, (17.5%)	5, (8.47%)	12, (12.12%)
45 to 54	10, (25%)	15, (25.42%)	25, (25.25%)
55 to 64	8, (20%)	23, (38.98%)	31, (31.31%)
64 to 75	6, (15%)	6, (10.16%)	12, (12.12%)
>75	2, (5%)	5, (8.47%)	7, (7.07%)

Table 9: Age group & gender wise distribution of Non-Diabetics among the population of Bharuch District

Age Groups (Years)	Male N (%)	Female N (%)	Total N (%)
15 to 24	29, (29%)	10, (6.62%)	39, (15.53%)
25 to 34	23, (23%)	29, (19.2%)	52, (20.71%)
35 to 44	14, (14%)	28, (18.54%)	42, (16.73%)
45 to 54	12, (12%)	35, (23.17%)	47, (18.72%)
55 to 64	14, (14%)	29, (19.2%)	43, (17.13%)
64 to 75	8, (8%)	14, (9.27%)	22, (8.76%)
>75	5, (5%)	6, (3.97%)	11, (4.38%)

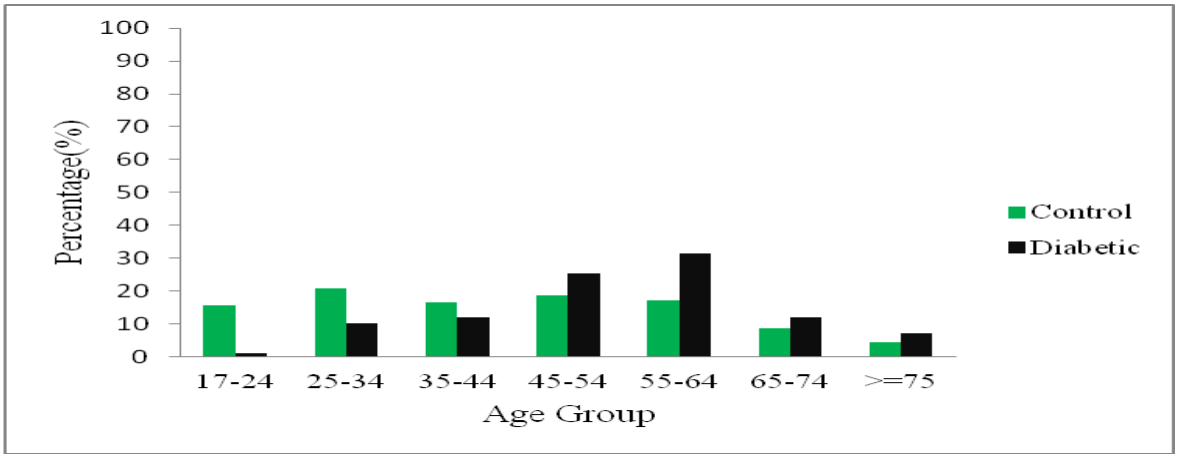


Figure 4: Age group wise distribution of prevalence of diabetes in Bharuch District

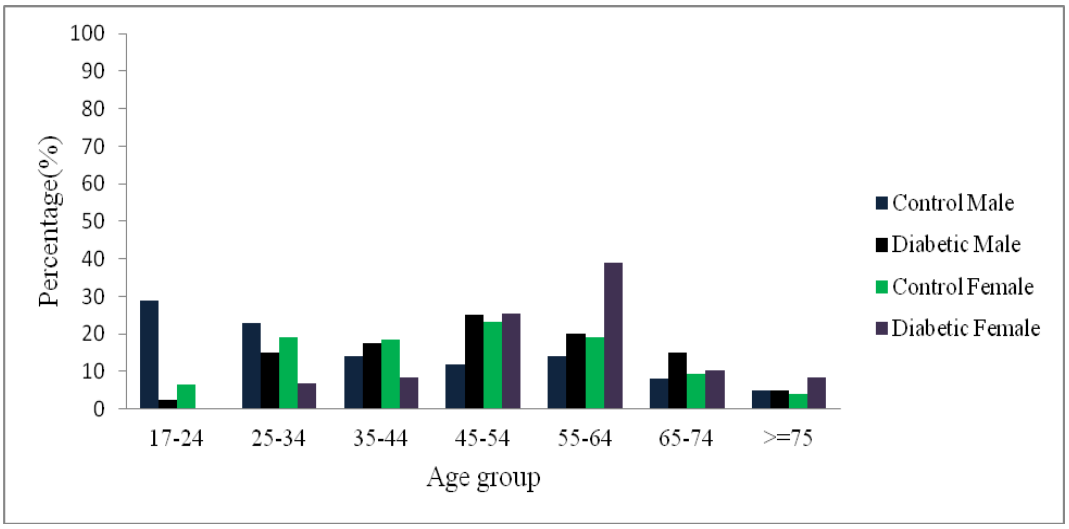


Figure 5: Gender specific distribution of prevalence of diabetes in Bharuch District

Table 10: Sociographic risk factors associated with Diabetes in Bharuch District

Risk Factor	Male N (%)	Female N (%)	Total N (%)
Marital Status			
Married	37, (92.5%)	48, (81.35%)	85, (85.85%)
Single	3, (7.5%)	11, (18.64%)	14, (14.14%)
Occupation			
Labour	2, (5%)	2, (3.38%)	4, (4.04%)
Business	19, (47.5%)	4, (6.77%)	23, (23.23%)
Service	9, (22.5%)	2, (3.38%)	11, (11.11%)
Student	1, (2.5%)	0, (0%)	1, (1.01%)
Housewife		51, (86.44%)	51, (51.51%)
Farmer	4, (10%)	0, (0%)	4, (4.04%)
Retired	5, (12.5%)	0, (0%)	5, (5.05%)

Table 11: Sociographic risk factors associated with Diabetes in Bharuch District

Social Factor	Male N (%)	Female N (%)	Total N (%)
Marital Status			
Married	55, (55%)	129, (85.43%)	184, (73.30%)
Single	32, (32%)	22, (14.56%)	54, (21.51%)
Occupation			
Labor	33, (33%)	3, (1.98%)	36, (14.34%)
Business	20, (20%)	6, (3.97%)	26, (10.35%)
Service	12, (12%)	12(7.94%)	24, (9.56%)
Student	2, (2%)	3(1.98%)	5, (1.99%)
Housewife	0, (0%)	17(11.25%)	17, (6.77%)
Farmer	8, (8%)	109(72.18%)	109, (43.42%)
Retired	5, (5%)	0, (0%)	5, (1.99%)

Table 12: Prevalence of food habit, addiction & migration in Diabetic population of Bharuch District

Addiction/ Food habit	Male N (%)	Female N (%)	Total N (%)
Smoking	0, (0%)	0, (0%)	0, (0%)
Alcohol	3, (4.41%)	0, (0%)	3, (3.33%)
Smoking	17, (25%)	1, (4.54%)	2, (2.22%)
Tobacco	20, (29.41%)	2, (9.09%)	22, (24.44%)
Diet			
Veg	19, (47.5%)	35, (59.32%)	54, (54.54%)
Non veg	19, (47.5%)	24, (40.67%)	43, (43.43%)
Wheat	40, (100%)	59, (100%)	99, (100%)
Rices	33, (82.5%)	57, (96.16%)	90, (90.9%)
Milk	24, (60%)	24, (40.67%)	48, (48.48%)
Migration			
Native	37, (92.5%)	55, (93.22%)	92, (92.92%)
Immigrant	0, (0%)	2, (3.38%)	2, (2.02%)
Physical Exercise			
Walking	4, (10%)	7, (11.86%)	11, (11.11%)
Jogging	2, (5%)	1, (1.69%)	3, (3.03%)
Meditation	2, (5%)	1, (1.69%)	3, (3.03%)

Table 10, 11, 12 shows the association of various sociodemographic factors with presence of diabetes. From studied population of diabetic patients, 85.85% are married and 14.14% are single. Maximum diabetics are Housewives (51.51%) from total studied diabetic population. Among diabetic population 54.54% patients were vegetarian and 43.43% patients were non vegetarian. In Bharuch, 11.11%, 3.03%, 3.03% of diabetic patients were following walking, jogging and meditation respectively. Also, among the diabetic population, 92.92% were native of Gujarat whereas, 2.02% were Immigrants thus indication of a genetic predisposition of a Gujarat.

Table 13: Anthropometric measurement of Diabetic subjects of Bharuch Distinct

Anthrop-ometric Factor	Male N (%)	Male Mean ± SD	Female N (%)	Female Mean ± SD	Total N (%)	Total Mean ± SD
BMI						
<18.5	3 (7.5%)	17.26 ± 1.30	8 (13.55%)	17.07 ± 1.42	11 (11.11%)	17.15 ± .26
18.5to 25	17 (42.5%)	22.79 ± 2.42	24 (40.67%)	22.29 ± 2.86	41 (41.41%)	22.47 ± 2.69
25.1to30	13 (32.5%)	26.56 ± 2.28	21 (35.59%)	27.39 ± 1.93	34 (34.34%)	27.02 ± 2.09
>30	7 (17.5%)	33.92 ± 3.34	6 (10.16%)	32.91 ± 1.91	13 (13.13%)	33.46 ± 2.72
Body Fat						
Low	0 (0%)		1 (1.69%)	18± 0	1 (1.01%)	18 ± 0
Normal	4 (10%)	16.07 ± 2.97	9 (15.25%)	27.83 ± 1.49	13 (13.13%)	23.91 ± 6.11
High	2 (5%)	29.2 ± 5.93	13 (22.03%)	33.45 ± 1.75	15 (15.15%)	32.60 ± 3.36
Very high	34 (85%)	30.88 ± 4.14	36 (61.01%)	39.73 ± 3.81	70 (70.70%)	35.30 ± 5.95
Visceral Fat						
Normal	15 (37.5%)	5.75 ± 3.27	40 (67.79%)	5.02 ± 2.47	55 (55.55%)	5.17 ± 2.63

High	10 (25%)	10.57 ± 2.92	13 (22.03)	14.54 ± 8.81	23 (23.23%)	12.39 ± 6.50
Very High	15 (37.5%)	18.83 ± 5.64	6 (10.16%)	18 ± 1.90	21 (21.21%)	18.62 ± 4.94
Weight						
41 – 50	4 (10%)	42.16 ± 6.22	10 (16.94%)	48.06 ± 8.82	14 (14.14%)	46.32 ± 8.42
51 – 60	8 (20%)	59.87 ± 9.60	17 (28.81%)	56.03 ± 3.11	25 (25.25%)	57.31 ± 6.14
61 – 70	8 (20%)	65.6625 ± 1.71	11 (18.64%)	65.83 ± 3.67	19 (19.19%)	65.76 ± 2.98
71 – 80	13 (32.5%)	75.20 ± 2.68	14 (23.72%)	74.83±2.7	27 (27.27%)	75.02 ± 2.58
81 – 90	5 (12.5%)	86.75 ± 1.25	3 (5.08%)	86.6 ± 4.04	8 (8.08%)	86.67 ± 2.77
91 – 100	0 (0%)		0 (0%)		0 (0%)	
>100	2 (5%)		0 (0%)		2 (2.02%)	117.5 ± 19.09

Height						
122 - 139	0 (0%)		1 (1.69%)	135 ± 0	1 (1.01%)	135 ± 0
140 - 154	3 (7.5%)	158.71 ± 6.70	12(20.33%)	145.88 ± 6.25	15(15.15%)	151.5 ± 9.05
155 - 170	30 (75%)	164.18 ± 3.83	43(72.88%)	159.15 ± 4.13	73(73.73%)	161.04 ± 4.68
171 - 185	6 (15%)	174.33 ± 4.22	3(5.08%)	171.33 ± 0.57	9(9.09%)	173.33 ± 3.67
186 - 200	0 (0%)		0(0%)	190 ± 0	0(0%)	190 ± 0
201 - 213	0 (0%)		0(0%)		0(0%)	
Blood Pressure						
Systolic		145.12 ± 18.63		145.39 ± 23.35		145.28 ± 21.45
Diastolic		90.3 ± 11.04		88.60 ± 13.82		89.29 ± 12.73
RBS	40 (100%)	227.5 ± 65.67	59 (100%)	243.08 ± 83.48	99 (100%)	236.72 ± 76.74

Anthropometric parameters among diabetic patients of Bharuch district were evaluated. Maximum no. of diabetic patients were found with, 41.41% of BMI with average and standard deviation 22.47059 ± 2.699577, 70.70% of Body fat 35.30 ± 5.95 and 55.55% of normal visceral fat.

Table 14: Anthropometric measurement of Non Diabetic subjects of Bharuch District

Anthropometric Factor	Male N (%)	Male Mean ± SD	Female N (%)	Female Mean ± SD	Total N (%)	Total Mean ± SD
BMI						
<18.5	14(14%)	17.21 ± 1.53	11(7.28%)	17.30 ± 1.55	25(9.96%)	17.26 ± 1.52
18.5to 25	56(56%)	21.53 ± 1.94	71(47.01%)	22.43 ± 1.85	127(50.59%)	22.02 ± 1.94
25.1to30	24(24%)	26.33 ± 1.23	48(31.7%)	27.02 ± 1.94	72(28.68%)	26.81 ± 1.77
>30	6(6%)	31.58 ± 0.91	21(13.90%)	32.55 ± 2.32	27(10.75%)	32.32 ± 2.10
Body Fat						
Low	4(4%)	8.75 ± 0.5	1(0.66%)		5(1.99%)	8.75 ± 0.5
Normal	24(24%)	15.15 ± 2.68	17(11.25%)	24.66 ± 3.39	41(16.33%)	18.32 ± 5.39
High	21(21%)	22.78 ± 1.44	42(27.81%)	32.35 ± 1.63	63(25.09%)	29.11 ± 4.82
Very high	51(51%)	31.28 ± 3.96	91(60.26%)	38.89 ± 3.95	142(56.57%)	36.39 ± 5.33
Visceral Fat						
Normal	65(65%)	4.66 ± 2.45	105(69.53%)	5.31 ± 2.51	170(67.72%)	5.07 ± 2.50
High	25(25%)	11.51 ± 3.61	30(19.86%)	11.74 ± 1.382	55(21.91%)	11.63 ± 2.69
Very High	10(10%)	19 ± 3.35	16(10.59%)	18.25 ± 3.67	26(10.35%)	18.5 ± 3.50

Weight						
41 - 50	23(23%)	48.04± 5.51	21(13.9%)	37.33± 1.52	44(44.44%)	45.59 ± 5.21
51 -60	30(30%)	54.80± 2.94	48(31.78%)	43.33± 3.76	78(78.78%)	55.23± 2.98
61 -70	25(25%)	65.57± 2.91	35(23.17%)	55.5± 2.99	60(60.6%)	65.42 ± 2.88
71 - 80	11(11%)	76.3 ± 3.21	29(19.20%)	65.30± 2.90	40(40.4%)	74.93 ± 2.90
81 – 90	2(2%)	81± 0	9(5.96%)	74.23 ± 2.51	11(11.11%)	84.03 ± 3.05
91 – 100	1(1%)		0(0%)	84.7 ± 2.98	1(1.01%)	92 ± 0
>100	14(14%)		2(1.32%)		16(16.16%)	118 ± 0
Height						
122 - 139	3(3%)		2(1.32%)	138 ± 2.64	5(1.99%)	138 ± 2.64
140 - 154	22(22%)	148.1 ± 3.31	37(24.5%)	150.34 ± 4.82	59(23.5%)	149.98 ± 4.67
155 - 170	59(59%)	162.48 ± 4.53	108(71.52%)	159.81 ± 3.41	167(66.53%)	160.94 ± 4.13
171 - 185	16(16%)	174.55 ± 6.57	3(1.98%)	178 ± 2.82	19(7.56%)	174.86 ± 6.36
186 - 200	0(0%)		0(0%)	172 ± 0	0(0%)	172± 0
201 - 213	0(0%)		0(0%)		0(0%)	

Blood Pressure						
Systolic		132.61 ± 18.42		131.40 ± 19.38		131.88 ± 18.93
Diastolic		83.27 ± 13.69		83.97386 ± 11.04		83.69 ± 12.13
RBS	100(100 %)	109.17 ± 14.44	151(100%)	112.65 ± 14.17	251(100%)	111.27 ± 14.35

In the sample studied from Bharuch, maximum no. of diabetics is founded under the normal range of BMI (41.41%) with average BMI 22.47%. Obesity in Type II diabetic patients is a very common phenomenon and often termed as Diabesity (Pandya and Patel, 2011). In current investigation for prevalence of diabetes in Bharuch, maximum number of diabetic patients are found with very high body fat (70.70%). Similarly, maximum no. of diabetic patients are found with normal visceral fat (55.55%).

Table 15: Prevalence of diabetes complications associated in diabetic population of Bharuch District

Complications	**Total Diabetics N (%)**	**Diabetic Males N (%)**	**Diabetic Females N (%)**
Blood pressure	76, (76.76%)	31, (77.5%)	45, (76.27%)
Neuropathy	35, (35.35%)	6, (15%)	29, (49.15%)
Hypoglycemia	2, (2.02%)	0, (0%)	2, (3.38%)
Cardiomyopathy	4, (4.04%)	0, (0%)	4, (6.77%)
Retinopathy	19, (19.19%)	7, (17.5%)	12, (20.33%)
Nephropathy	10, (10.1%)	4, (10%)	6, (10.16%)
Obesity	11, (11.11%)	1, (2.5%)	10, (16.94%)

From studied diabetic population of Bharuch, maximum (76.76%) diabetic patients were having blood pressure and 35.35% of diabetic patients were having neuropathy and 19.19% diabetic patient having retinopathy.

3.1.2 Prevalence of Diabetes in Narmada

Table 16: Prevalence of Diabetes among the population of Narmada District

Population	Male N (%)	Female N (%)	Total N (%)
Diabetic	61, (34.66%)	65, (29.01%)	126, (31.5%)
Non-Diabetic	115, (65.34%)	159, (70.98%)	274, (68.5%)

In demographic survey study of Narmada, 31.5% of people have found to be diabetic. A total of 400 individuals falling in four age groups viz. 15-24, 25-34, 35-44, 45-54, 55-64, 65-74 and >75. The maximum diabetic patients (28.57%) are found in age group of 45-54. The present study shows highest no. of females (32.3%) are found to be diabetic in age group of 45-54 whereas highest no. of males (29.52%) are found to be diabetic in age group of 55-64.

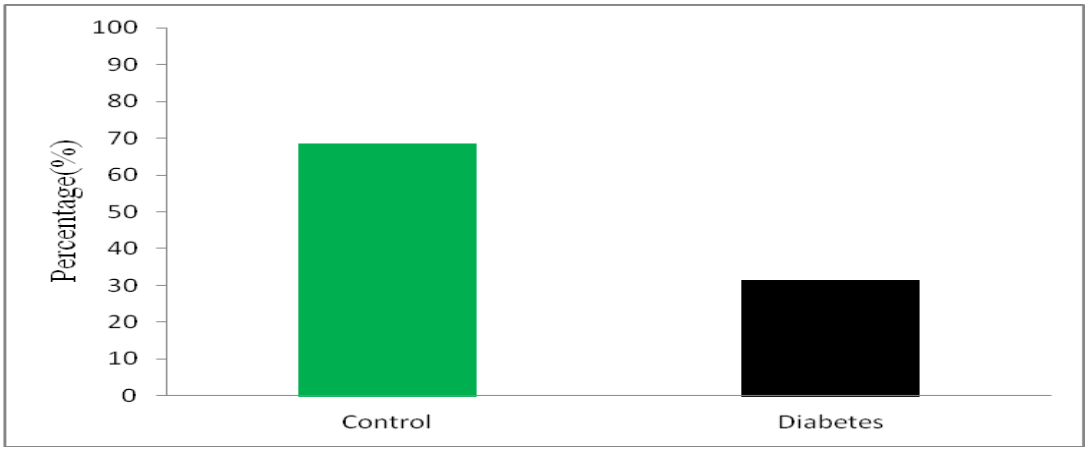


Figure 6. Prevalence of Diabetes in total samples of studied population in Narmada District

Table 17: Age group & gender wise distribution of Diabetics among the population of Narmada District

Age Groups (Years)	Male N (%)	Female N (%)	Total N (%)
15 to 24	3, (4.92%)	0, (0%)	3, (2.38%)
25 to 34	2, (3.28%)	2, (3.07%)	4, (3.17%)
35 to 44	11, (18.03%)	11, (16.92%)	22, (17.46%)
45 to 54	15, (24.03%)	21, (32.3%)	36, (28.57%)
55 to 64	18, (29.52%)	17, (26.15%)	35, (27.78%)
64 to 75	6, (9.84%)	10, (15.38%)	16, (12.69%)
>75	6, (9.84%)	4, (6.15%)	10, (7.94%)

Table 18: Age group & gender wise distribution of Non-Diabetics among the population of Narmada District

Age Groups (Years)	Male N (%)	Female N (%)	Total N (%)
15 to 24	15, (13.04%)	16, (10.06%)	31, (16%)
25 to 34	20, (17.39%)	24, (15.09%)	44, (11.27%)
35 to 44	27, (23.48%)	38, (23.89%)	65, (25.66%)
45 to 54	19, (16.12%)	36, (22.64%)	55, (20%)
55 to 64	16, (13.91%)	22, (13%)	38, (13.4%)
64 to 75	16, (13.91%)	15, (9.43%)	31, (11.27%)
>75	8, (6.96%)	8, (5.03%)	16, (5.03%)

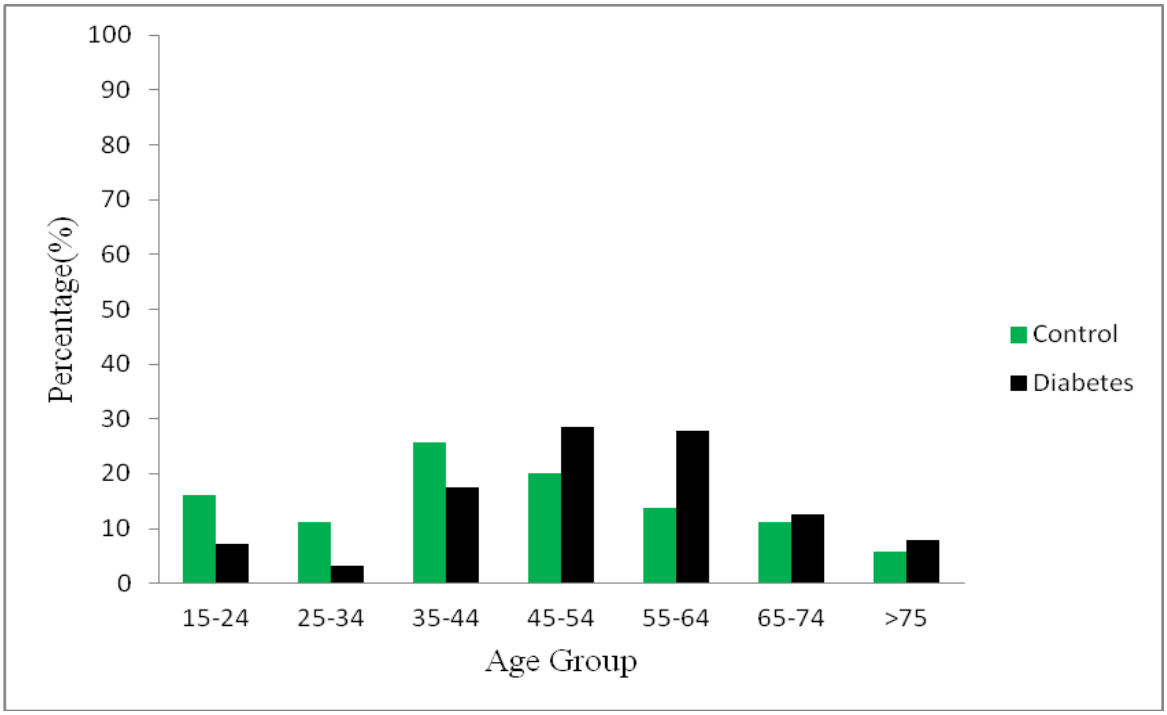


Fig. 7 Age group wise distribution of total control and Diabetic population

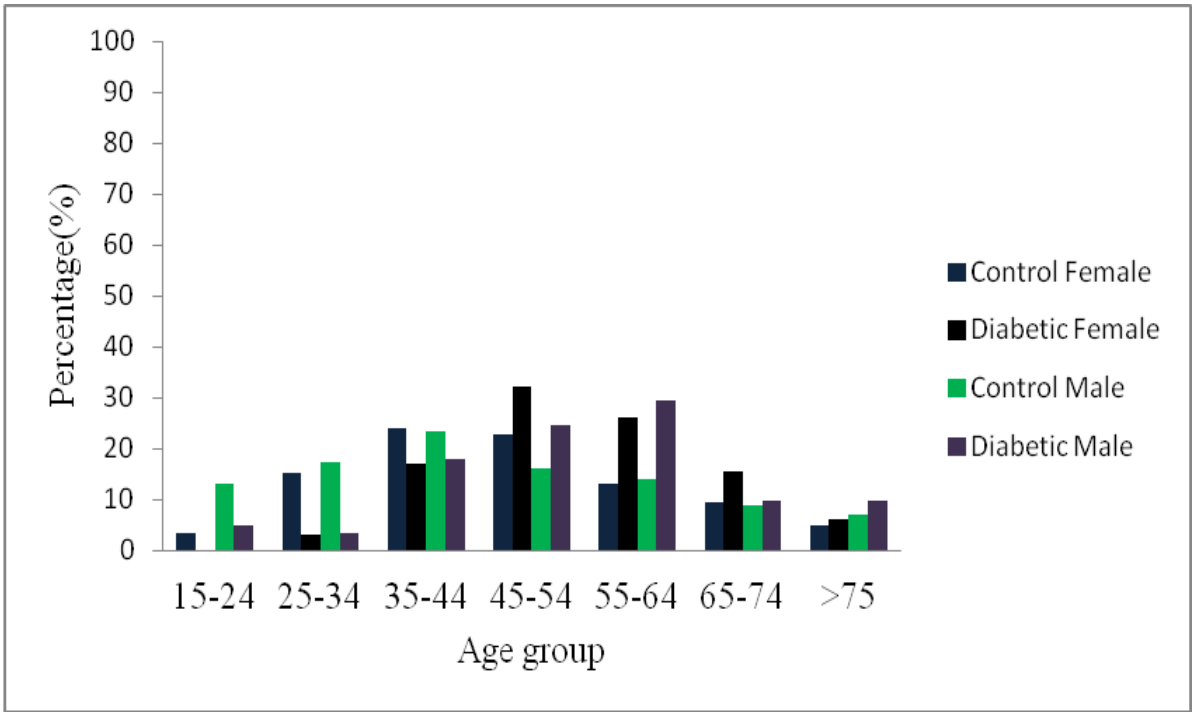


Fig. 8 Age wise and Gender specific distribution of Control and Diabetic patients

Table 19: Sociographic risk factors associated with Diabetes in studied Diabetic population of Narmada District

Risk Factor	Male N (%)	Female N (%)	Total N (%)
Marital Status			
Married	53, (86.88%)	59, (90.76%)	112, (88.89%)
Single	8, (13.11%)	6, (9.23%)	14, (11.1%)
Occupation			
Labor	3, (4.92%)	1, (1.54%)	4, (3.17%)
Business	1, (1.64%)	0, (0%)	1, (0.79%)
Service	6, (9.84%)	1, (1.54%)	7, (5.56%)
Student	3, (4.92%)	0, (0%)	3, (2.38%)
Housewife	0, (0%)	59, (90.77%)	59, (26.82%)
Farmer	30, (49.18%)	2, (3.07%)	32, (25.39%)
Retired	18, (29.51%)	2, (3.07%)	20, (15.87%)

Table 20: Sociographic risk factors associated with Diabetes in studied Non-Diabetic population

Social Factor	Male N (%)	Female N (%)	Total N (%)
Marital Status			
Married	96, (83.47%)	122, (76.72%)	218, (79.56%)
Single	19, (16.52)	37, (23.27%)	56, (20.43%)
Occupation			
Labor	13, (11.3%)	13, (8.17%)	26, (9.49%)
Business	9, (7.83%)	0, (0%)	9, (3.28%)
Service	19, (16.52%)	1, (0.62%)	20, (7.29%)
Student	11, (9.57%)	5, (3.14%)	16, (5.47%)
Housewife	0, (0%)	131, (82.38%)	131, (47.81%)
Farmer	44, (38.26%)	8, (5.03%)	52, (18.98%)
Retired	19, (16.52%)	1, (0.62%)	20, (7.29%)

Table 21: Factors associated with Diabetes in studied Diabetic population

Factor	Male N (%)	Female N (%)	Total N (%)
Smoking			
Alcohol	6, (9.83%)	0, (0%)	6, (4.76%)
Smoking	1, (1.64%)	0, (0%)	1, (0.79%)
Tobacco	1, (1.64%)	0, (0%)	1, (0.79%)
Diet			
Veg	16, (26.23%)	62, (95.38%)	78, (61.90%)
Non veg	45, (73.77%)	3, (4.62%)	48, (38.09%)
Wheat	61, (100%)	64, (98.46%)	125, (99.20%)
Rices	41, (67.21%)	60, (92.30%)	101, (80.16%)
Pulses	41, (67.21%)	62, (95.38%)	103, (81.74%)
Milk	50, (81.97%)	46, (70.77%)	96, (81.74%)
vegetables	61, (100%)	64, (98.46%)	125, (99.20%)
Migration			
Native	61, (100%)	62, (95.38%)	123, (97.62%)
Imigrant	0, (0%)	3, (4.61%)	3, (2.38%)
Physical Exercise			
Walking	10, (16.39%)	25, (38.46%)	35, (27.77%)
Jogging	0, (0%0	0, (0%)	0, (0%)
Meditation	1, (1.64%)	4, (6.15%)	5, (3.96%)

Table 19, 20, 21 shows the association of various socio demographic factors with presence of diabetes. From studied population of diabetic patients, 88.89% are married and 11.1% are single. Maximum diabetics are Housewives (26.82%) from total studied diabetic population. Among diabetic population 61.9% patients were vegetarian and 38.09% patients were non vegetarian. In Narmada, 27.77%, 0%, 3.96% of diabetic patients were following walking, jogging and meditation respectively. Also, among the diabetic population, 97.62% were native of Gujarat whereas, 2.38% were Immigrants thus indication of a genetic predisposition of a Gujarat.

Table 22: Anthropometric measurement of study group of Diabetic subjects of Narmada population

Anthropometric Factor	Male N (%)	Male Mean ± SD	Female N (%)	Female Mean ± SD	Total N (%)	Total Mean ± SD
BMI						
<18.5	0, (0%)	0	2, (3.22%)	0	2, (1.58%)	0
18.5to 25	25, (40.98%)	22.96 ± 1.56	25 (40.32%)	22.26 ± 1.89	50, (39.68%)	22.52 ± 1.79
25.1to30	29, (47.54%)	27.73 ± 1.45	22, (35.48%)	26.71 ± 1.39	51, (40.47%)	27.17 ± 1.49
>30	7, (11.47%)	33.11 ± 1.78	13, (20.96%)	32.82 ± 2.87	20, (15.87%)	32.92 ± 2.51
Body Fat						
Low	0, (0%)	0	1, (1.61%)	0	1, (0.79%)	-
Normal	3, (4.91%)	0	1, (1.61%)	0	4, (3.17%)	-
High	8, (13.11%)	23.76 ± 0.90	14, (22.58%)	32.75 ± 1.60	22, (17.46%)	-
Very high	50, (81.96%)	33.08 ± 3.86	46, (74.19%)	38.95 ± 3.53	96, (76.19%)	-
Visceral Fat						
Low	0, (0%)	0	0(0%)	0	0(0%)	0
Normal	14, (22.%)	6.75 ± 1.63	43, (69.%)	4.75 ± 2.29	57, (45.23%)	6.22 ± 1.91
High	22, (36.06%)	12.3 ± 1.53	10, (16.12%)	11.10 ± 1.68	32, (25.39%)	11.48 ± 1.67
Very High	25, (40.98%)	18.67 ± 3.13	9, (14.51%)	18.71 ± 2.57	34, (26.98%)	19.20 ± 3.54
Weight	61, (48.41%)	72.10 ± 15.83	65, (51.58%)	59.01 ± 7.65	126, (31.5%)	69.43 ± 15.42
Height	61, (48.41%)	164.76 ± 5.09	65, (51.58%)	157.89 ± 3.41	126, (31.5%)	160.65±5.35

Blood Pressure	**Sys**	61, (48.41%)	147.96 ± 18.96	65, (51.58%)	143.26 ± 22.98	126, (31.5%)	144.93±21.62
	Dia	61, (48.41%)	90.89 ± 10.21	65, (51.58%)	89.53±10.63	126, (31.5%)	90.02±10.45
RBS		61, (48.41%)	110.02±5.33	65, (51.585)	190.19±69.02	126, (31.55)	191.30±10.45

Anthropometric parameters among diabetic patients of Narmada district were evaluated. Maximum no. of diabetic patients were found with, 40.47% of BMI with average and standard deviation 27.17 ± 1.49, 76.19% of Body fat and 45.23% of normal visceral fat.

Table 23: Anthropometric measurement of study group of Non Diabetic subjects of Narmada population

Anthropometric Factor	**Male N (%)**	**Male Mean ± SD**	**Female N (%)**	**Female Mean ± SD**	**Total N (%)**	**Total Mean ± SD**
BMI						
<18.5	11, (9.57%)	0	25, (16.02%)	16.12±1.58	36, (13.28%)	16.62±1.54
18.5to 25	61, (53.04%)	22.96±1.56	88, (56.41%)	21.49±2.34	149, (53.87%)	21.81±2.16
25.1to30	36, (31.3%)	27.73±1.45	34, (21.38%)	26.79±1.27	70, (25.83%)	26.91±1.31
>30	7, (6.09%)	33.11±1.78	9, (5.76%)	32.26±1.69	16, (5.9%)	32.45±1.90
Body Fat						
Low	0, (0%)	0, (0%)	6, (3.84%)	17.53±2.51	6, (2.21%)	-
Normal	17, (14.78%)	14.59±2.86	39, (25%)	26.49±2.74	56, (20.15%)	-
High	20, (17.39%)	23.14±1.85	51, (32.69%)	32.64±1.52	71, (26.19%)	-

Very high		78, (67.82%)	31.17±4.53	60, (38.46%)	38.53±2.42	138, (50.92%)	-
Visceral Fat							
Low		0, (0%)	0, (0%)	9, (5.76%)	0, (0%)	9, (3.32%)	0, (0%)
Normal		59, (33.91%)	5.46±2.54	120, (76.12%)	6.14±1.96	179, (29.15%)	5.00±2.40
High		40, (34.78%)	11.82±1.69	19, (12.17%)	11±1.61	59(21.77%)	11.59±1.71
Very High		16, (13.91%)	18.77±2.91	8, (5.12%)	20.18±4.53	24(8.85%)	18.76±2.77
Weight		115, (65.43%)	65.27±11.58	159, (70.98%)	57.18±12.71	274, (68.5%)	60.98±12.82
Height		115, (65.43%)	165.87±5.66	159, (70.98%)	158.30±3.54	274, (68.5%)	161.72±5.96
Blood Pressure	**Sys**	115, (65.43%)	139.40±20.69	159, (70.98%)	132.64±22.01	274, (68.5%)	135.65±21.66
	Dis	115, (65.43%)	91.69±12.68	159, (70.98%)	85.30±11.63	274, (68.5%)	88.17±12.52
RBS		115, (65.43%)	118.88±21.98	159, (70.98%)	110.83±16.61	274, (68.5%)	114.51±19.63

In the sample studied from Narmada, maximum no. of diabetics are found under the high range of BMI (40.47%). Obesity in Type II diabetic patients is a very common phenomenon and often termed as Diabesity (Pandya and Patel, 2011). In current investigation for prevalence of diabetes in Bharuch, maximum number of diabetic patients are found with very high body fat (76.19%). Similarly, maximum no. of diabetic patients are found with normal visceral fat (45.23%).

Table 24: Prevalence of Complications associated with diabetes

Complications	Total Diabetics N (%)	Diabetic Males N (%)	Diabetic Females N (%)
Blood pressure	98, (76.19%)	48, (78.69%)	48, (73.84%)
Neuropathy	55, (43.65%)	19, (31.15%)	36, (55.38%)
Hypoglycemia	8, (6.35%)	2, (3.28%)	6, (9.23%)
Cardiomyopathy	8, (6.35%)	2, (3.28%)	6, (9.23%)
Retinopathy	21, (16.67%)	10, (16.39%)	11, (16.92%)
Nephropathy	41, (35.54%)	8, (13.11%)	33, (50.76%)
Obesity	4, (10.32%)	4, (6.56%)	9, (13.845)

From studied diabetic population of Narmada, maximum (73.84%) diabetic patients were having blood pressure and 55.38% of diabetic patients were having neuropathy and 16.92% diabetic patient having retinopathy.

3.2 Blood Glucose estimation and Body weight measurement

Table 25: Blood Glucose estimation

	5th Day (mg/dl)	10th Day (mg/dl)	15th Day (mg/dl)	20th Day (mg/dl)	25th Day (mg/dl)	30th Day (mg/dl)	35th Day (mg/dl)	40th Day (mg/dl)
C	130±7.4	110.4±6.7	117.4±5.4	121±7.1	134.5±10.05	132.16±8.13	132.6±7.12	117±6.12
D	327.4±28.7 ***	395.5±69.6 ***	579.8±88.1 ***	445.4±20.7 ***	488.4±107.02 ***	566.2±47.36 ***	571.6±25.55 ***	364.6±93.57 ***
D+I	134.7±31.1 @@@	138.5±25.7 @@@	144.7±6.8 @@@	159±4.7 @@@	158 ± 5.6 @@@	163 ± 10.6 @@@	145 ± 11.4 @@@	131 ± 5.1 @@@

Value are mean ± SD separate experiments (n= 6 rats per group)
ANOVA followed by Student's-Newman-Keul's Test.

C=control, D=Diabetes, C+IIH=Insulin induced Control Hypoglycemic Rats, D+IIH=Insulin induced Diabetic Hypoglycemic Rats.

***p<0.001 When compare to C
@@@ p<0.01 When compare to D

A significant increase in blood glucose level of D was observed from 3rd day of model induction with streptozotocin when compared to C. In D+I group there was a significant decrease in blood glucose level when compared to D. Blood glucose level of D group was 200mg/dL-300mg/dL and blood glucose level of D+I group was 80-120 mg/dL.

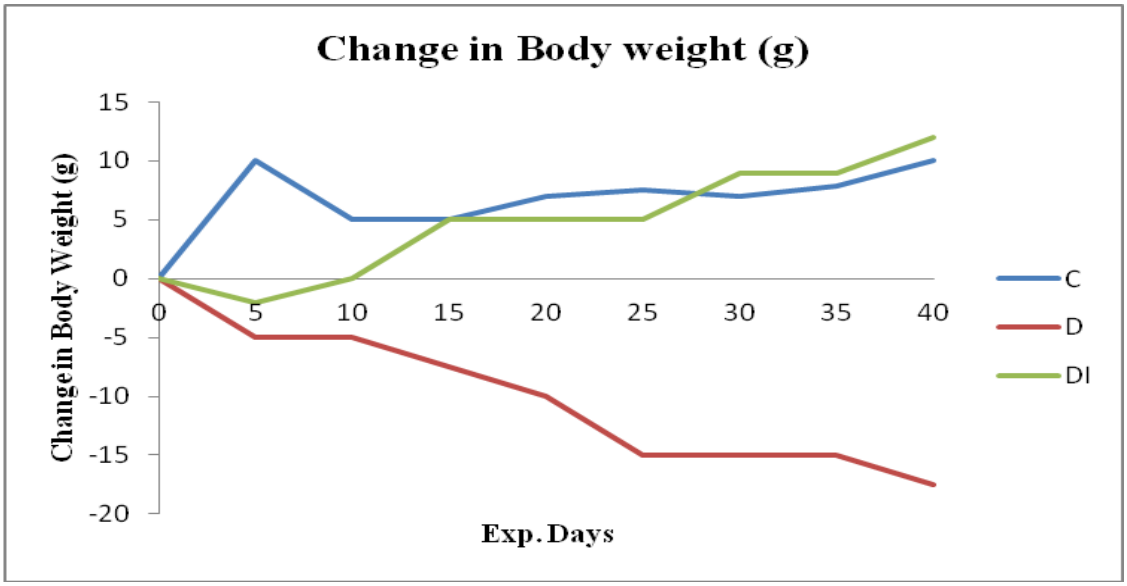


Figure 9. Change in Body weight of control and experimental group of rats

C=control, D=Diabetes, DI=Insulin treated diabetic

A significant decrease in body weight of D was observed when compared to C from 3rd day of experiment corresponding to high blood sugar resulting in polyuria which led to dehydration and loss of body fluids and electrolytes. During diabetes, lack of insulin prevents transporters mediated glucose entry into the cell. Hence initiating alternative mechanism for glucose production in cell via glycogenolysis and gluconeogenesis. Glycogenolysis leads to depletion of glycogen reserves signalling for lipolysis causing this unhealthy weight loss in D group (Wood et al., 2004). Insulin treatment prevents excessive fat metabolism and thus an increase in body weight of D+I group when compared to D from 5th day of experiment was observed. A similar effect of insulin has previously been reported by the studies of Mahmoud et al., (2009) and Akbarzadeh et al., (2007).

3.3 Gene Expression

3.3.1 Agarose Gel Electrophoresis of total RNA

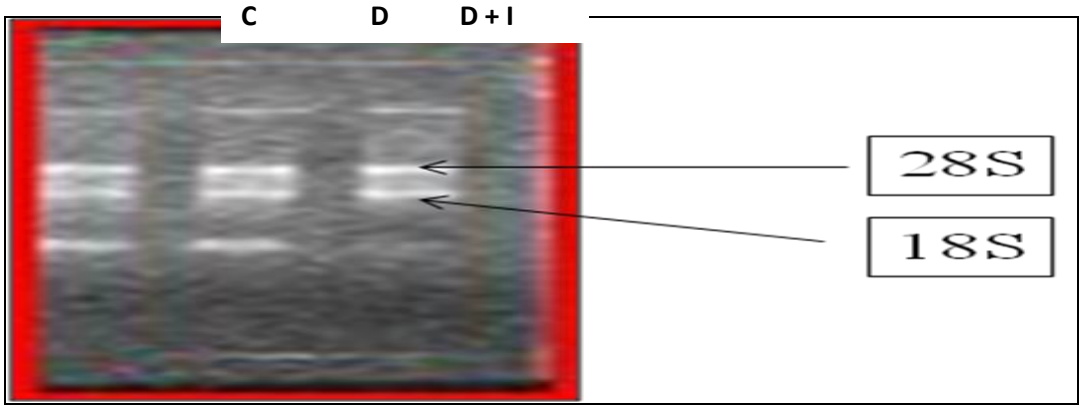


Fig. 10: Agarose gel electrophoresis of Total RNA Isolation from Cerebral Cortex

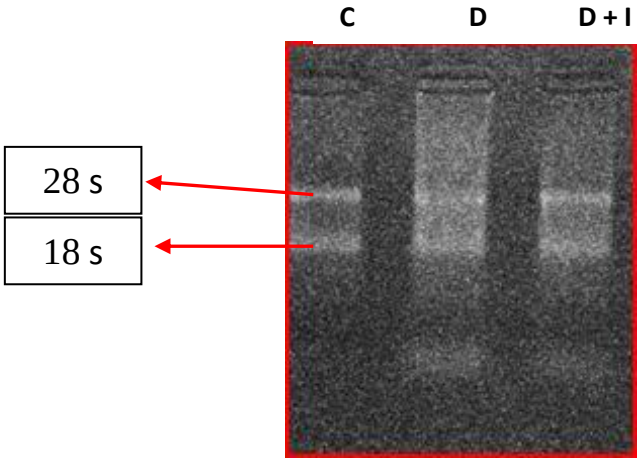
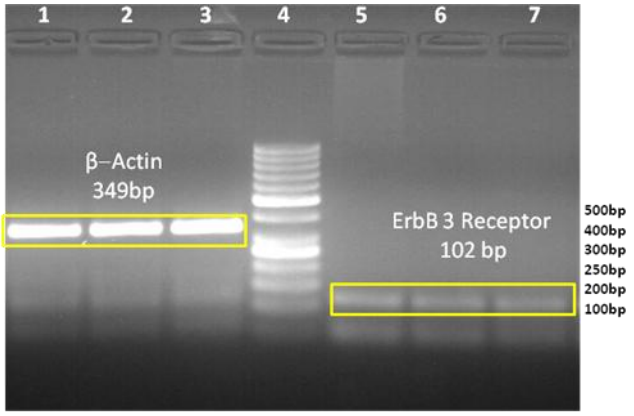


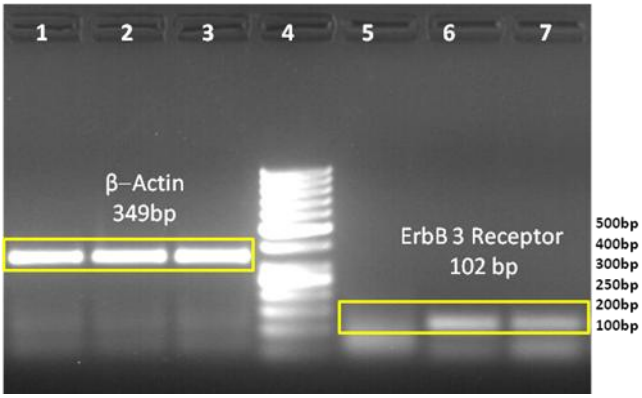
Fig. 11: Agarose gel electrophoresis of Total RNA Isolation from Corpus Striatum

Intact RNA was observed on agarose gel. Two different components of rRNA – 28S, 18S could be resolved in the gel and could be distinguished.



Well No.	Sample
1	Diabetic + Insulin treated, β–Actin
2	Diabetic, β–Actin
3	Control, β–Actin
4	DNA ladder
5	Control, ErbB 3 Receptor
6	Diabetic, ErbB 3 Receptor
7	Diabetic + Insulin treated, mErbB 3 Receptor

Figure 12. PCR analysis of ErBb 3 receptor in CC



Well No.	Sample
1	Diabetic + Insulin treated, β–Actin
2	Diabetic, β–Actin
3	Control, β–Actin
4	DNA ladder
5	Control, ErbB 3 Receptor
6	Diabetic, ErbB 3 Receptor
7	Diabetic + Insulin treated, mErbB 3 Receptor

Figure 13. PCR analysis of ErBb 3 receptor in CS

The gene expression studies of ErbB 3 receptor in CC and CS was one time experiment and needs to be repeated.

4. Summary

The main of this study is to create a diabetic map of South Gujarat. To attain the objective, two districts were selected of South Gujarat: Bharuch and Narmada. The typical risk factors of physical inactivity, unhygienic eating habits, urbanization integrated with inherent genetic attributes and changes in body composition are the reasons for increase in cases of diabetes. Therefore, we've tried to spot the several variables like obesity, adiposity, high body fat percentage, BMI, family history, dietary pattern, and lifestyle in diabetes. The present study states 28.28% prevalence of diabetes in Bharuch district and 31.5% from Narmada district. The utmost prevalence of diabetes has been observed in adults and retired persons in both the districts. The current investigation of shows that 70.70% diabetic patients of Bharuch and 50.92% of diabetic patients of Narmada have very high body fat. In term of visceral fat, maximum no. of diabetic patients from Bharuch (55.55%) and Narmada (45.23%) districts were normal. But, in case of BMI, 41.41% of diabetic patients from Bharuch district have normal BMI and 40.47% of diabetic patients from Narmada district have high BMI. Decreased physical activity leads to obesity which is one of the risk factor of diabetes. Poor control of hyperglycemia for years results in multiple complications like Neuropathy, Cardiomyopathy, Retinopathy, and Nephropathy. Our study shows Neuropathy and Hypertension are main complications associated with diabetic as well as non-diabetic patients which is more than 50% in both the regions. Long lasting neuropathy affects „oligodendrocyte" – a myelin producing counterpart of Schwann cell in brain leading to cognitive dysfunction, can induce an altered mental state, changes in personality, memory lapses, or severe impairment like dementia. Today, no medical intervention exists to promote total neurological recovery after demyelination. Therefore, present investigation aims to evaluate the effect of insulin treatment on ErbB mediated myelination in cerebral cortex and corpus striatum of diabetic rats. Diabetes was induced in rats with streptozotocin and blood glucose and change in body weight of experimental group of rats were recorded regularly throughout the experiment. Diabetic rats with insulin treatment showed a significant decrease in blood glucose and increase in body weight whereas the weight of diabetic rats was decreased continuously from the day of experiment. Gene expression studies show that ErbB 3 receptor amplifies at 102 bp in both the regions i.e; cerebral cortex and corpus striatum.

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