Effect of green tea (*Camellia sinensis*) extract on testicular activity in streptozotocin induced diabetic male rat model

Thesis Submitted to

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In partial fulfillment of the requirements

For the degree of

Master of Science

In Clinical Biochemistry

Submitted by
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Under the Supervision of
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LOVELY PROFESSIONAL UNIVERSITY, PUNJAB, INDIA
2015

DECLARATION

This is to submit that this written submission in my thesis entitled **"Effect of green tea (*Camellia sinensis*) extract on testicular activity in streptozotocin induced diabetic male rat model"** represents original ideas in my own words and where others' ideas and words have been included, I have adequately cited and referenced the original sources. I also declare that I have stuck to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the school and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when required.

This thesis encompasses the information generated by me based on experimental work carried out in the Lovely School of Physiotherapy and Paramedical Sciences, Lovely Professional University, Punjab. I assure and hold full responsibility for its genuineness.

Dated: 05-05-2015   Kamaldeep Singh

Place: Punjab, India   (Reg. No.11312489)

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CERTIFICATE

This is to certify that the present thesis entitled " **Effect of green tea (*Camellia sinensis*) extract on testicular activity in streptozotocin induced diabetic male rat model** " is the outcome of the original piece of work carried out by Mr. Kamaldeep Singh (Registration No: 11312489) himself under my guidance and the contents of his thesis did not form a basis of the award of any previous degree to his and to the best of my knowledge to anybody also. The thesis has not been submitted by the candidate for any research degree in any other University.

The dissertation is fit for submission to the partial fulfillment of the conditions for the award of M.Sc. in Clinical Biochemistry. Further, certified that the candidate in habit and character is a fit and proper person for the award.

(Dr. PranayPunj Pankaj)

Supervisor

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Place ; LPU, Punjab   Kamaldeep
Singh

DEDICATION

- ✓ I dedicate my dissertation work to my family and many friends. A special feeling of gratitude to my loving parents, whose words of encouragement and push for tenacity ring in my ears.

- ✓ I also dedicate this dissertation to my many friends, who have supported me throughout the process.

- ✓ I dedicate this work and give special thanks to my best friend Savita Devi.

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Abstract

Study has been based to estimation the Assessment of green tea (*Camellia sinensis*) extract on testicular activity in streptozotocin induced diabetic male rat model. The aim of study is to assess histo-architecture and biochemical effects of oral administration green tea extracts and streptozotocin induces diabetic male albino rats on reproductive function. Male rats were administered the green tea extract orally for 21 days, while the control group received only water. In this assay, twenty four (24) male albino rats of Wistar strain weight (150-200 g), age 3 months, were obtained from Lovely Professional University. The rats (24) were divided into four groups of 6 each rats according to the design of experiment and treatment. Animals in group (1) are Non-diabetic rats with Normal diet and consider as a normal control. Animals in group (2) Non- diabetic rats were treated with orally green tea extract+ CMC 5% as vehicle200mgkg^{-1} body weight. Animals in group (3) diabetic rats were treated with single dose of Streptozotocin (120mgKg bw) Animals in group (4) 6 rats each were administered with Single dose of Streptozotocin with green tea extract. Treatment design was selected to evaluate histo-architecture and biochemical effects of green tea extracts on reproductive function in male albino rat. Data were analyzed by ANOVA test and T test. At the end of experimental period, animals were sacrificed and their blood and testes samples were collected for the analysis, testes were removed for histopathology. Result of this study showed that green tea having the hypoglycemic and hypotriglycerdima effects. After the 14th day there were seen reduction in body weight of group 2 and group 4 animals. This study also shows that diabetes having effects on male reproductive system. Treated animal having the low level of reproductive hormones.

List of abbreviations

DM	Diabetes Mellitus
T1DM	Type one diabetes mellitus
T2DM	Type two diabetes mellitus
GDM	Gestational Diabetes Mellitus
IDDM	Insulin dependent diabetes mellitus
NIDDM	Non insulin dependent diabetes mellitus
WHO	World health organization
ESRF	End stage renal failure
GFR	Glomerular filtration rate
CNS	Central nervous system
ED	Erectile dysfunction
CVD	Cardiac vascular disorder
MND	Motor neuron dysfunction
FSH	Follicle stimulating hormones
LH	Luteinizing hormones
DNA	Deoxyribonucleic acid
mtDNA	Mitochondrial deoxyribonucleic acid
MSD	Male sexual dysfunction
RE	Retrograde ejaculation
PKA	Protein kinase A
GTE	Green tea extract
GTLE	Green tea leaf extract
AGE	Advanced glycatiom end product
cGMP	Cyclic guanosine monophospate

DIED	Diabetes induced erectile dysfunction
NO	Nitric oxide
EC	Epicatechin
EGC	Epigallocatocatechin
EGCG	Epigallocatocatechin-3-gallate
IP	Intra-peritoneal
^{o}C	Degree Centigrade
Hr	Hours
Mg	Milligrams
G	Gram
Mm	Millimeter
Cm	Centimeter
L	Liter
Ml	Milliliter
OD	Optical Density
Rpm	Revolutions per Minute
UV	Ultra Violet
HPLC Chromatography	High Performance Liquid
µl	Microlitre
C	Catechin
GC	Gallo Catechin

CHAPTER 1

Introduction

1.1 Diabetes mellitus

Diabetes mellitus (DM) is primary or idiopathic disease that deals with the chronic metabolic disorder involving carbohydrate, lipid and protein by occurrence of insulin deficient or disorder. Other conditions like arteriosclerosis (hardening and loss of elasticity of the wall of arteries), nephropathy (kidney damage), neuropathy (peripheral nerves dysfunction) and micro-anginopathy (thickening and weakening of capillary walls) can also devoted through DM (Camilleri, 2008). The characteristics symptoms may present with DM are polyuria, polydipisa, blurring of vision and body weight loss (WHO, 1999). It causes retinopathy, nephropathy and neuropathy. It is also associated with an increased incidence of cardiovascular disease, reduced life expectancy, significant morbidity due to specific diabetes related micro-vascular complications and diminished quality of life (Pickup, 1991).

It has become a principal cause of morbidity and mortality in human populations (Yenigum, 1997). Many other organs also cause DM, the harshness of disease depend on the how long presence of disease inside the body and how much time it took to controlled. DM characterised by acute and chronic hyperglycaemia with disturbance of metabolic disorder caused from fault or improper secretion of insulin and action of insulin or both (Kasiam, 2009; Gohl, 2008; Alebiosu, 2003). In modern societies, the DM is one of the most leading public health threats and in whole world. Its prevalence increased more rapidly day by days. According to WHO (World Health Organization in 2000), 171 million people were recorded with DM which showed that there were 60% elevation comparatively to 1995 (WHO, 2002).

1.2 History of diabetes mellitus

DM has been known to mankind for 10th centuries. Now days the classical diabetes can be diagnose genuinely. Patients under evidence of DM are often subject to screening tests supported by blood (serum and plasma) and urine analysis. In earlier times, diabetes must have been extremely complicating to physicians trying to understand the disease, which manifests with strange symptoms and signs. That all information was before knowledge of glucose

metabolism and the role of insulin was elucidated. Arataeus of Cappadocia is credited with naming the disease in the first century AD on the basis of excessive urination. He coined the name diabetes from the Greek word for 'syphon' ('dia-' through, 'bainein' to go).1 some years earlier Celsus is understood to have reported the same feature. Many centuries ago the key initial discovery of sweet urine in diabetes had been made, India, China and Arabia having been recorded in antique. The term (quaint by today's standards) 'sweet pissing disease' was used in Hindu Sanskrit. Thomas Willis, whose name is associated with the arterial circle at the base of the brain, made the same observation in Oxford in the 17th century (Medvei, 1993). He noted the sweet taste of urine in the most common form of diabetes (mellitus from the Greek for honey) and contrasted that sweetness with the lack of sweetness in the urine of a person with diabetes insipidus. A further step forward in the understanding of the disease was the discovery of sugar as the cause of the sweetness in diabetes by Dobson in the 18th century.1 He also made the key discovery of excess sugar in the blood. An English surgeon, Rollo, correctly noted the smell of acetone on the breath of some diabetics (ketoacidosis) and even attempted treatment with dietary restriction. (Medvei, 1993). In the mid 1800s, Traube made the connection between carbohydrate intake and urinary secretion of sugar, with the corollary that reduction in carbohydrate reduced glucose in the urine. The Islets of Langerhans (discovered by a German medical student) were suspected to be the production site of this antidiabetic substance as this group of cells differed from the rest of the pancreas, in that there were no tubules

Still research and development is continue on long-acting forms of insulin, including continuous infusion pumps and synthetic insulin. However, many diabetics insulin injection was not the required treatment. In patients where pancreatic insulin was deficient, but not totally absent (type 2 or non-insulin dependent diabetes), other methods of controlling hyperglycaemia were needed. In the 1940s, an accidental discovery by Janbon in France indicated that a chemical tested on dogs for typhoid treatment (a sulphonylurea compound) caused a side-effect of hypoglycaemia. (Medvei, 1993). When tested on diabetic patients, it caused a significant drop in blood glucose levels.

The effect was to simulate the secretion of insulin from the Islets, but this was no substitute for insulin when pancreatic production was minimal or non-existent. This fortuitous finding led to the development and refinement of hyperglycaemic agents that could be taken orally. Oral hypoglycaemic medications have become the mainstay of treatment for many with noninsulin dependent diabetes, at least in the early stages.

1.3 Classification of diabetes mellitus
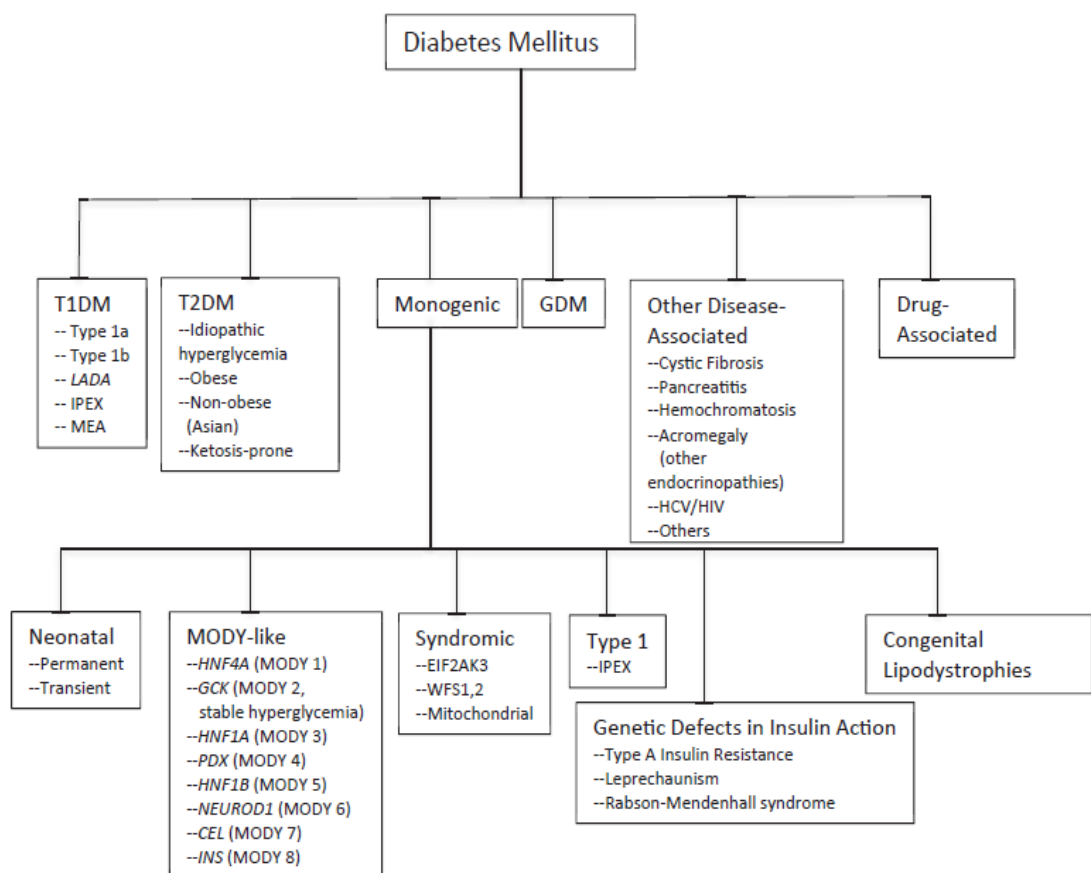
Table no. 1.1 Diabetes Classification.

1.3.1 Type 1 diabetes

Type 1 DM condition deals with the deficiencies production/ secretion of insulin from destruction of beta cells in the Islets of Langerhans (pancreas). Most commonly type 1 DM an immune mediated disorder, although in some occurrence this cannot be confirm. Therefore, some cases or conditions are

considered idiopathic. It is also known as insulin dependent diabetes mellitus (IDDM) and was often thought of as juvenile onset diabetes (Ian, 1999).

1.3.2 Type 2 diabetes

Type 2 DM may result from resistance to insulin action a cellular/receptor, which may be Intensify by deficient compensation in insulin secretion to decreased insulin action. Approximetly 90 per cent of all patients having the type 2 DM and before diagnosis it may be present at an asymptomatic level for long periods. It was also called as non-insulin dependent diabetes mellitus (NIDDM) or adult onset diabetes (Ian, 1999).

1.3.3 Gestational diabetes mellitus

GDM disease related with the development of any glucose intolerance with first recognition during pregnancy or its onset. This intolerance condition subsequently boldness in the great majority of cases after delivery of pregnancy. Approximately 4 per cent of all pregnancies patients having GDM complications in the USA, (Engelgau, 1995) although this may vary in other countries. Women having GD have higher risk of later developing diabetes (Harris, 1988).

1.4 Major areas contributing to diabetic complications

As the diabetes disease process several organs or organ systems are affected. Some effects are acute and direct, such, as diabetic ketoacidosis and hypoglycaemia.
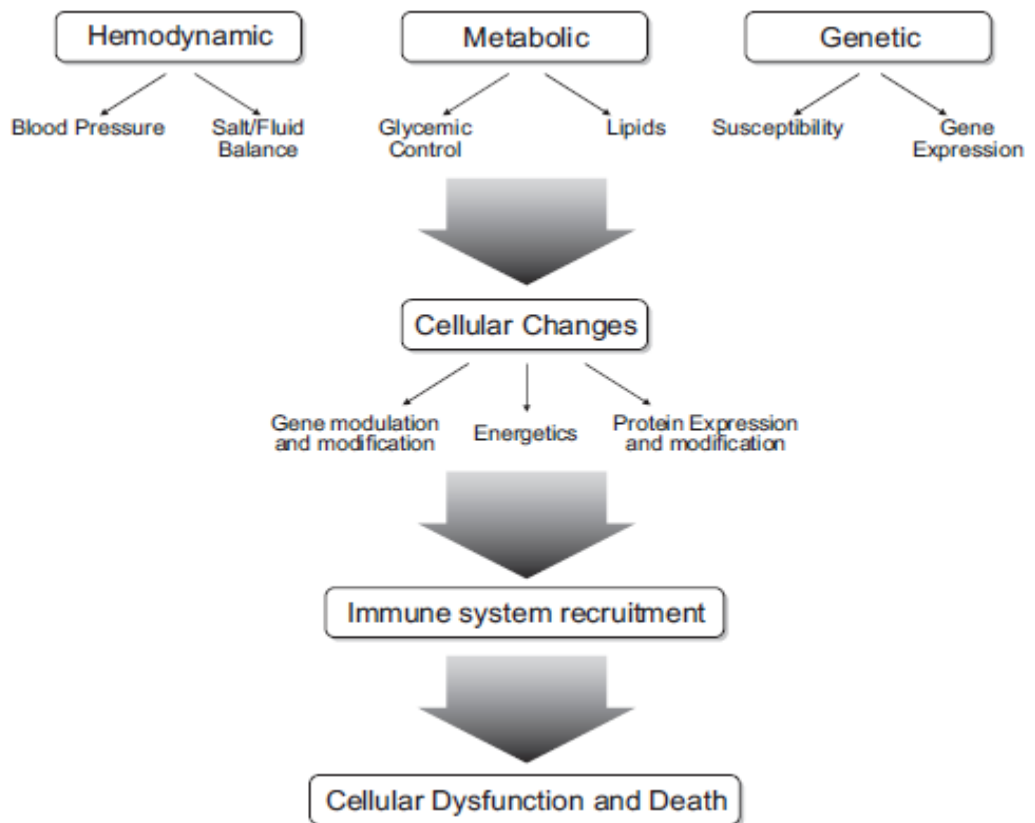
Fig no.1.1 overview of the major areas contributing to diabetic complications.

1.4.1 Nephropathy

Diabetic nephropathy is one of the most responsible causes of ESRF (End Stage Renal Failure). (Gilbertson et al., 2015) ESRF Clinically characterized by the proteinuria which caused by the impaired functioning of GRF (glomerular filtration rate) and impairment function of GFR subsequently decline proteinuria and progression caused from a long duration upto 10-20 years. (Mogensen, 1983). Additionally kidney disease is also a major risk factor for the   macro-vascular complications like Hypertension (UK Prospective Diabetes Study Group, 1998) strokes and heart attacks (Lancet, 2010) and poor glycemic control (UKPDS, 1998) resident kidney cells affects by the high concentration of glucose and damage the cells like endothelial cells, smooth muscles and podocyte and mesangial cells(Amico, 1981).

1.4.2 Retinopathy

Muscles loss or blindness is another major complication of the DM. Retinopathy characterized by a spectrum of lesion in the retina which results blindness (Frank, 2004; Hirai et al., 2011). Blindness results vascular permeability and degradation and neovascularisation (formation of new blood vessels). Due to the necrosis of the cell leads to the dysfunctioining of the neural ratina which alert electrophysiology and loss of ability to discriminate between colour (Frank, 2004).

1.4.3 Neuropathy

DM neuropathy is another major complication of DM (Abbott et al., 2011). D neuropathy envelope autonomic and somatic damage the spinal cord and CNS. (Wessels et al., 2006) Vascular abnormality causes profession of diseases in diabetic neuropathy. Which impaired another ED, wound healing and CVD. These vascular abnormalities can also leads to hyperplasia of endothelial and thinking of capillary basement membrane results oxygen tension and hypoxia. Necrosis is the important part of the brain and size of neuron is affected which leads to motor neuron dysfunction. MND impaired function of autonomic system. These can seen in DM individuals (Josephine et al., 2013).

1.5 Male Sexual Dysfunction (MSD)

A link of DM with sexual dysfunction has been perceived earlier in 10th century. When Avicenna reported that "collapse of sexual function" as a precise problem of DM (Macfarlane et al., 1997) In both men and female the Sexual dysfunctions have been associated from DM. It is supposed that, pathogenesis involve are vascular insufficiency, neuropathy and some psychological problems, that develops some conditions such as impotence or erectile dysfunction(ED), ejaculation disorder (pre-mature or delayed ejaculation) and decreased libido (Zarzycki et al., 2005; Clark, 2004; Berardis et al., 2007).

DM is responsible for biochemical variation and other pathological changes that adapt male fertility. The sexual dysfunction such as spermatogenesis (Daubresse et al., 1978; Baccetti et al., 2002), retrograde ejaculation (Bourne et al., 1971; Fedele, 2005) or erectile dysfunction (Sexton, 1997) occurs and it

end up with decreased sexual appetite in diabetic individuals (Kolodny et al., 1974). DM affect on pathways by multiple molecular mechanisms with dramatic consequences to male reproductive functions. DM decreased the sperm quality and functioning competitively to normal. Alteration in testicular cells is concerned with glucose metabolism. Specific mechanisms hormonal control and glucose sensing machinery may also play critical role in sub fertility and fertility correlated to DM. many other reports also identify that the diabetes mellitus correlate with degradation of hormones particularly sex hormones(steroids hormones) (Stanworth et al., 2011; Maric et al., 2010)

Insulin dependent diabetes mellitus (IDDM) is correlated to decrease the motility and vitality of semen and decreased semen ejaculation without change in viscosity of sperm (Miralles, 2004). Alteration in insulin can change the primary sexual glands functions and testicular functions. Which cause concentrate seminal insulin than that serum insulin (Chandrashekar, 2005).

Microscopic examination of semen analysis that includes semen value, sperm count, motility and its morphology are done to inspect male fertility with sperm nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) quality. These have been given 'proxy' signal of male fertility status (Agarwal, 2005; Agarwal, 2003).

Report on relation between DM and male infertility with relation to plasma levels of testosterone, FSH, LH are still infancy(Ballester et al., 2004). Modern societies concede a deeper look into rate of fertility that shown highly increased frequently of DM have been correlate with falling fertility and birth rate (Hamilton, 2006; Lutz, 2006). Most of the complications due to DM have been studied widely but sexual dysfunction is still incompletely understood (Zarzycki et al., 2005).

1.6 Classification of MSD
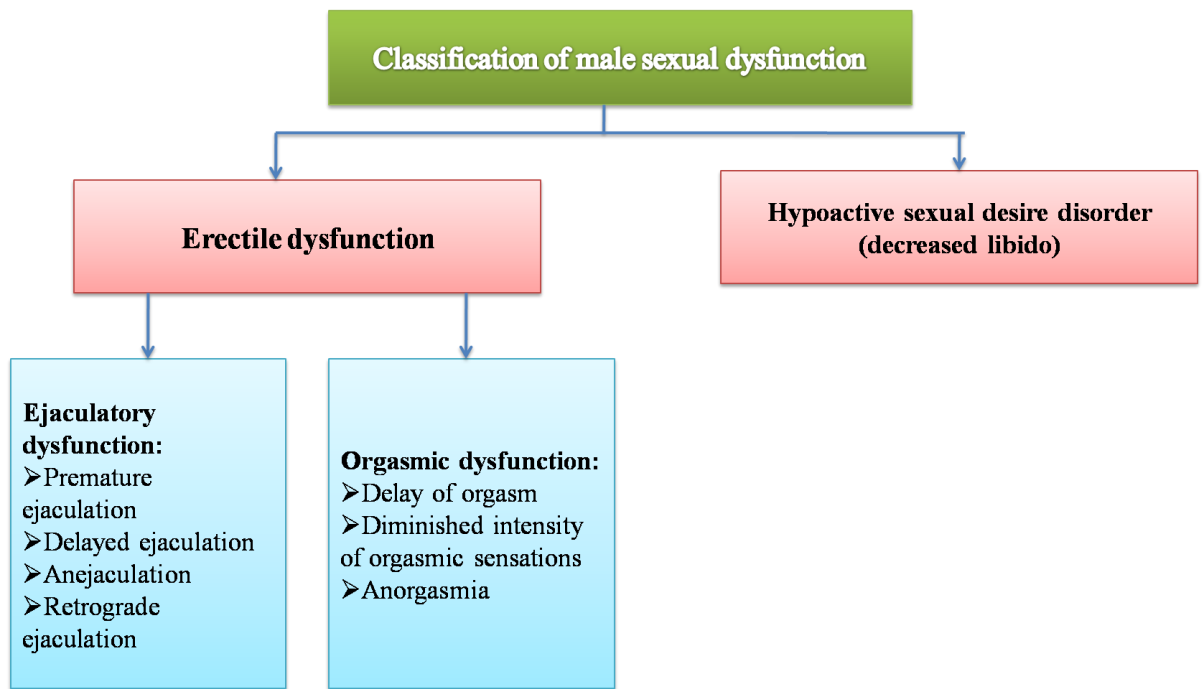
Fig no. 1.2 overview on classification of male sexual dysfunction.

1.7 Prevalence of DM associated MSD

The worldwide frequency of IDDM can be differ from its low range 1-2 per 100,000 in Japan to a more than 40 per 100,00 in Finland country part (Atkinson, 1994). The prediction of date for NIDDM is more difficult due to its heterogeneity in appearance of disease. Approximately 5% to 7% prevalence rate world widely noted (Yki-Jarvinen, 1994).

Greenberg et al., (1978) reported the causes of infertility in 425 successive men aged in between 26-44 identified for sub fertility and only 0.7% (3 men) had IDDM patients. Two of these infertile men had complained for retrograde ejaculation and one of these had sexual dysfunction.

It was hypothesized that DM has very little complication upon male infertility or reproductive system functions. The majority of vast dysfunction (less than 90%) of patient from Europe in the year are diagnosed with DM previously the age of 30 (Williams, 2004).

Mecculloch et al., recorded that prevalence of ED studies indicate that patients who have this disorder and hospitalized are increased with age. Man with age

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of 20-24 years was recorded prevalence of 5.7% and this rose up to 52.4% in aged 55-59 years of men (Klein et al., 1996).

Johannes et al. (2000) estimated ED to occur in more than or equal to 50% of man with DM worldwide. Most probably ED comes within 10 years diagnosis of DM. The percentage of ED was observed in male with DM is to be higher than diabetic male without DM. The 12% of men who having ED, observe that previously undiagnosed from DM.

According to epidemiological studies both type 1 and type 2 diabetes are involved to increased risk of ED(Giuliano et al., 2004). In type 1 DM occurs at younger age in men as compare to normal population. The percentage of insulin resistance is 3 time more prone in men with ED. Men with type 2 DM had highly risk of ED as the increased duration diagnosis since, specifically for those men that was diagnosed more than 20 years previously (Guay, 2007). The prevalence of ED showed much higher in DM as compare with general population counterpart. ED is correlated with poor metabolic control (Roth, 2003) increase in age (Fedele et al., 2000; McCulloch et al., 1980) neurological damage, consumption of alcoholic beverages (Lundberg et al., 2001) time of evolution of DM (Klein et al., 1996) use of some drug (Kleinman et al., 2001) and complication arises are micro and macro vascularpathy and other factor. It is predicted, prevalence rate ranging from 35%-75% of men having DIED based on age and general metabolic state of men patients observation. It is reported that men with diabetes may develop ED within 5-10 years (Romeo et al., 2000). In a cross section study survey by feldiabetesan in 541 men of DM between age 20-59 years more prevalence of ED rate of 35% increasing progressively. The prevalence increscent based on age, the 6% were seen in age group of 20-24% years and 52% were seen in age group of 55-95% years. The 55-95% of men with DM was recorded as affected with ED after the age of 60 years as compared with unplanned population in Massachusetts Aging Male Survey (Feldiabetesan et al., 1994; Kaiser, 1999).

In another study done by Richardson 2002 in patients suffering from Type 1 DM since from 10 years, ED were observed in range of 1.1% of men in age

group of 21-30 years, 55% of affected men in the age group of 50-60 years and 75% affected men reported with the age more than 60 years (Richardson, 2002).

1.7.1 Prevalence of ED among patients with type 1 and type 2 DM

It is still doubtful that ED caused in men due to type 2 diabetes (non insulin dependent diabetes, T2DM and late onset) or those ED affected men caused by type 1 (insulin dependent diabetes, T2DM and early onset). A very few literature are available on prevalence of ED by which type of diabetes. On that basis, one literature show that men with increased BMI (body mass index) and T1DM observed having high risk of ED as comparatively with men increased BMI and T2DM. In same literature estimated that the age adjusted prevalence of ED was in more range around about 51% with T1DM counterpart with T2DM (Fedele et al., 2000). But on other hand the other literature survey described that insulin dependent is 40% and non insulin dependent DM 52% engage in impotence (Miccoli et al., 1987). In this study survey they found that percentage of ED men in Italia with DM was more in T2DM as comparatively T1DM (Fedele et al., 2001). Explanation on prevalence of ED in diabetes through studies or literature still did not differentiate between type 1 and type 2diabetes. According to Bacon et al., (2002) and kalter et al., (2005) they reported, prevalence of ED similar from both type 1 and type 2 diabetes. On other hand the second studies report Shown that high risk from diabetes to induce ED in men with type 1 diabetes (Fedele et al., 1977)

1.8 Clinical features

Two major type of sexual problem are observed in diabetic patients (Kolodny et al., 1979).

major type of sexual problem

- characterized by reduction in sexual appetite
- degree of erectile failure
- lethargy, tiredness and malaise
- associated with hyperglycaemia

second type of sexual problem

- diabetis impotence
- determined disturbance of sexual function
- progressive and irreversible decline in sexual function
- physchological stress

1.8.1 Erectile dysfunction

Undoubtedly in diabetes, the most prominent sexual problem is difficulty in maintaining or obtaining an erection (Glazerman et al., 1976). Erectile failure is the most common clue of neuropathy in DM (Martin, 1953). Most information of erectile disorder in diabetes men is hidden or they try to hide, that causes continuous and progressive degradation of energy in trying erection activity. Erections that developed continuously throughout the day while walking or masturbating may lead to erectile failure (Rnbin, 1958). Alteration in maintaining erection is first sign of erectile failure along with premature ejaculation; affect the ability to obtain erection. Severity of erectile failure rises day by day with change in the shape of penis and penile trauma during erection (Fairburn et al., 1982).

1.8.2 Ejaculation

Ejaculation alteration is a common problem in diabetes. The most probably disorder is retrograde ejaculation in which semen flows backward direction in to the bladder instead of flowing through anterior urethra (Greene, 1968). This usually result in the patient which allow the pumping or pushing sensation correlated with ejaculation without semen come up from his penis. After ejaculation the urine which passes is appear cloudy. For conformation the

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diagnosis is based on finding of high number of spermatozoa in a orgasmic specimen of urine. These same clinical symptoms are developing from complication of bilateral lumber sympathectomy, bladder neck surgery and a person how regularly taking a medicine i.e. guanethidine and phenoxybenzamine (act as adrenergic blocking agents). Presently, there has not been prevalence study of ejaculatory alteration induces from diabetes. Only 2 person have noted with retrograde ejaculation out of 185 diabetic men and on other hand 85 person noted were impotent (Kolodny et al., 1979).

1.8.3 Spermatogenesis

The untreated and poorly controlled diabetic men lead to complication of infertility and testicular atrophy (Rodriguez, 1983). Spermatogenesis is a metabolically active process which deals with the production of haploid geno type with spermatozoa inside the seminiferous tubule through the process of mitotic cells division of spermatogonial stem and meiotic cells division of spermatocytes. Patients that having mild carbohydrate intolerance or controlled diabetes also having a less fertility and sperm density not has much affected (Klebanow, 1960). The alteration on sperm motility from diabetes is still debated (Hicks et al., 1973). In diabetic men the spermatogenic infertility is not as much common among those how having a retrograde ejaculation to induce ejaculation infertility. Other numerous abnormalities were observed that includes tubules diameter decreased, hyalinized walls of tubule, cellular debris or occluded lumina from epithelial cells, exfoliated germ cells, abnormalities in sertoli cells and leydig interstitial cells and micro-vascular changes (Kolodny et al., 1979).

1.8.4 Impotence

During sexual intercourse insufficient or failure to maintain a erection through penis is called erectile impotence. Approximately 30-60% of diabetic men have been suffer from erectile impotence and it prevalence depend up on its age factor. The jurisdiction perceive impotence in diabetes have divided into two main categories, first type usually occurs when diagnosis of erectile failure and during the treatment of poor glycemic control. It consists of loss of

sexual drive in erectile failure from various degrees of induction. On other hand second type is characterized with progressive and irreversible decline in sexual function occurs in longer standing diabetes (Fairburn et al., 1982).

1.9 Risk factors for MSD

The risk factors involved in development of MSD such as advanced age and longer time period of diabetes has been correlated to increases the chances for develop the ED in diabetic men patients (Giugliano et al., 2010; Lewis et al., 2010; Fedele et al., 1998). The hyperglycemia is one of the other risk factor to develop ED in diabetic men but it is still not clear yet. The some studies finding shown that the relationship between poor glycemic control, expressed by increased level of glycated HbA_{1c} (haemoglobin $A_{1c)}$ (Penson et al., 2003; Giugliano et al., 2010).   On other hand other studies literature did not report or find the any relationship between that (Siu et al., 2001; Hunayan et al., 2007) More over other common related risk factor which are recognized as to develop ED in diabetic men includes hyperglycemia, hypertension, obesity and body overweight, sedentary lifestyle, smoking, metabolic syndrome and autonomic neuropathy (Ponholzer et al., 2005; Bortolotti et al., 2001; Giuliano et al., 2004; Nicolosi et al., 2003; Chew et al., 2008) some diabetic complication such as micro vascular (Vinik et al., 2003; Chew et al., 2013) and macro vascular (Heruti et al., 2007; Chew et al., 2008) also play important rule to increases the risk for develop ED in diabetic men.For managing the DM patients may use large number of drug it may cause sexual dysfunction either by effect on ejaculatory through penis function or sex drive and an effect up on erectile. Some medication is used to treat diabetes and some of them are used to treat it secondary complication or other associated condition such as anxiety, depression and hypertension. So the use of those medicines rarely can develop ED by themselves (Eardley, 2010). The consumption of too much alcoholic content as per required amount per day may lead to develop ED in both general and diabetic men (Nicolosi et al., 2003; Kalter et al., 2005).

Factors associated with the development of SD in diabetic men patients

Erectile Dysfunction
- Age
- Duration of diabetes
- Metabolic control
- Hypertension
- Atherogenic dyslipidemia
- Obesity
- Sedentary lifestyle
- Drugs:,
- Comorbidities: hypogonadism, depression

Ejaculatory dysfunctions

Premature ejaculation
- Longer duration of diabetes
- Worse metabolic control
- CVD
- ED
- Diabetic neuropathy

Delayed ejaculation and anejaculation
- Diabetic autonomic neuropathy

Retrograde ejaculation
- internal sphincter of the bladder
- diabetic neuropathy

Hypoactive sexual desire disorder
- Duration of diabetes
- Metabolic control
- Erectile dysfunction
- cardiovascular disease, diabetic
- neuropathy
- Psychogenic factors

Fig no.1.3 list of factors associated with MSD in diabetes.

Premature ejaculation can develop under influence of following factors i.e. longer duration of diabetes, occurrence of CVD, ED (Sakka, 2003) and poor or worse metabolic control. Other involvement of factors is psychogenic and organic specifically autonomic neuropathy play important rule to develop PS (vinik et al., 2003) Still no article studying the risk factors for association of Delayed ejaculation in diabetic men has been published. Risk factor for develop anejaculation are involved diabetic autonomic neuropathy which occur due to lack of peristalsis of the vas (Sexton, 1997). Retrograde ejaculation problem occurs due to alteration in internal sphincter of the bladder. Other factor that can cause RE is diabetic neuropathy which changes the sympathetic fibres of bladder neck (Sexton, 1997; vinik et al., 2003). There are very less literature studies on risk of hypoactive sexual desire disorder associate with decreased libido in diabetic men. In two studies, the presence of an reverse relationship between age and sexual interest was found. An association between ED and decreased libido has been reported (Malavige et al., 2008; Bancroft & Gutierrez, 1996). Some other rsik factors which associate with diabetes that reduced the sexual interest are depression (Lustman & Clouse, 2005; Maraldi et al., 2007; Mezuk et al., 2008)

hypogonadism (Bhasin et al., 2010; Kapoor et al., 2007; Dandona et al., 2009) coronary artery disease, renal failure and the use of certain drugs (antidepressants, certain antihypertensive therapies).

1.10 Treatment of MSD
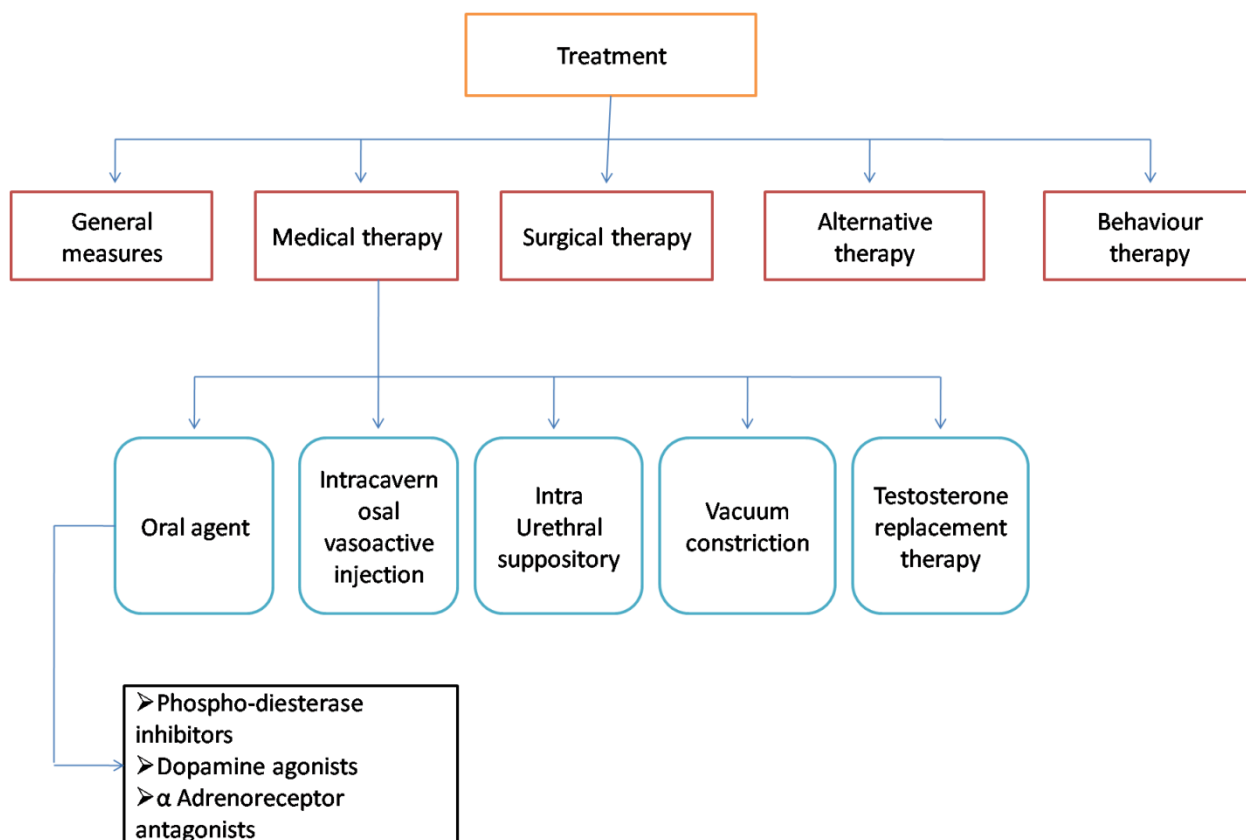
Fig no.1.4 treatment available for MSD

1.11 Green tea (*Camellia sinensis*)

World widely the Green tea (*Camellia sinensis*) is one of the most consumed beverages commonly. Green tea have several biological prosperities in there active components that including cancer chemoprevention, tumour cell suppressor, antiviral and anti-inflammatory activities (Yang et al., 2000) antioxidant activity (Morel et al., 1993; Guo et al., 1996). They also having the suppressor effects on several enzymes, like aromatase (Satoh et al., 2002; Goodin & Rosegren, 2003) angiotensin converting enzyme (Actis et al., 2006) and thyroid peroxidase (Divi & Doerge, 1996). Green tea leaves has 4 to 5 cm long roots. Characteristically green tea has yellowish-white flowers and when

the plant size is not more then or less than two meter long they has to cut for harvesting. Dried leaves of green tea *C. sinensis* contain high content of active component like polyphenols (30%–36%), principally flavanols are present in high amount which mostly known as catechins (Ahmad & Mukhtar, 1999). The sub cetagory of catechins are epigallocatechin-3-gallate (EGCG), epicatechin-3 gallate (ECG), epigallocatechin (EGC) and epicatechin (EC).

Scientific Classification

Kingdom	Plantae
Order	Ericales
Family	Theaceae
Genus	*Camellia*
Species	*C. sinensis*
Binomial name	*Camellia sinensis* (L.) Kuntze

Table no.1.2 scientific information of GT

Fig no. 1.5 natural leaf of green tea

1.12 History of green tea

The accurate date is still not available for the firstv cultivation of green tea *Camellia sinensis*. According to Chinese culture approximately 4,000 years ago, there were one person named Emperor Shen Nung warming or boiling the pot of water under a tree. The tree leaves fell into the water and mixed in it. He drank the whole pot and felt enliven. On that time, he finalized to promote or increases its cultivation throughout the world.

Tea was first processed in the United States in 1650 although the *Camellia sinensis* genus entered the country in 1744 in the gardens of Georgia. The first attempts at cultivation were without success, so further attempts were needed for successful cultivation (1). *Camellia sinensis* is currently cultivated in tropical and subtropical climates in many parts of the world (2).

1.13 Green tea components

The variety of components in green tea is very large. Its components include gallocatechin gallate (GCG), gallocatechin (GC), catechin gallate (CG), catechin (C), and flavonoids such as kaempferol, quercetin and myricetin. Other important components include theanine, derived from amino acid, the xanthine alkaloid caffeine, theophylline, theobromine, saponins, and tannins. Green tea has more than 300 other substances (Cooper et al., 2005).However, the main active components to which both beneficial and adverse health effects have been attributed are the four polyphenolic catechins: epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC), epicatechin (EC) ()(Vuong et al., 2010). A very important benefit that is attributed to catechins, especially to epigallocatechin gallate (EGCG), is the property of decreasing or maintaining body weight due to its capacity to induce and stimulate thermogenesis and the oxidation of fats (Dulloo et al., 19920).

1.12.1 Chemical composition of green tea leaves

Constituent	Percentage (% of dried leaf)
Polyphenols	37.0
Carbohydrates	25.0
Caffeine	3.5
Protein	15.0
Aminoacids	4.0
Lignin	6.5
Organic acids	1.5
Lipids	2.0
Ash	5.0
Chlorophyll	0.5

Table no. 1.3 composition of GTL

CHAPTER 2

REVIEW OF LITERATURE

2.1 Review of study

Luis et al., (1980) were observed that DM in man is frequently associated with SD. It is now generally agreed that retrograde ejaculation and impotence in diabetic men are caused in the majority of cases by diabetic neuropathy of the pelvic autonomic nervous system. Occasionally, impotence may be related to diabetic vascular disease. Decreased androgen production is an extremely rare cause of impotence in diabetic men. Thus, testosterone should not be used empirically in the treatment of these patients. Reports on the effects of diabetes on androgen and gonadotropin secretion, testicular histology, semen quality, and fertility of men have often been contradictory, with some authors suggesting that diabetes results in severe disturbances and others reporting no detrimental effects effects.

Christopher et al., (1982) were basically determined that diabetic men are prone to a variety of sexual problems. It is probable that most of these sexual difficulties are the result of a progressive physical disorder upon which a psychological reaction is superimposed. Assessment needs to take account of both the physical and psychological components of the problem. The aim of management is to help the patient and his partner enjoy sexual function to the full within whatever limits are set by irreversible factors. Using this approach it may be possible to improve the prognosis of what in the past has been dismissed as an intractable clinical problem.

Wade et al., (1997) were observed that DM is a well-known cause of ED in the diabetic male. The impact of diabetes upon male fertility appears to be limited to only a small number of patients who seek reproductive assistance. The majority of male patients with both NIDDM and IDDM are fertile, but this issue has never been properly assessed in a prospective fashion in a young population, It is plausible that diabetes leads to an acceleration of the normal degeneration of the testis seen with aging, thereby explaining some of the nonspecific abnormalities reported in the various animal models and human clinical studies. However, it appears that most of the semen abnormalities

reported in the controlled human studies can be explained by neuropathology with subtle or overt ejaculatory dysfunction. Reduced ejaculate volume and peripheral neuropathy were observed in almost all men with semen abnormalities. Disturbances in ejaculation may lead to low ejaculate volume, reduced sperm motility, and reduced sperm count.

Charles et al., (2006) were estimated that ED affects between 32% and 46% of people with diabetes. The patho-physiology of diabetes is multifactorial and no single aetiology is at the forefront. Treatment ranging from medical management to surgical implantation of a penile prosthesis is the standard at this time. Gene therapy using vectors appears to offer interesting and novel approaches to the treatment of the underlying pathophysiology of diabetic ED. However, further study in gene therapy is needed to fully ascertain its safety and utility in humans.

Hiroshi et al., (2004) provides present study evidence that green tea has an antidiabetic effect. Although we could not find simple reversed effect of green tea on the diabetes-induced modifications of the levels of several serum proteins, we found that the 4211 (4212) Da protein level that was decreased in the diabetic state was further decreased after green tea administration. This is the first report demonstrating that a certain serum protein may be involved in the antihyperglycemic effect of green tea. The contribution of this protein should be further studied.

Lasantha et al., (2009) were described that ED in men with diabetes is likely to become a more serious problem in the future with the rapidly increasing prevalence and earlier onset of diabetes. The aetiology of ED in diabetes is multifactorial. Most of the risk factor associations with diabetic ED have been identified in cross-sectional studies. A holistic approach is needed. Optimal glycaemic control, management of associated comorbidities, and lifestyle modification should be recommended to all patients. Psychosexual and relationship counseling would be beneficial for men with such coexisting problems. Hypogonadism should be identified and may need

treatment.Premature ejaculation and reduced libido are commonly associated conditions with diabetic ED and should be identified and treated.

Marina et al., (2009) in this study investigated the acute effects of green tea extract (GTE) and its polyphenol constituents, (−)-epigallocatechin- 3-gallate (EGCG) and (−) epicatechin (EC), on basal and stimulated testosterone production by rat Leydig cells *in vitro*. Leydig cells purified in a Percoll gradient were incubated for 3 h with GTE, EGCG or EC and the testosterone precursor androstenedione, in the presence or absence of either protein kinase A (PKA) or protein kinase C (PKC) activators. The reversibility of the effect was studied by pretreating cells for 15 min with GTE or EGCG, allowing them to recover for 1 h and challenging them for 2 h with human chorionic gonadotropin (hCG), luteinizing hormone releasing hormone (LHRH), 22(*R*)-hydroxycholesterol or androstenedione. GTE and EGCG, but not EC, inhibited both basal and kinase-stimulated testosterone production.

Vrushali et al., (2011) were mostly focsed on prevalence of ED in diabetic patients is increasing day by day. The pathophysiology of diabetes induced erectile dysfunction is multi factorial and no single etiology is at the forefront. However, in the past few years, our knowledge on the pathophysiology of ED and on male sexual problems in general has expanded enormously. However, there are still many unanswered questions that need to be addressed and more efforts need to be made in order to improve drug design and therapy. Treatment ranging from medical management to surgical implantation of a penile prosthesis is the standard at this time. In addition, even though DIED is not a life-threatening disease, it is an important factor to be considered in the global assessment of quality of life.

 Shyamal et al., (2014) they were done Experimental work to show the effect of Green Tea Leaf Extract (GTLE) on male reproductive system and also justify its effect to use GTLE as a castrative agent. The extract was given to the two different experimental animal groups with two different doses. After applying the doses in 26 consecutive days, it was found that the weight of the

testis and epididymis was markedly decreased in highly treated group. The sperm count and its motility was also reduced drastically. Result of this study showed that GTLE, relatively at high dose has its castrative activity on male reproductive system. Histological examination showed inhibition of spermatogenesis as evidence by disintegration of seminiferous tubules of testis.

2.2 Possible aetiology factors for MSD

The aetiology of MSD mainly based on two possible factors i.e. physical and psychological factors which further sub divided into different factors. Most probably it is observed that MSD is developing in a complex psychosomatic fact in which physical and psychological factors are involved. One factor and more than one factor are responsible for particular subject.
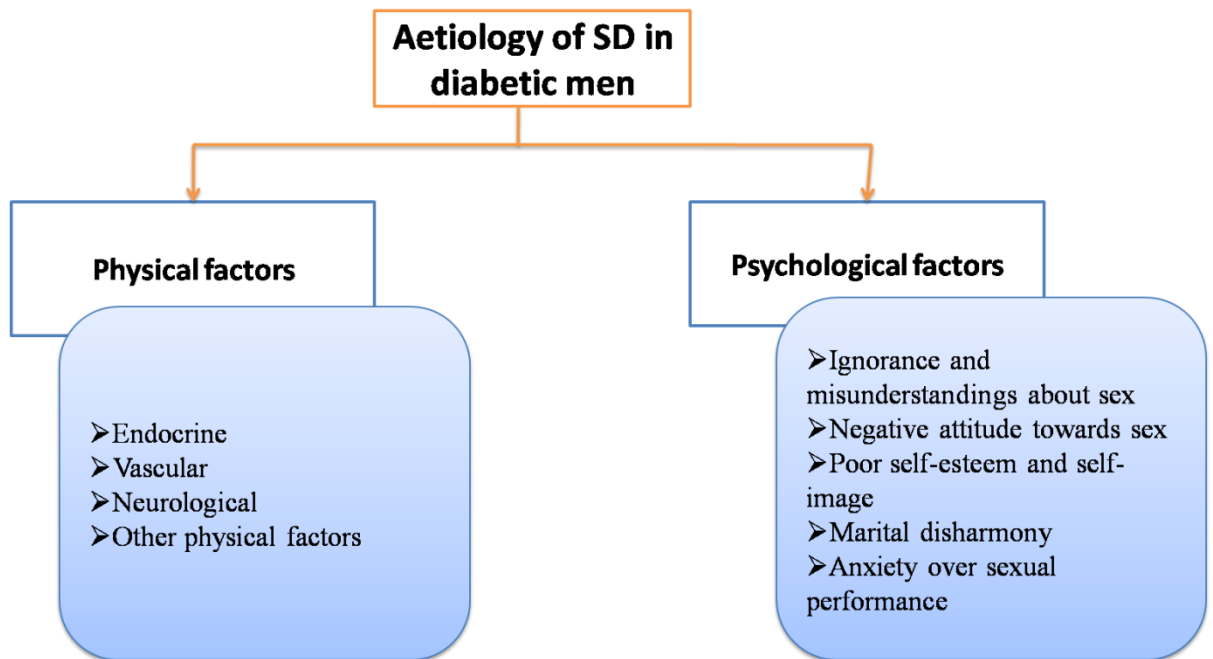
Fig no.2.1 major aetiological factors of MSD in diabetic man

2.2.1 Physical factors

It is still uncertain, whether MSD of diabetic men associated from endocrinal or hormonal abnormalities or not. According to study literature some

hormonal abnormalities have been identified but there were no evidence of surety. One study reported that diabetes had damaging effects on testosterone production (Schoffling et al., 1963). Jensen et al (1979) reported that reduced in impotent diabetics have effect on local endocrine transmitters and also observed impaired LH response to GnRH stimulation of basal normal gonadotropin levels. Distiller et al (1975) reported that young diabetic men with good glycemic control had increased basal LH level and GnRH stimulation response elevated. It also found that subject having normal total plasma testosterone levels but mordantly elevated SHBG binding capacity.

Recent evidence on vascular factor has been suggesting that abnormalities in internal pudendal arteries which associated with stenosis and atheroma, altered the blood supply to erectile tissue and cells which can raises to impotence or SD in diabetic men (Michal, 1982). Neurological autonomic nerve damage is widely associated symptom of SD in diabetes men. Generally, its depends upon the epidemiological and clinical relationship between symptoms of autonomic neuropathy and altered erectile function. There are some evidence to suggest that pelvic autonomic neuropathy have contribution to erectile dysfunction of many diabetic men. (Clarke 1979). In diabetic men who have symptoms of autonomic neuropathy is inversely observe occurs of impotence in it. After development of impotence the other clinical symptoms is autonomic damage (Ewing et al., 1980). MSD have other physical factors that includes some physical disease, heavy consumption of alcohol and ingestion of verity of medicines or hypoglycaemic agents or insulin. (furlow1979). In recent report suggest that thiazide diuretic is one of the cause of development of impotence in hypertensive men. [106] It is widely observed that psychological factors are the primer of development of impotence in diabetic or aetiology of most MSD.

2.3 Patho-physiology of ED in diabetic men

The patho-physiology of ED in diabetic men is multifactorial and no single cause is reasonable. The following are possible mechanism of ED in diabetic men.
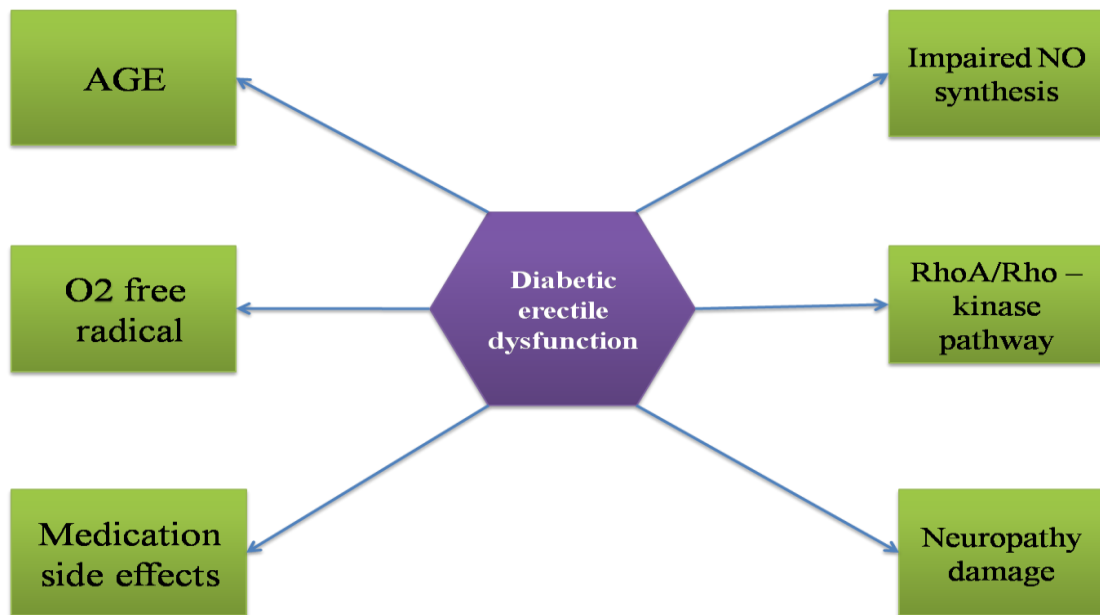
Fig no. 2.2 patho-physiology of DED

2.3.1 AGEs (advanced glycation end products) and high level of O_2 free radicals

Diabetes condition leads to hyperglycemia that further rises to development of AGEs. Generally, the formation of AGEs is based upon the non enzymatic reaction between glucose and lipid protein or nucleic acid (Cartledge et al., 2001). Between AGEs and vascular collagen the covalent bond occurs that leads to decreased thickening, elasticity atherosclerosis and conversion in endothelial function (Singh et al., 2001). High amount of AGEs found in corpus cavernosum in diabetic men, impling a precise effect of the AGEs (Giuseppe et al., 2006). Depositions of AGEs in aging and diabetic tissue leads to increases glucose level (Bucala et al., 1991). The relationship between AGEs with diabetic ED based on generation of oxygen free radical(react with NO) which comes from quench NO and oxidative cell damage, changes in relaxation of cavernosal smooth muscle, peak decreased in cGMP (cyclic guanosine monophosphate). Various channels and receptor may effected at molecular level from AGEs, which are mostly found on cavernosal smooth muscle cell. High level of effect particularly seen on the potassium channel which is mainly responsible for subsequent relaxation of cavernosal smooth muscles and interacellularly facilitate the release of calcium. Early

development of DIED take place on damage of potassium channels (Cartledge et al., 2001).
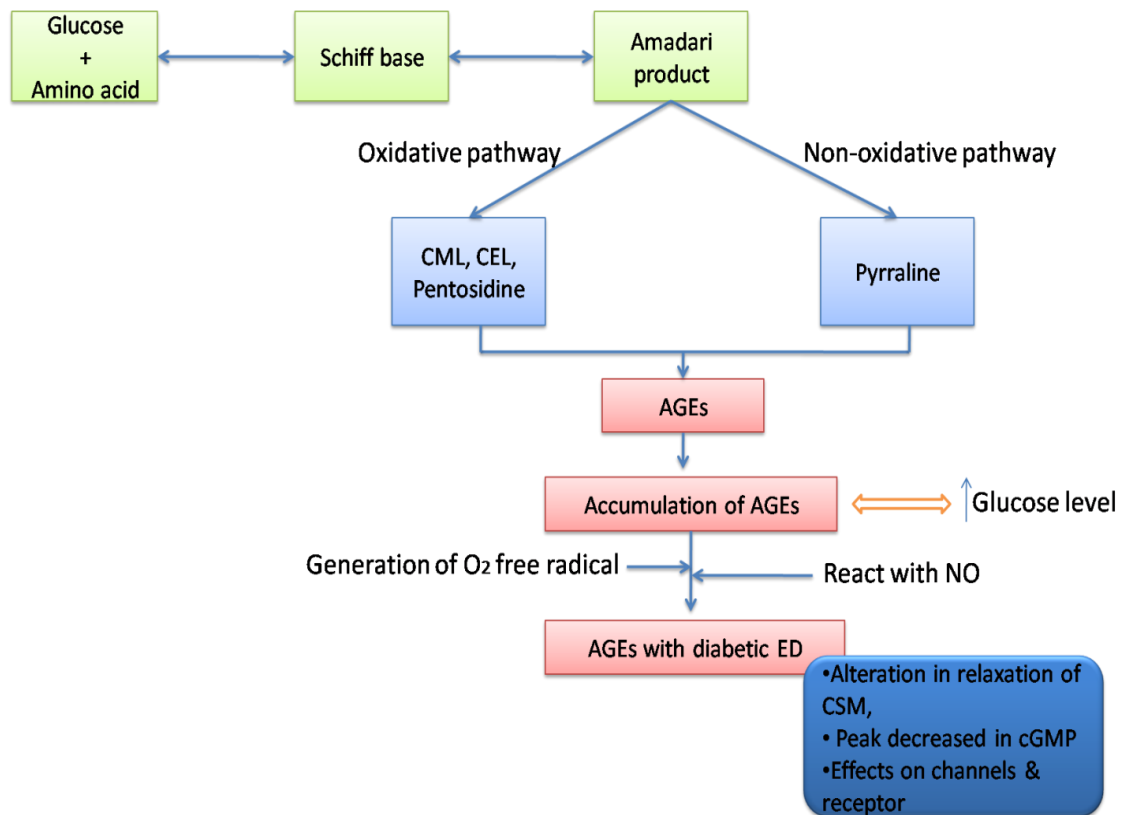
Fig no. 2.3 action of AGE on DIED

2.3.2 Impaired NO synthesis

Production of NO take place from the endothelium of the arteries of the penis and nitrergic neurons utilizing endothelial nitric oxide synthase (eNOS) and respectively from neuronal nitric oxide synthase. The formation of cGMP does not going to interfare NO of relaxation of the corpus cavernosum (Cellek et al., 1999). The high level of superoxide radicals of is particularly persent at cavernosal tissue. The men with DIED having the decreased level of NO synthase., resulting another possible pathways leading to smooth muscle and cavernosal dysfunction (Christ et al., 1995). It is also hypothesized that guanylyl cyclise activity impairs in diabetes impairs, thereby less the production of cGMP. Furthermore, ineffective endothelial dysfunction rapidly deliver the functional syncytium of the corpora cavernosa. That leads to decreased NO and, cGMP, participate significantly in the development of DIED (Costabile, 2003).

2.3.3 RhoA/Rho-kinase

Recent research ecidence has shown that the transduction pathway for the ET and according to that its receptor might play a role in development diabetic ED. The pathway is containing following products i.e. GTP-binding protein, RhoA, and its effector agent, Rho-kinase. The relationship between ET-1 with RhoA/Rho-kinase pathway to induced vasoconstriction has been shown (Park et al., 2002; Wang et al., 2002; Buyukafsar, 2003). The involment of activation the pathway two things are responsible i.e. quench eNOS and less the production of NO (Ming et al., 2002). Rat, rabbit, and human cavernosal tissue that all are containing Rho-kinase, and it has been shown to be upregulated in diabetic rats. It is suggested that the RhoA/Rho-kinase pathway interfare ED through decreased production of NO in the penis (Rees et al., 2002; Bivalacqua et al., 2004; Chua et al., 2006).

2.3.4 Neuropathic damage

DIED can also caused by Neuropathic damage mechanism (Costabile, 2003). Micro-vascular and macro-vascular prominent complication are arises in diabetes particularly from peripheral neuropathy and autonomic neuropathy (Agarwal et al., 2003). Most of the literature survive shown early autonomic and somatic nerve dysfunction were seen in diabetic patients recorded by longer discontinuation in the recall potential of pudendal nerves and in urthroanal reflexes and altered bulbar urethral (Vernet et al., 1995). That all neuropathic pathway will seen when it occurs early in the mechinsm of development of DIED A central neuropathic mechanism may also play critical role to the neuropathy induced by DIED (Costabile, 2003).

2.4 Traditional medical uses of Green tea (*Camellia sinensis*)
2.4.1 Anti cancer activity

The anticancer activity already has been reported that polyphenol and di and tri terpens tea compound having this activity. Two steroidal saponins tea compound named TS1 and TS2 and tea root extract having the cytotoxic and

apoptogenic effect on human cell lines and on cells from leukemia patients. It was found that TRE caused apoptosis and TRE TS1 and TS2 significantly decreased cell count (Ghosh et al., 2006).

2.4.2 Lipid lowering activity

Tea contain supplement like vitamin E, it has been helpful for decreased the plasma LDL (low-density lipoprotein), cholesterol concentrations, LDL oxidation, and early atherosclerosis compared to the consumption of tea alone by the hamsters. Tea also having the anti- oxidant activity through the incorporation of vitamin E into the LDL molecule (Hashimoto et al., 1987).

2.4.3 Anti carcinogenic activity

In vitro and in vivo green tea polyphenols having the antimutagenic and anticarcenogenic properties. It was found that the 50% mutagenicity effect was seen at a conc. of 5 mg/plate on *Salmonella typhimurium* strains reacted with 50 mg/plate of aqueous tobacco extract. An inhibition of tobacco induced urinary mutagenicity in rats was also found, indicating that green tea has some considerable action on reducing the mutagenic and carcinogenic effect of tobacco (Santhosh et al., 2005).

2.4.4 Neuromuscular-blocking action

The black tea was examinee, they have botulinum neurotoxin types A, B, and E neuromuscular-blocking action in the mouse phrenic nerve-diaphragm preparationns (Higuchi et al., 1995).

2.4.5 Antibacterial

Braun et al (2007) in vitro research studies shown that tea have action to inhibited the growth of such bacteria each 7 strains of *Stapholococcus* species and *Streptococcus* species, *Corynebacterium sins*, along with that they have inhibitory action on 19 strains of *Escherichia coli* and 26 strains of *Salmonella* species (Braun, 2007).

2.4.6 Antiviral

In vitro, the methanol extract of *Camellis sinensis* classes of Nonfermented and semifermented have inhibitory effects on *Helicobacter pylori* and its colonisation should be done with enzyme urease (Hassani et al., 2009). 3.5 mg/mL of semifermented extract and 2.5 mg/mL of nonfermented extract shows best complete inhibition result of two subunits of the urease enzyme. On otherhand the most bactericidal effects of *H. pylori* were found on 4 mg/mL of nonfermented and 5.5 mg/mL of semifermented extract. Thus as compera to other tea the *Camellia sinensis* nonfermented extract reduces more number of *H. pylori* colonies and also at lower concentration doses inhibits urease production, this all action mainly due to presence the polyphenol and catechin content in green tea (Hassani et al., 2009).

2.4.7 Diabetes

Tsuneki et al (2004) reported that antidiabetic effects have been obtained from green tea. They were observed that, without any affecting on insulin levels it can lower the glucose levels which generally present in bloodstream. In normal healthy human the dosage upto 1.5 g/body of green tea increases glucose metabolism (Tsuneki et al., 2004). Iso et al (2006) study report compare that consumption of green tea of six cups or more per day as comparatively one cup was lower the 33% of development of diabetic from CVD and cancer.

After oral administration of *Camellia sinensis,* the aqueous green leaf extract at concentration 450 mg kg-1 showed a strong glucose lowering action in rats (Shokrzadeh et al., 2006).

2.4.8 Immunomodulatory effect

In culture green tea had action to lower the alloresponsiveness and also had immunosuppressive effects. The highly effect of immunosuppressive tea achieved through lowering in IL-2 production. For determination the effect of green tea in vitro lymphocyte proliferation tests by the use of phytohemagglutin culture assey of mixed lymphocyte on transplant related immune function. (Yoshida et al., 1996).

2.4.9 Anti Spasmodic activity

The contraction of intestine in rabbit and rats model was seen after the induction of Hot water green tea extract and tannin fraction of the dried entire vs. pilocarpine-induced spasms and barium (Riso et al., 2002).

2.4.10 Antioxidant activity

After administration of black tea leaves in human red blood cells, was effective against damage by oxidative stress. The oxidative stress induced by phenylhydrazine, $Cu2+$-ascorbic acid, and xanthine/xanthine oxidase systems which all of them act as inducer. Black tea extract mostly eliminate the peroxidation of lipid in pure erythrocyte membrane and of whole red blood cell. Similarly, the degradation of membrane proteins can also be completely protected by black tea (Sagesak et al., 1994).

2.4.11 Antigenotoxic effect

The antigenotoxic effect of green tea extract against genotoxic damage induced by two anabolic steroids Trenbolone and Methyltestosterone in cultured human lymphocytes, both in absence and presence of metabolic activation. The results prove the antigenotoxic potential of green tea extract. Because the epidemiologic studies and research findings in laboratory animals have shown the antigenotoxic potential of tea polyphenol, the usefulness of tea polyphenol for various human diseases like cancer and coronary heart disease etc should be evaluated in clinical trials (Gupta et al., 2009)

2.4.12 DNA effect

Green tea extract, in cell culture at a dose of 10 mg/L corresponding to 15 mmol/L EGCg for 24 hours, did not protect Jurkat cells against $H2O2$-induced DNA damage. The DNA damage, evaluated by the Comet assay, was dose-dependent. However, it reached plateau at 75 mmol/L of $H2O2$ without any protective effect exerted by the extract. The DNA repair process, completed within 2 hours, was unaffected by supplementation (Murakami et al., 1999).

2.4.13 Weight loss

Green tea extract standardised to 8.35% caffeine and 24.7% catechins has been shown to stimulate brown adipose tissue in vivo, with thermogenesis greater than the effect the caffeine content accounts for (Dulloo et al., 2000). Long term ingestion of tea catechins stopped the accumulation of body fat in mice with high fat diet induced obesity, possibly due to the activation of hepatic lipid metabolism (Tokimitsu, 2004). This effect was also found in non obese rats (Ito et al., 2008).

2.4.14 Arthritis

Severity of arthritis symptoms in rats was significantly reduced by green tea polyphenols at a dose of 8 mg/L for nine days (Kim et al., 2008). A prospective cohort study of 31 336 women aged 55-69 drinking three or more cups a day of tea had a reduced risk of developing rheumatoid arthritis compared with those who drank no tea (Mikuls et al., 2002).

2.4.14 Dental caries

Green tea effectively prevents dental caries (Koo & cho, 2004). Both semifermented and nonfermented *Camellia sinensis* extracts (black and green teas) prevent the growth of oral *Streptococci* responsible for dental caries and bacteremia following dental work, such as *Streptococcus mutans, S. mitis* and *S. sanguis*, with black tea showing a greater effect due to a higher content of volatile components (Hassani et al., 2008).

2.5 Effect of EGCG on diabetes

A study by Waltner-Law et al. provided compelling in vitro evidence that EGCG decreases glucose production of H4IIE rat hepatoma cells (Waltner et al., 2002). The investigators showed that EGCG mimics insulin, increases tyrosine phosphorylation of the insulin receptor and the insulin receptor substrate, and reduces gene expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase. Recently, green tea and green tea extracts were demonstrated to modify glucose metabolism beneficially in experimental models of type II diabetes mellitus (Tsuneki et al., 2004; Wu et al., 2004). In addition, EGCG ameliorates cytokineinduced b cell damage in

vitro (Han , 2003) and prevents the decrease of islet mass induced by treatment with multiple low doses of streptozotocin in vivo (Song et al., 2003). Lambert et al. showed that intragastric administration of EGCG at a dose of 75 mg/kg resulted in a Cmax of 128 mg/l total plasma EGCG and a terminal half-life of 83 minutes (Lambert et al., 2003). Furthermore, in humans an oral intake of EGCG at a dose of 50 mg (0.7 mg/kg) resulted in a Cmax of 130 mg/l total plasma EGCG and a terminal half-life of 112 minutes. These results indicate that rodents must be orally administered 100- to 600- fold more EGCG (depending on whether they are administered by gavage or by feed admixture) to achieve similar plasma concentrations as those found in humans. Total plasma EGCG concentrations shown to be efficacious in mice and rats can be reached by an intake of low to moderate doses of EGCG in humans (Ullmann et al., 2003).

2.6 Effect of green tea extract on Male reproductive system

The effects of green tea extract compound catechins on the male reproductive system have been described. Epidemiological and laboratory studies suggest an association between diet and androgens that can alter prostate cancer risk (Ripple et al., 1997; Clinton, 1998). It has been shown that parenteral injection of EGCG can suppress human prostate and breast tumour growth in athymic mice (Liao et al., 1995) and reduce the weight of testes and accessory reproductive organs, as well the circulating level of luteinizing hormone (LH) and testosterone in the intact rat (Kao et al., 2000). Although the antigonadotropic effect of catechins is explained as a secondary effect of EGCG on food intake (Kao et al., 2000) or on aromatase activity (Satoh et al., 2002; Goodin et al., 2003), a modulatory function could be present even at the gonadal level.

Currently, there is no evidence for a direct effect of green tea catechins on testicular steroidogenesis or on the enzymes involved in androgen production. Although the involvement of the protein kinase A (PKA) and protein kinase C (PKC) signalling pathways in testicular androgen production is well known

(Dehejia et al., 1982; Wanderley et al., 1996), it is unclear whether green tea catechins modulate these pathways in Leydig cells. There is evidence that EGCG and other flavonoids can modulate the PKC (Lin, 2002; Levites et al., 2003) and PKA signalling pathways in other animal models . The aim of this study was to investigate the direct *in vitro* effects of green tea extract (GTE) and its purified catechins on the basal and the PKA and PKC-stimulated testosterone production by rat Leydig cells (Lin, 2002; Lorenz et al., 2003).

2.7 The mechanism action of streptozotocin

Streptozotocin (STZ, 2-deoxy-2-(3-(methyl-3- nitrosoureido)-D-glucopyranose has a molecular formula of $C_8H_{15}N_3O_7$, molecular weight of 265 g/mol and the structure is composed of nitrosourea moiety with a methyl group attached at one end and a glucose molecule at the other end.(Donal1997).STZ has been one of the chemical agents is widely used for the induction of diabetes in experimental animals to induce both insulin-dependent and non-insulin-dependent diabetes mellitus in the experimental diabetes in animals and causes a state of insulin-dependent diabetes mellitus by inhibits the insulin secretion.STZ is cytotoxic to pancreatic β-cells and its effects can be seen within 72 hours after administration and also depending on the dose administered (Junodet al., 167). The induction of experimental diabetes in the rat using chemicals which selectively destroy pancreatic B cells is very convenient and it is simple to use. Their mechanism of action in B cells of the pancreas has been intensively investigated .The most usual substances to induce diabetes in the rat are alloxan and streptozotocin(. T. szkudelski).

Inhibition of insulin secretion by streptozotocin
Streptozotocin effects on glucose and insulin homeostasis reflect the toxin-induced abnormalities in beta cell function. Initially, insulin biosynthesis and there, secretion and glucose metabolism (both glucose oxidation and oxygen consumption) are all affected (Nukatsuka M et al., 1990). The toxic action of STZ involves its uptake into cells. STZ has no immediate, direct inhibitory effect upon glucose transport (Elsner M et al., 2000). STZ is taken up by

pancreatic B cells via glucose transporter GLUT2 where it causes β-cell death by DNA fragmentation due to the nitrosourea moiety. A reduced expression of GLUT2 has been found to prevent the diabetogenic action of STZ (Schnedl et al., 1994, Thulesen et al., 1997). Wang and Gleichmann (1995, 1998) observed that STZ itself restricts GLUT2 expression in vivo and in vitro when administered in multiple doses or upon glucose phosphorylation by glucokinase

Three major pathways associated with cell death are

    (i)   DNA methylation:)

    (ii)   Nitric oxide (NO) production

    (iii)   Generation of free radicals as hydrogen peroxide
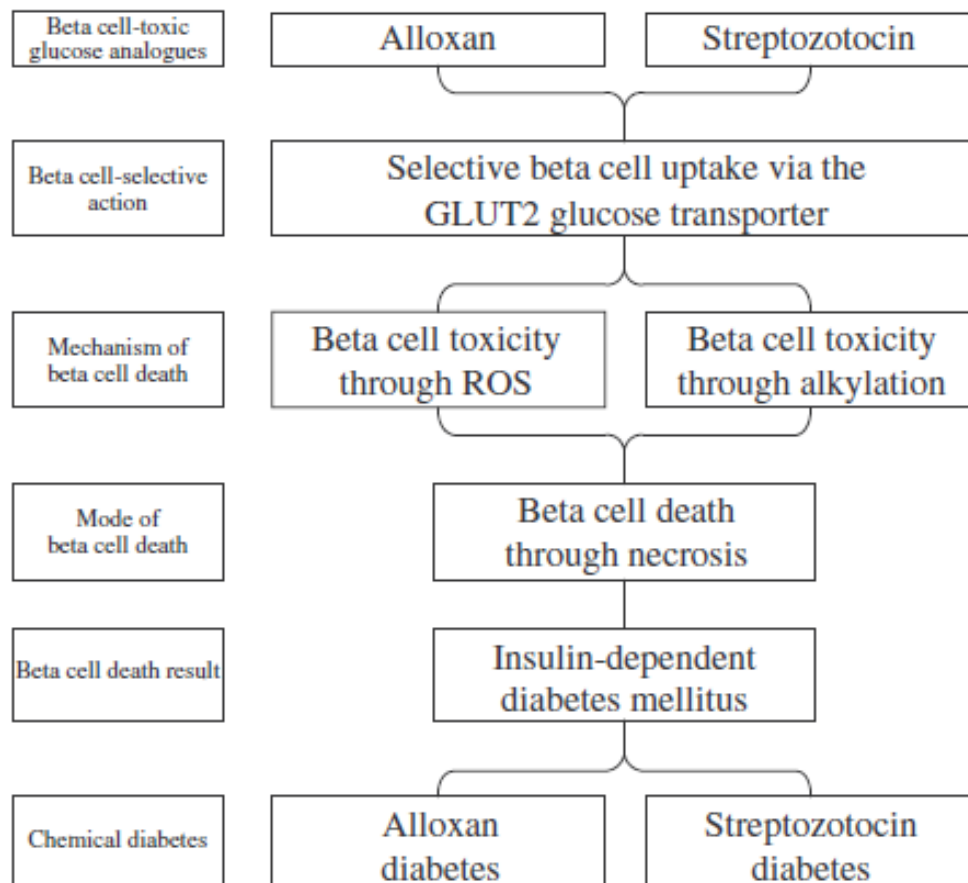
Fig. no. 2.4 Schematic representation of the toxic effects of the glucose analogues streptozotocin and streptozotocin in beta cells, which produce chemical diabetes

CHAPTER 3

Research Envisage

3.1 Hypothesis

It has been hypothesised that Green tea extract due to its high anti-oxidant and anti-diabetic effect, supposed to inhibit alteration of gonadal function in diabetes mellitus.

3.2 Aim & Objectives

- Protective effects of Green tea extract on reproductive function in albino antidiabetic streptozotocin-diabetic male rats**.**

- Green tea extracts effect on testicular part of diabetic male albino rat.

- Study of the histological and biochemical changes in the testis after treatment with green tea extract.

- To perform biochemical testes in streptozotocin induced diabetic male rat

CHAPTER 4

Comprehensive Plan

4.1 Plan of work :

Title	(Months)						
	0-1	1-2		2-3		3-4	
Induction of diabetes to rat	▓						
Diagnosis of diabetic rat	▓						
Administration of GTE	▓	▓					
Checking of urinary protein	▓	▓					
Sacrificing of rats			▓	▓	▓		
Analysis of biochemical parameters on serum			▓	▓	▓		
Analysis of testes tissue sample			▓	▓	▓		
Analysis of data and application of statistical tools						▓	
Writing a report of research	▓	▓	▓	▓	▓	▓	
Presentation of result of research							▓

CHAPTER 5

Material and Methodology

5.1 Plant material green tea extract

5.2 The extract material was collected from A.M LABS New Delhi, India in the month of April 2011. General specification Green Tea Extract Powder (98% polyphenols / 40% EGCG)

Composition specification

Total polyphenols (UV)	min98%
Total catechins (HPLC)	min70%
Content EGCG (HPLC)	min40%
Content caffeine (HPLC)	max5%

Physical Property

Solubility	water soluble fig no. 5.1 green tea extract
Particle Size	100% through 60 mesh
Loss on Drying	max 5%

Storage: - Store in a cool dry place, avoiding sunlight directly

5.1.1 Manufacturing process flowchart

Green Tea Extract Powder (98% polyphenols / 40% EGCG)
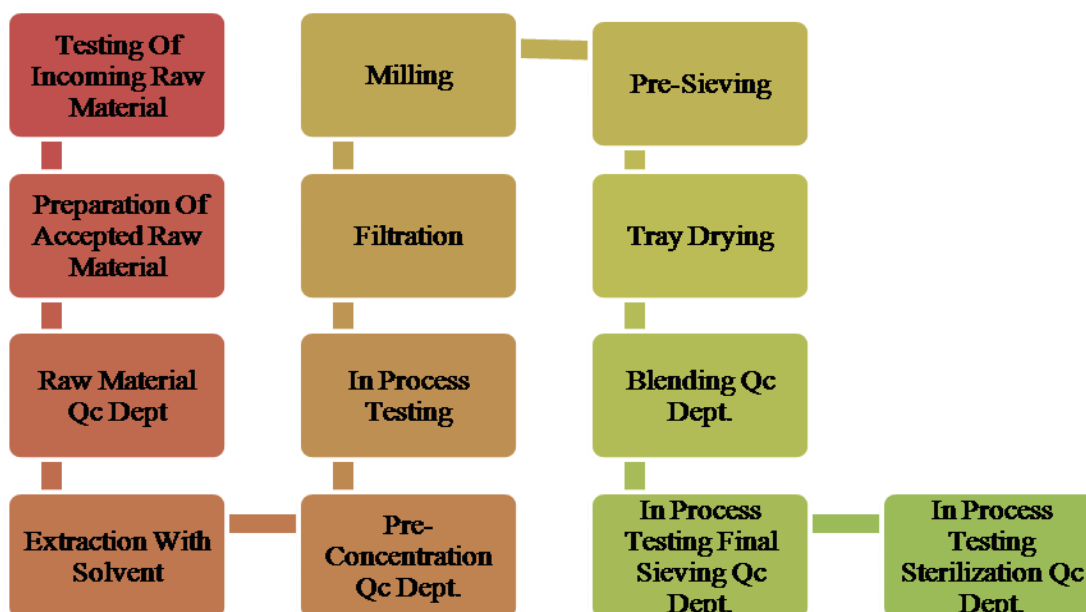
Fig no. 5.2 step by step processing of manufacturing the green tea extract.

5.2 Experimental Animals

In this assay, thirty six (36) male albino rats of wistar strain weight (190-300 g), age 3 months, were obtained from Lovely Professional University. The rats (36) were divided into six groups of 6 each rats according to the design of experiment and treatment. The animals were housed in the steel cages each cage consists of not more than 3 rats in an air condition room (22 ± 3°C, 55 ± 5% humidity and a 12-h light/dark cycle) and were maintained with free access to water and standard laboratory diet. After the in induction of first single dosage of STZ in fasting rats, there was death of 4 rats due to high motility rate of STZ. So they were act as or used as normal control study. After that we were design the study according to remaining rats, they were divided into 4 groups and each group having 6 rats. Know study were conducted int o 4 groups and four groups are Group 1 normal control, Group 2 normal + green tea, Group 3 diabetic control and Group 4 were diabetic + green tea. The study was conducted at the Lovely professional University, Punjab after obtaining Institutional Animals Ethical Committee clearance bearing the number   LPU/LSPS/IAEC/CPCSEA/Meeting No 5,   Jan 2015 Protocol No. 7

Fig no 5.3 animal placed in cages with different grouping

Fig no 5.3.1 air conditioner to provide accurate environment.

5.2.1 Grouping of animals

Groups	Treatment	Sex of animal	Required animal	Dose (mgkg-^{1}bw)/ route
1	Non-diabetic rats with Normal diet (Normal Control)	Male	6	Nil
2	Non- diabetic rats + green tea extract	Male	6	200mgkg^{-1} bw; Orally
3	Diabetic rats + normal diet	Male	6	Single dose of streptozotocin (55mgKg^{-1}bw) i.p dissolved in normal saline)
4	Diabetic rats + green tea extract	Male	6	Single dose of streptozotocin (55mgKg^{-1}bw) i.p dissolved in normal saline +200mgkg^{-1} bw; green tea extract Orally

Table no 5.1. four groups of animals

5.4 Chemical preparation

Streptozotocin

Green tea extract

5.3.1 General of Streptozotocin & preparation

Streptozotocin was obtained from lovely professional university. It is prepared by dissolving in normal saline at RT and after preparation place the solution over the ice or ice pack. It is always prepared freshly for immediate use within 5 min was injected by intra-peritoneal routes, in overnight fasted male rats, The doses were determined according to the body weight of animals weight of streptozotocin (45mg/kg) required in mg.vol of STZ (5%W: V) required in ml.

Healthy adult albino rats (150-200 g) were used in the study. Determination of dose by applying following formula:-

Dosage in mg and ml = B.W of animal (grams) × weight of streptozotocin(mg)

$$\overline{\qquad\qquad 1000 \qquad\qquad}$$

Streptozotocin induced a dose-dependent mortality in 60%, 50% and 20% of rats receiving 60 mg/kg, 50 mg/kg, and 45 mg/ kg respectively of streptozotocin within 15 days of injection, respectively. Streptozotocin induced diabetes in 60% of rats receiving either 120 mg/kg or 150 mg/kg of streptozotocin and 60% of rats receiving 45 mg/kg dose

5.3.2 Green tea extracts preparation & dosages

Green tea extract prepared 200mg/ kg body weight dissolved in distilled water.

Fig no. 5.4 bottles of green tea extract

5.4 Administration of streptozotocin to induce Diabetes
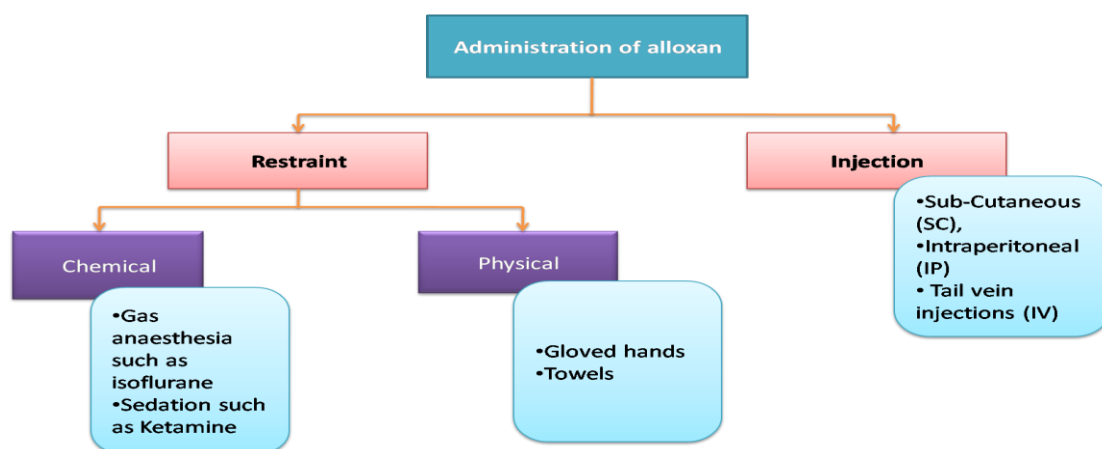
Fig no. 5.5 different type of induction the streptozotocin

5.4.1 Procedure for IP injection

When making an IP injection good restraint and good injection technique will help minimize any secondary problems that may occur with this type of injection. Restrain your animal using either the scruff and holding the tail with pinky or ring finger in mice. If using rats gently grabbing them over the shoulders causing the legs to cross over the chest to help prevent getting bit is common restraint. ` Once animal is restrained turn over so abdomen is exposed. Please monitor chest movements to make sure the animal is doing ok. On the mouse you want to make your IP injection in the lower right or left quadrant of abdomen trying to avoid hitting bladder, liver, or other internal organs. Then inject the solution with slow pressure applying on syringe plug.Gently remove the needle from abdomen of rats.

5.4.2 Selection of area to inject IP

Fig no. 5.5.1 shown the ventral side of rat Inject the IP injection for preparation of diabetic model.
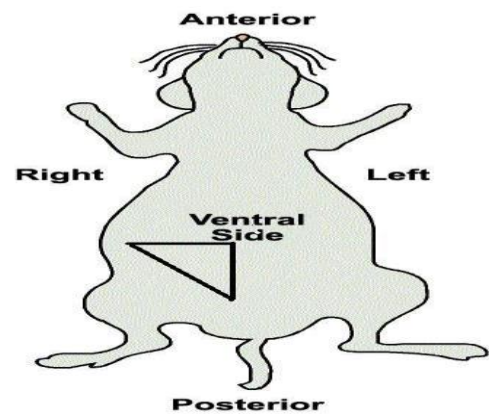
Fig no. 5.6 normal handling   fig no. 5.6.1 control on rat and   fig no. 5.6.3
IP
 rat of rat from the neck   exposed the area for injection   injection to
rat

5.5.3 Characteristics of normal and Streptozotocin induced diabetic rat
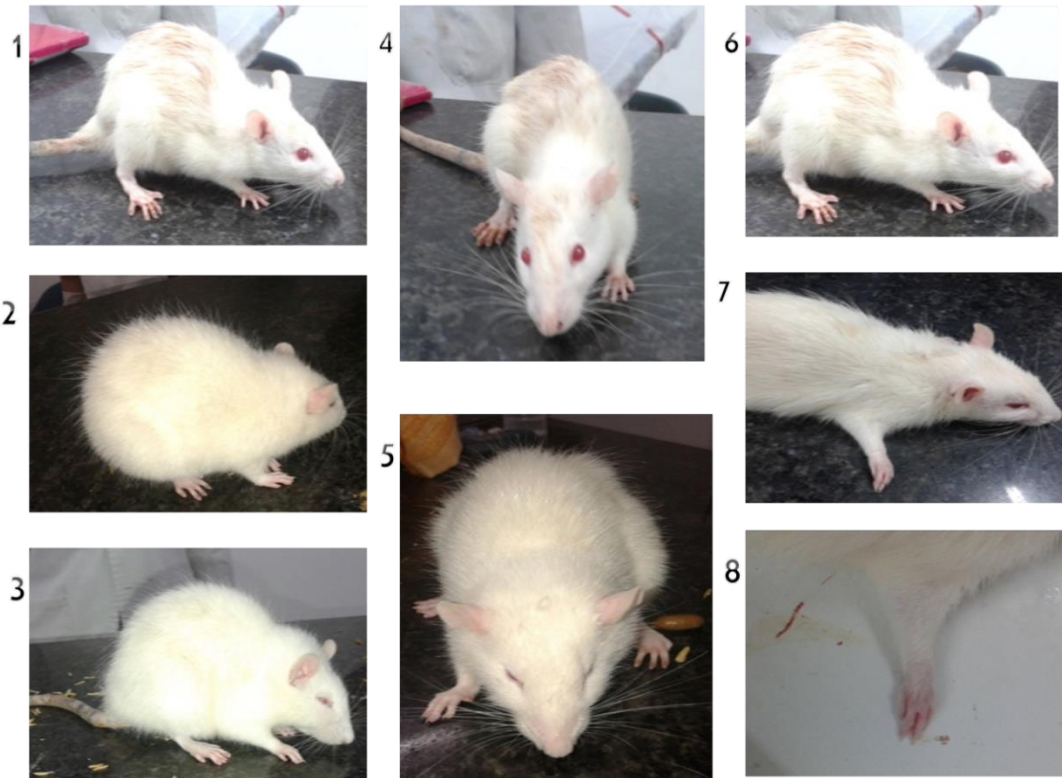
Fig no. 5.7 1. Normal rats without Streptozotocin induction. 2&3. Diabetic rat model having straight hair on body. 4. Normal rat with normal face and head feature. 5. Diabetic rat with peak and triangular shape face. 6. Nomarl rat with normal feet and structure. 7&8 highly hyperglycaemic rat having paralysed feet, not able to walk properly and not such movement present in it.

5.5 Biochemical parameters

5.5.1 Kits for biochemical analysis

The assay kits were obtained from modern surgical house C-38, sportrs and surgical complex basti bawa khel, Jalandhar, Punjab.

Parameters for testing

1. **Blood glucose**
2. **Lipid profile**
   - **Cholesterol**
   - **Triglycerides**
3. **Total protein**
   - **Albumin**
   - **Globulin**
4. **Testosterone (hormone assay)**

5.5.2 Biochemistry Analysis

Sample preparation: After the last treatment, blood samples (2ml) were taken from each animal by cardiac puncture. The samples were immediately transferred into plain vial tubes.

5.5.3 Sample collection

1. **Blood sample from eye**
2. **Blood sample from tail**
3. **Collection of blood sample by cardiac puncture**

5.5.3.1 Blood Collection from the Orbital Sinus (eye puncture vein)

Lay the anesthetized mouse on its side on a table or hold it in your hand with its head pointing down (**Fig. 5**). With your first finger and thumb (finger above and thumb below the eye) pull the skin away from the eyeball, above and below the eye, so that the eyeball is protruding out of the socket as much as possible. Take care not to occlude the trachea with your thumb. Insert the tip of a fine-walled Pasteur   pipette (o.d. of from the orbital sinus to prevent blood from spilling out of the tube.

Bleeding usually stops immediately and completely when the pipette is removed. It

may be necessary to apply gentle pressure on the eyeball for a brief moment by closing the skin above and below the eye using your first finger and thumb. It is recommended that sample collection not be repeated on the same eye for at least two weeks.

Caution: Blindness can occur if the optic nerve is damaged as a result of the blood collection tube coming into contact with the nerve, which attaches to the

middle of the ventral surface of the eye. Ocular ulcerations, puncture wounds, loss of vitreous humor, infection, or keratitis may occur as a result of poor technique or uncontrolled movement of the animal.
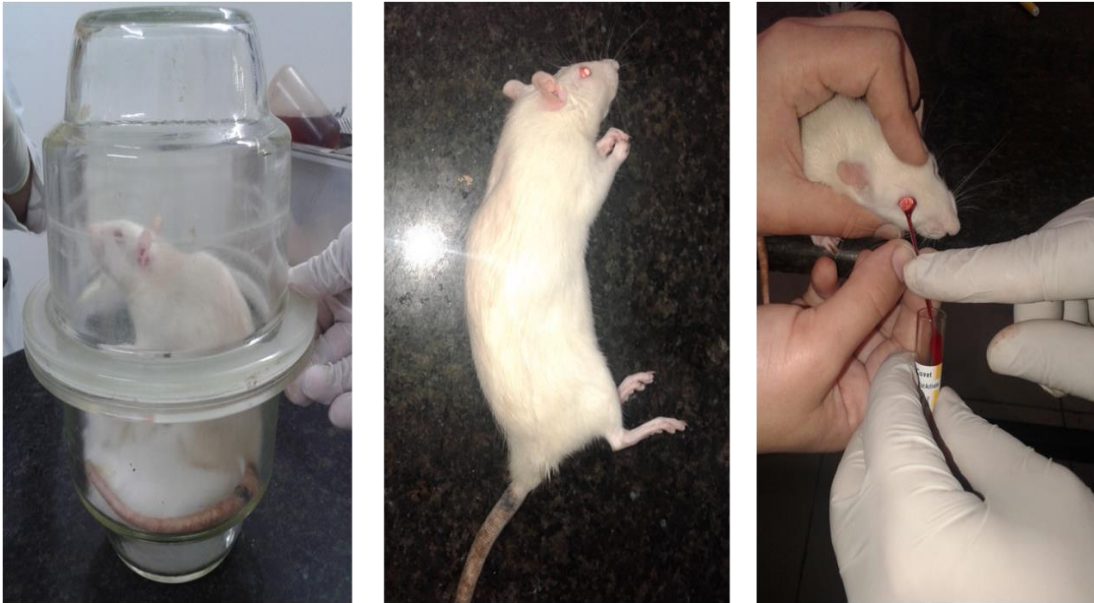
Fig no. 5.8 rat for anaesthesia fig no.5.8.1 aneasethesia rat   fig no.5.8.3 collection of blood in glass camber.   from eye with capillary'

5.5.3.2 Blood Collection from the Tail

Warm the mouse and place it in a restraining tube as described above. Do not attempt to increase blood flow by rubbing the tail from the base to the tip, as this will result in leukocytosis (increased white blood cell count). Using a scalpel, straight edge razor, or sharp scissors, quickly removes up to 1 cm of the tail. Collect blood in a capillary tube as drops appear. Apply pressure or use a cauterizing agent such as a styptic pencil (silver nitrate) to stop the bleeding.

Fig no.5.9 collection of blood from tail vein

When several samples are needed within a short time period, the original wound can be reopened by removing the clot. When additional samples are

needed at a later date, blood samples can be obtained by removing just 2-3 mm of additional tail. Cutting the tail too short may result n trauma to the cartilage and ultimately to the coccygeal vertebrae.

5.5.3.3 Blood collection technique from cardiac puncture

Anesthesia Technique:

1. Anesthetize animal. (Surgical plane of anesthesia is required!)

2. Test for reaction by corneal reflex and toe pinch.

3. Blood may be obtained through a ventral, left lateral, or open approach.

Ventral approach (closed)

a. Place animal on back (dorsal recumbency)

b. Palpate heart

c. Insert needle slightly left of and under sternum, directed toward animal's head.

d. Needle and syringe should be held 20-30 degrees off horizontal.

e. Insert into heart

Left Lateral approach (closed)

a. Place animal on right side (right lateral recumbency)

b. Palpate heart on left lateral thoracic wall (approximately at point of flexed elbows, between ribs 5 and 6)

c. Insert needle slowly (between ribs and perpendicular to the body) and into heart

Open approach

a. Place animal on back (dorsal recumbency)

b. Wet skin on the abdomen with 70% alcohol

c. Make a V-cut through the skin and abdominal wall ~1cm caudal to the last rib

d. Move internal organs to the side

e. Insert needle through the diaphragm and into the vena cava or heart

7. Gently apply negative pressure on syringe plunger. Heart chamber may collapse if negative pressure is too great.

8. Never move the needle side-to-side as this could lacerate the heart or vena cava!

9. If no blood appears, slowly withdraw the needle so that it remains just under the skin or the diaphragm and redirect in a slightly different direction.

10. If blood stops flowing, slowly rotate needle or move it slightly in or out.

11. Withdraw needle after blood has been collected.

Fig no. 5.10 shows that firstly anesithised the rat. Placed in dissected rack and put the pines over it. Cut the first layer of skin with plan scissor and after that cut the skin with pin scissor. Hold the skin with the help of forceps and exposed the heart. Inject the syringe into heart and collect the blood sample.

5.5.4 Sample Preparation (serum)

1. Blood was collected in plan vial without using an anticoagulant.

2. Blood was allowed to clot for 30 minutes at 25°C.

3. Blood was centrifuged at 2,000 x g for 15 minutes at 4°C. Top yellow serum layer was pipette off without disturbing the white buffy layer. Serum was stored on ice.

5.6 Biochemical analysis:

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At the end of the experimental period, the rats were anesthetized by light ether and fasting blood samples were drawn directly from cardiac. Then blood samples were centrifuged at 3000 rpm for 15 minutes and serum was separated and used for the biochemical analysis. Serum glucose, triglycerides (TG), total cholesterol (TC) and high-density lipoprotein-cholesterol (HDL-c) levels were determined enzymatically using the standard methods. Low-density lipoprotein-cholesterol (LDL-c) level was calculated by Friedwald formula as follows: LDL-cholesterol.

The hormonal assays (testosterone hormone) were determined by enzyme linked immunosorbent assay (ELISA) method using AccuBind ELISA kit(Monobind Inc. CA, USA). Total plasma protein and the lipid profile.

5.6.1 Determination of blood glucose by the glucose assay kit

Fasting blood glucose level was determined on the 1st, 7th, 14th and 21st day

Principle:

Glucose oxidase (GOD) catalyzes the oxidation of glucose to gluconic acid. The formed hydrogen peroxide (H2O2), is detected by a chromogenic oxygen acceptor, phenol-aminophenazone in the presence of peroxidase (POD):

Principle: (Trinder's method)

α-D-glucose $\xrightarrow{\text{Mutarotase}}$ β-D-glucose

β-D-glucose +H2O+O2 $\xrightarrow{\text{Glucose oxidase}}$ D-gluconicacid+H_2O_2

H_2O_2+ 4-aminophenazone+phenol $\xrightarrow{\text{Peroxidase}}$ Quinonemine + 4 H_2O
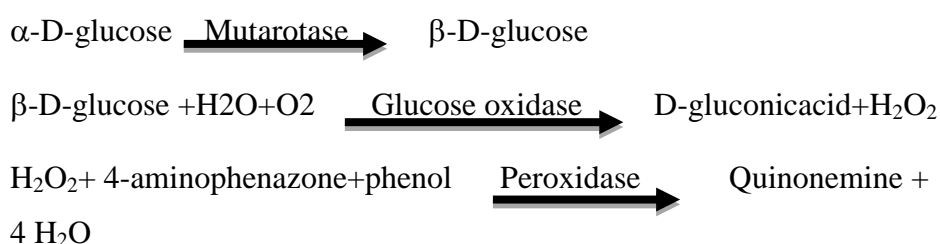
The intensity of the color formed is proportional to the glucose concentration in the sample.

In this assay, glucose is oxidized to δ-gluconolactone with concomitant reduction of the Flavin Adenine Dinucleotide (FAD) dependent enzyme glucose oxidase. The reduced form of glucose oxidase is regenerated to its oxidized form by molecular oxygen to produce hydrogen peroxide. Finally,

with horseradish peroxidase as a catalyst, hydrogen peroxide reacts with 3, 5-Dichloro-2-hydroxybenzenesulfonic acid and 4-Aminoantipyrine (also called 4-Aminophenazone) to generate a pink dye with an optimal absorption at 520 nm. The intensity of the colour produced is directly proportional to the glucose concentration in the sample (Trinder, 1969). The serum glucose concentration was expressed as mg/dl.

Apparatus – Plane vial, Micropipette.

Procedure

Reagents	Blank	Standard	Test
Working reagent	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	--	--
Standard	--	10 µl	--
Test serum		--	10 µl

Mix well and incubate for 15 minutes at 37 c . read the absorbance of standard and each test tube against reagent blank at nm on bio chromatic analyzer.

Table no 5.2 procedure for glucose.

It was mixed thoroughly that stops coagulation of the blood solution. It was then centrifuged at 3000 rpm for 5 to 10 minutes. Supernatant was discarded and plasma was stored. Now in each test tube 1ml L_1 i.e. glucose reagent is taken. In blank B test tube 10 µl distilled water, in standard S test tube 10 µl standard glucose which was supplied and test T test tube 10 µl plasma was added. All the three test tubes are incubated at 37 ^{0}C for 10 minute or kept at room temperature for 30 minute. Now optical density of all three solutions is taken by spectrophotometry at 520 nm.

Calculation –

Let O.D. of blank = B

O.D. of standard = S

O.D. of test   = T

$$\frac{(T - B)}{(S - B)} \times 100 \text{mg/dl}$$

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Formula=

5.6.2 Biological assays of lipid profile

The serum was used for the estimation of lipid profile. Total Cholesterol (TC) and HDL were obtained by standard procedure (direct method). LDL and VLDL level were obtained by calculating using formula. Very Low Density Lipoprotein and Low Density Lipoprotein were calculated by as per Fridevald's equation (Fridevald *et al.*, 1979).

5.6.2.1 Estimation of Total Cholesterol assays (CHOD/POD Method)

Principle:

Cholesterol esterase hydrolyses esterifies cholesterol to free cholesterol. The free cholesterol is oxidized to form hydrogen peroxide which further reacts with phenol and 4- aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoeimine dye complex. Intensity of the colour formed is directly proportional to the amount of cholesterol present in the sample.

Reaction:

Cholesterol esterase $+H_2O \longrightarrow$ cholesterol+ fatty acids

Cholesterol $+O_2 \longrightarrow$ cholestenone+H_2O_2

H_2O_2+Phenol+4 amino anti-pyrine$\longrightarrow$ red quinonimine dye+H_2O_2
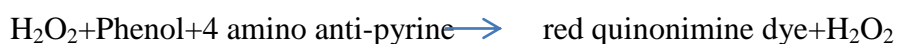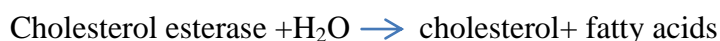
Apparatus – Test tubes, Micropipette

Chemical – Total Cholesterol assays kit contains

Reagent 1: Cholesterol enzyme reagent 1

Reagent 2: cholesterol standard reagent 200mg/dl.

Reagent 3: Cholesterol precipating reagent.

Procedure

Reagents	Blank	Standard	Test
Enzyme reagent	1000 **μl**	1000 **μl**	1000 **μl**
Serum			10 **μl**
Standard		10 **μl**	

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Distilled water	10 μl		

Table no 5.3 procedure for cholesterol.

Calculation –

O.D. of blank = B

O.D. of standard = S

O.D. of test = T

$$\text{Formula} = \frac{(T - B)}{(S - B)} \times 200 \text{ mg/dl}$$

Reaction mechanisms

1. Cholesterol + Oxygen (enzyme cholesterol oxidase)→ Cholestenone + Hydrogen peroxide

2. Hydrogen peroxide + 4-Aminophenazone + Phenol (enzyme peroxidase) → Colored complex.

Fig no.5.11 estimation of glucose tubes   fig no. 5.11.1 estimation of cholesterol tubes

5.6.2.2 Estimation of Serum Triglyceride (GPO/POD method)

Apparatus – Test tube, micropipette & pipette.

Chemical – Kit contains

Reagent 1Triglyceride enzyme reagent 1

Reagent 2 : standard 200mg/dl

Procedure

Reagents	Test	Standard	Blank

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Reagent 1	1000 μl	10 μl	10 μl
Standard		10μl	
Serum	10 μl		
Distilled water			10 μl

Table no 5.4 Procedure for triglyceride.

Mix well and incubate for 10 minutes at 37 °c. Final color is stable for 30 min. mix well and measure the absorbance of standard and sample against the reagent blank at 505nm.

Calculation–

Let O.D. of blank = B

O.D. of standard   = S

O.D. of test   = T

$$\text{Formula=} \frac{(T-B)}{(S-B)} \times 200 \, \text{mg/dl}$$

Reaction mechanism

a) Triglycerides + Water (Enzyme esterase)→ Glycerol + Carboxylic Acid

b) Glycerol + ATP (Enzyme glycerol kinase) → glycerol-3-phosphate + ADP

c) Glycerol-3-phosphate + Oxygen (Enzyme glycerol-3-phosphate oxidase) → Dihydroxyacetone phosphate + Hydrogen peroxide

d) Hydrogen peroxide + 4-Aminophenazone + 4-Chlorophenol (Enzyme peroxidase) → Colored complex

5.6.3 Estimation of total protein (Biuret method)

Principle:

The peptide bonds of protein react with copper II ions in alkaline solution to form a blue-violet ion complex, (the so called biuret reaction), each copper ion complexing with 5 or 6 peptide bonds. Tartrate is added as a stabiliser whilst iodide is used to prevent auto-reduction of the alkaline copper complex. The colour formed is proportional to the protein concentration and is measured at 546 nm (520-560).

Reagent Composition

Reagent1. Copper II Sulphate 12 mmol/l

 Potassium Sodium Tartrate 31.9 mmol/l

Potassium Iodide 30.1 mmol/l

Sodium Hydroxide 0.6 mol/l

Reagent2. Standard

Reagent Preparation: Reagents are liquid, ready to use.

Procedure

Reagents	Blank	Standard	Test
Working reagent	1000µl	1000 µl	1000 µl
Distilled water	20 µl	--	--
Standard	--	20 µl	--
Test serum		--	20 µl

Mix and incubate for 10 minutes (in case of automatic procedure incubate for 5 minutes) incubation in the dark. Absorbance of the sample A1 and the standard (calibrator) A2 against reagent blank is read in interval 30 minutes. Table no 5.5 Procedure for total protein.

Calculation: $\dfrac{\text{Absorbance of Total protein (g/dl)}}{\text{Absorbance of standard}}$ = x standard concentration

Fig no.5.12 Triglyceride tubes   fig no.5.12.Ttotal protein tubes

5.6.3.1 Estimation of albumin (BCG Method)

Principle:

Albumin binds with the dye Bromocresol green in buffered medium to form green coloured complex. The intensity of the colour formed is directly proportional the amount of albumin present in the sample.

Reaction

Acidic medium

Albumin + Bromocresol $\xrightarrow{\hspace{2cm}}$ Green albumin BCG complex

Procedure:

Reagents	Blank	Standard	Test
BCG reagent	1000 µl	1000 µl	1000 µl
Distilled water	10µl	-	-
Standard	-	10 µl	-
Serum	-	--	10 µl

Mix well and incubate for 1 minute at RT. Read the absorbance of standard and each test tube against reagent blank at630 nm on bio chromatic analyzer.

Table no 5.6 Procedure for albumin.

Calculation:

Albumin conc. (gm./dl) = $\dfrac{\text{Abs T} \times 4}{\text{Abs S}}$

Fig no. 5.13 Albumin tubes &analyzer

fig no. 5.13.1 instrument colorimeter

5.7 Histopathology of tissue

- At the end of the experiment, animals were sacrificed and their organs were removed.

- After the extraction of the testis from the animal's body, they were weighed and then promptly treated with 10% formaldehyde (fixation) in order to preserve its structure and molecular composition.

- After overnight fixing, organs samples were dehydrated; the piece of testis was dehydrated by bathing it successfully in acetone three changes one hour each.

- The acetone was then replaced with a solvent chloroform three changes one hour each miscible with the infiltration (clearing).

- As the tissues were cleared with chloroform, they became transparent (clearing).

- Once the tissue has been cleared by chloroform it was placed in melted paraffin in an oven maintained at 58°-60°C (infiltration).

- The heat caused the solvent to evaporate and the spaces within the tissues became filled with paraffin. The tissue together with its impregnating paraffin hardened after it had been taken out of the oven.

- The hard block containing the tissue was then taken to the microtome and sectioned by the microtome steel paraffin wax-embedded and cut in sections (5μm) then mounted on slides.

- The sections were then floated on water and transferred to a glass slide and after deparaffinization in xylene; sections were rehydrated through a graded ethanol series, stained with Haematoxylin-Eosin (HE) and dehydrated through xylene.

Fig no. 5.14 Testis tissue for histopathology from diabetic control rat

Fig no. 5.15 Pair of testis from diabetic + green tea rat

CHAPTER 6

Data Analysis and Result

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6.1 Statistical data analysis

Result of effect of GTE (200mg/kg B.W) on body weight, different biochemical parameter and Histo-architecture are presented below table no Statistical analysis were carried out by Microsoft excel 2007. All data of initial day, 7th day and 14th day were expressed in as Mean ± SD. Statistical analysis was performed using two samples assuming unequal variance t-test and ANOVA Analysis of Variance two factors without replication test for compared differences between two groups that were compared each by using one way ANOVA, with *P*<0.05 considered statistically significant.

6.1 Effect of GTE after 7th day determined by different biochemical analysis and data

Groups	FBS	Protein	Albumin	Glob	TC	TG
Normal control	78±17	7.8±0.09	4.5±0.75	3.25±0.68	91.8±13.97	49±4
Normal + GT	92±11	7.7±0.24	5.3±0.33	2.38±0.47	77.2±12	44±5.1
Diabetic control	151±15	7.4±0.6	4.2±0.25	3.25±0.50	93.5±13.9	60±7.3
Diabetic + GT	137±5	7.1±0.8	4.1±0.45	3±0.52	74.66±13.4	57.16±12

Table No.6.1: Data has been reported as Mean ± SD in all four groups after 7th day.

Mean ± SD

Effects of green tea extract on different parameters are reported as mean ± SD of blood sugar, Total protein, albumin, globulin, TG and cholesterol in all 4 groups at the 7[th]day are presented in table No 6.1 (1). Biochemical parameter was not significant different between groups because *p value* is more than 0.05, when it compared to normal. One way Analysis of Variance was used for statistical significance assessment, with 95% confidence interval shown in table no 6.1 (2).

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Comparison of different biochemical parameters of different 4 groups are presented in graphical form fig no 6.1(a).
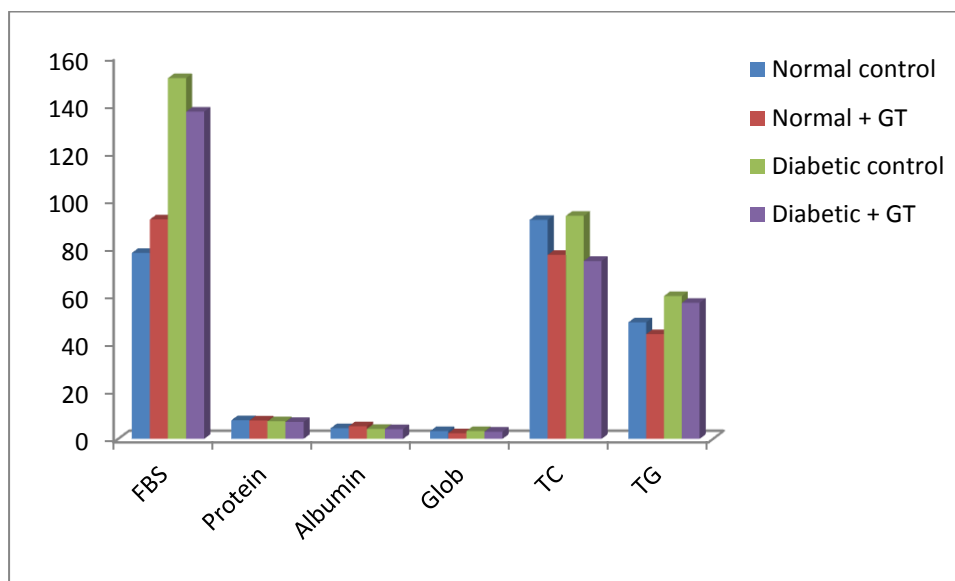
Fig no. 6.1 (a) comparison graph for biochemical data after 7th day

6.2 Effect of GTE after 14th day determined by different biochemical analysis and data

Effects of green tea extract on different parameters are shown in Table 4.3 (1). The mean of blood sugar, Total protein, albumin, globulin, TG and cholesterol in all 4 groups at the 14[th]day are presented in table no. 6.2.2.Biochemical parameter was not significant different between groups because *p value* is more than 0.05, when it compared to normal. One way Analysis of Variance was used for statistical significance assessment, with 95% confidence interval shown in table no 6.2.2. Comparison of different biochemical parameters of different 4 all groups are

Groups	FBS	protein	Albumin	Globulin	Cholesterol	TG
Normal control	80±16	6.5±0.6	4.5±0.1	2.1±0.62	92±23	**49±6**
Normal + GT	78±14	6.4±0.3	5.3±0.26	2±0.24	70±5.2	**42±7**
Diabetic control	154±12	6.7±0.4	4.2±0.20	3.2±0.29	89±2.4	**78±14**
Diabetic + GT	122±5.7	5.6±0.5	4.1±0.4	2.6±0.78	76±13.5	**55±24**

Table No. 6.2.1: Data has been reported as Mean ± SDin all four groups after 14th day.

Graphically present in graph no. 6.2 (a)
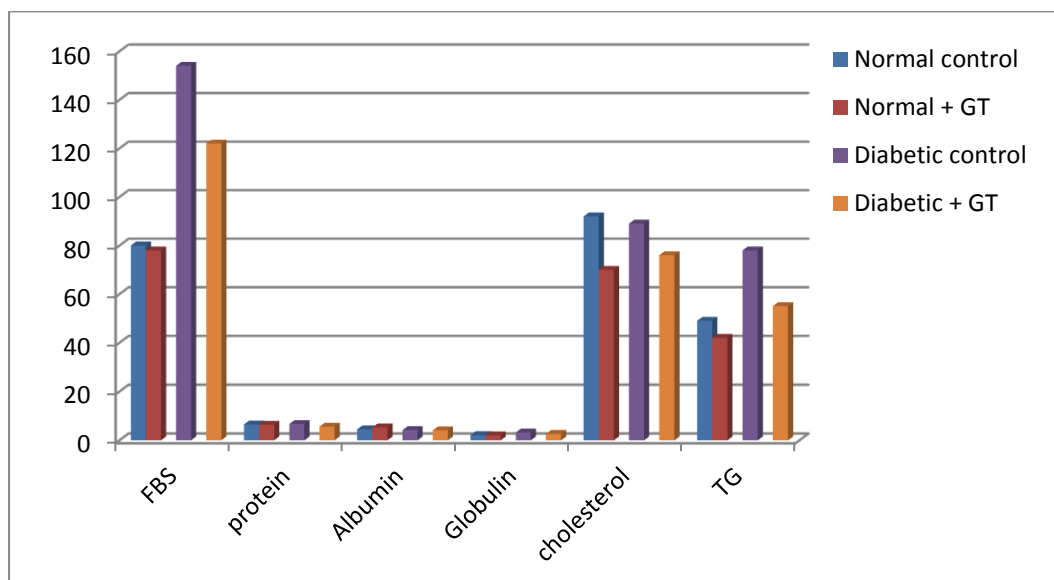
fig no. 6.2 (a) comparison graph for biochemical data after 14th day

ANOVA: Two-Factor Without Replication
For 7th days data

SUMMARY	Count	Sum	Average	Variance
Normal control	4	462.8479	115.712	7559.939
Normal +GT	4	472.1276	118.0319	7840.938
Diabetic control	4	495.8071	123.9518	3393.159
Diabetic+ GT	4	477.8283	119.4571	4705.769
B.W	4	889.1633	222.2908	740.4046
FBS	4	459.5824	114.8956	1219.205
TG	4	210.5	52.625	55.8588
Cholesterol	4	349.3652	87.34129	74.62999

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Groups	144.5807	3	48.19356	0.070807	0.974116	3.862548
Variables	64373.7	3	21457.9	31.52629	4.19E-05	3.862548
Error	6125.716	9	680.6351			

Total	70643.99	15		

Table No. 6.1.2 One way ANOVA was used for statistical significance assessment, with 95% confidence interval p value (>0.05) of both groups and variables was not significant.

ANOVA: Two-Factor Without Replication
for 14th days data

SUMMARY	Count	Sum	Average	Variance
Normal control	4	470.4421	117.6105	8015.848
Normal + GT	4	458.2384	114.5596	8349.921
Diabetic control	4	509.0251	127.2563	2681.753
Diabetic +GT	4	464.9349	116.2337	4741.479
B.W	4	894.9667	223.7417	931.0745
FBS	4	433.6828	108.4207	1350.428
TG	4	226.5303	56.63258	247.3582
Cholesterol	4	347.4608	86.8652	49.98852

ANOVA

Source of Variation	SS	Df	MS	F	P-value	F crit
Groups	389.7486	3	129.9162	0.15915	0.921122	3.862548
Variables	64020.2	3	21340.07	26.14208	8.92E-05	3.862548
Error	7346.8	9	816.3111			
Total	71756.75	15				

Table No. 6.2.2 One way ANOVA was used for statistical significance assessment, with 95% confidence interval p value (>0.05) of both groups and variables was not significant.

6.3 Effect GTE on serum glucose value

Effect of green tea extract observed after 7th and 14 day it is shown graphicallyfig no 6.3 (a). Serum glucose level is significant ($p > 0.05$) when compared to normal as it is shown in table no 6.3.2. Data of 7th and 14th day has been reported in mean ±SD are shown in table no 6.3.1.

Groups	Day 7th	Day 14th
Normal control	78±17	80±16
Normal + GT	92±11	78±14
Diabetic control	151±15	154±12
Diabetic +GT	137±5	122±5.7

Table no. 6.3.1: Data has been reported in mean ±SD day 7th and 14th.

Mean ± SD

Fig no 6.3(a) : comparison of serum glucose data from 7th to 14th days b/w different 4 groups.

T-Test: Two-Sample Assuming Unequal Variances

	Diabetic control	Diabetic green tea
Mean	151.0752688	137.6666667
Variance	230.6625043	25.06666667
Observations	6	6
Hypothesized Mean Difference	0	
Df	6	
t Stat	2.053851288	
P(T<=t) one-tail	0.042891726	
t Critical one-tail	1.943180281	
P(T<=t) two-tail	0.085783452	
t Critical two-tail	2.446911851	

Table no 6.3.2: Blood glucose level is significant p vale (<0.05) when compare to diabetic control.

6.4 Effects of GTE on total protein

Effect of GTE observed after 7th and 14 day it is shown graphically fig no 6.4
(a). Serum total protein level is significant ($p > 0.05$) when compared to normal
as it is shown in table no 6.4.2. Data of 7th and 14th day has been reported in
mean $\pm$SD are shown in table no 6.4.1.

Groups	Day 7th	Day 14th
Normal control	7.8±0.09	6.5±0.60
Normal + GT	7.7±0.24	6.4±0.39
Diabetic control	7.4±0.63	6.7±0.45
Diabetic +GT	7.1±0.80	5.6±0.59

Table no. 6.4.1: Data has been reported in mean $\pm$SD day 7th and 14th.
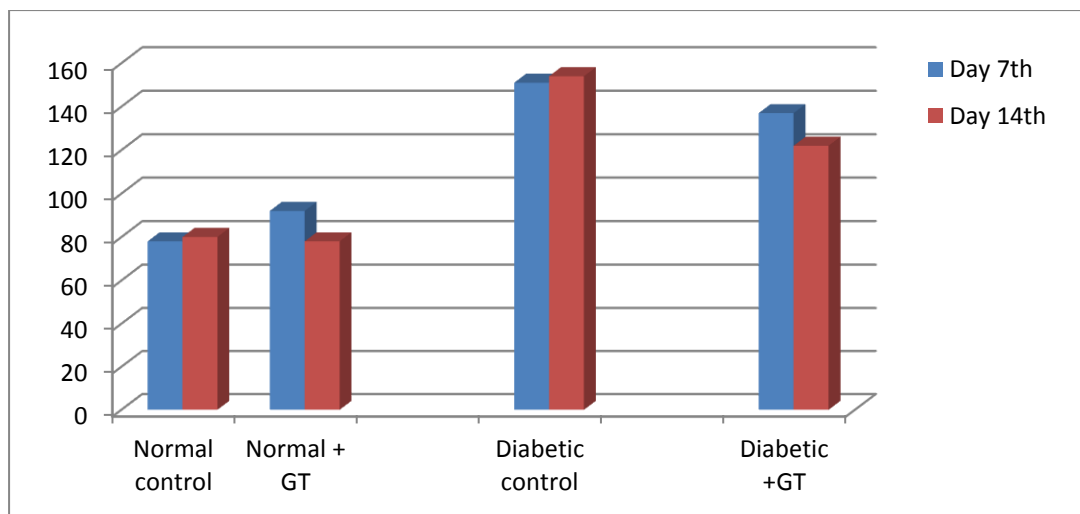
Mean $\pm$ SD
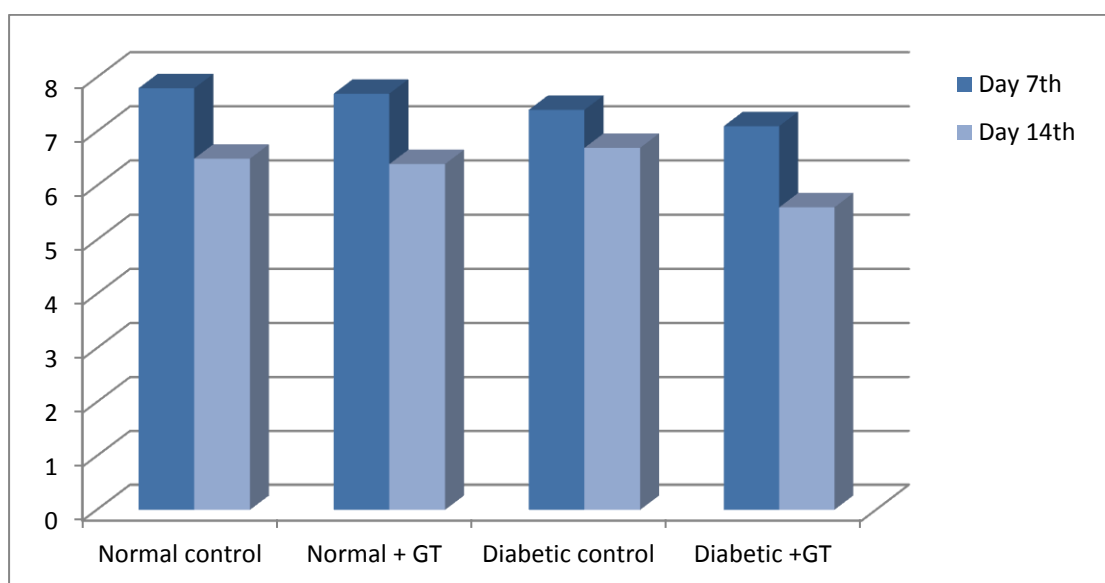
Graph no 6.4(a) : comparison of serum total protein data from 7th to 14th days
b/w different 4 groups.

t-Test: Two-Sample Assuming Unequal Variances

	Diabetic control	Diabetic Green tea
Mean	6.714286	5.69047619
Variance	0.204082	0.354421769
Observations	6	6
Hypothesized Mean Difference	0	
Df	9	
t Stat	3.35569	
P(T<=t) one-tail	0.004223	
t Critical one-tail	1.833113	
P(T<=t) two-tail	0.008447	
t Critical two-tail	2.262157	

Table no 6.4.2**:** Blood total protein level is significant p vale (<0.05) when compare to diabetic control.

6.5 Effects of GTE on albumin

Effect of GTE observed after 7th and 14 day it is shown graphically fig no 6.5 (a). Serum albumin level is significant (p>0.05) when compared to normal as it is shown in table no 6.5.2. Data of 7th and 14th day has been reported in mean ±SD are shown in table no 6.5.1.

Groups	Day 7th	Day 14th
Normal control	4.5±0.75	4.3±0.18
Normal + GT	5.3±0.33	4.4±0.26
Diabetic control	4.2±0.35	3.5±0.20
Diabetic +GT	4.1±0.45	2.9±0.41

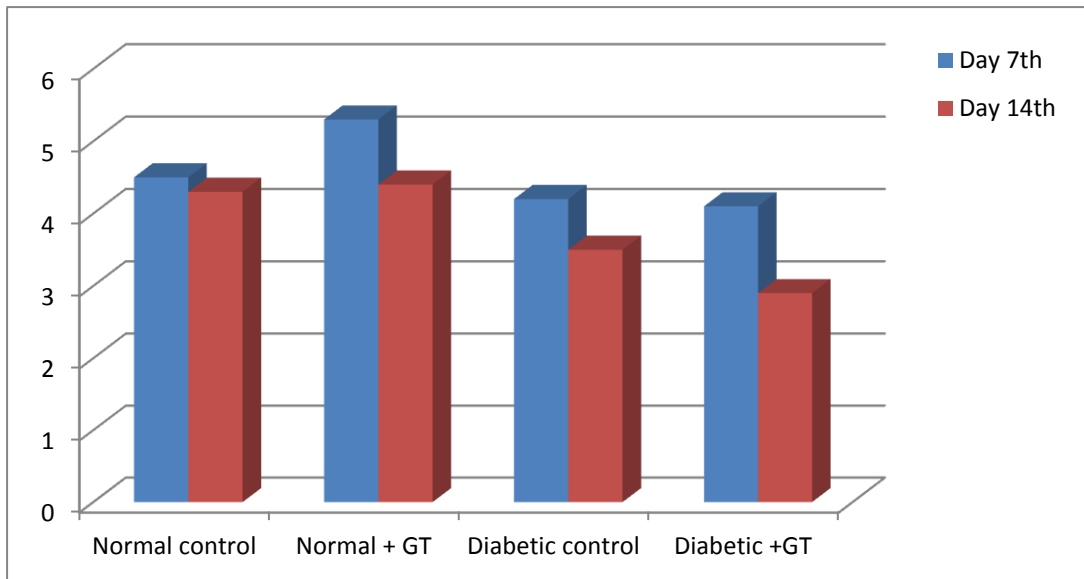Table no. 6.5.1: Data has been reported in mean ±SD day 7th and 14th.

Mean ± SD

Fig no 6.5(a) : comparison of serum albumin data from 7th to 14th days b/w
different 4 groups.

t-Test: Two-Sample Assuming Unequal Variances

	Diabetic control	Diabetic Green tea
Mean	3.5	2.922222
Variance	0.040444	0.171407
Observations	6	6
Hypothesized Mean Difference	0	
Df	7	
t Stat	3.074824	
P(T<=t) one-tail	0.008973	
t Critical one-tail	1.894579	
P(T<=t) two-tail	0.017945	
t Critical two-tail	2.364624	

Table no 6.5.2: Total protein level is significant p vale (<0.05) when compare
to normal

6.6 Effect of GTE on cholesterol

Effect of GTE observed after 7th and 14 day it is shown graphically fig no 6.6
(a). Serum cholesterol level is significant ($p>0.05$) when compared to normal

as it is shown in table no 6.6.2. Data of 7th and 14th day has been reported in mean ±SD are shown in table no 6.6.1.

Groups	Day 7th	Day 14th
Normal control	91.8±13.97	92±23
Normal + GT	77.2±12	70±5.2
Diabetic control	93.5±13.9	89±2.4
Diabetic +GT	74.66±13.4	76±13.5

Table no. 6.6.1: Data has been reported in mean ±SD day 7th and 14th.

Mean ± SD

Fig no 6.6 (a) : comparison of serum cholesterol data from 7th to 14th days b/w different 4 groups.

t-Test: Two-Sample Assuming Unequal Variances

	Diabetic control	Diabetic Green tea
Mean	89.2778	76.4379085
Variance	6.24074	184.8276304
Observations	6	6
Hypothesized Mean Difference	0	
Df	5	
t Stat	2.27532	
P(T<=t) one-tail	0.03598	
t Critical one-tail	2.01505	

P(T<=t) two-tail	0.07196	
t Critical two-tail	2.57058	

Table no 6.6.2 : cholesterol level is significant p vale (<0.05) when compare to diabetic control.

6.7 Effects of GTE on Triglyceride

Effect of GTE observed after 7th and 14 day it is shown graphically fig no 6.7 (a). Serum triglyceride level is significant ($p>0.05$) when compared to normal as it is shown in table no 6.7.2. Data of 7th and 14th day has been reported in mean $\pm$SD are shown in table no 6.7.1.

Groups	Day 7th	Day 14th
Normal control	49±4	49±6
Normal + GT	44±5.1	42±7
Diabetic control	60±7.3	78±14
Diabetic +GT	57.16±12	55±24

Table no. 6.7.1: Data has been reported in mean $\pm$SD day 7th and 14th.
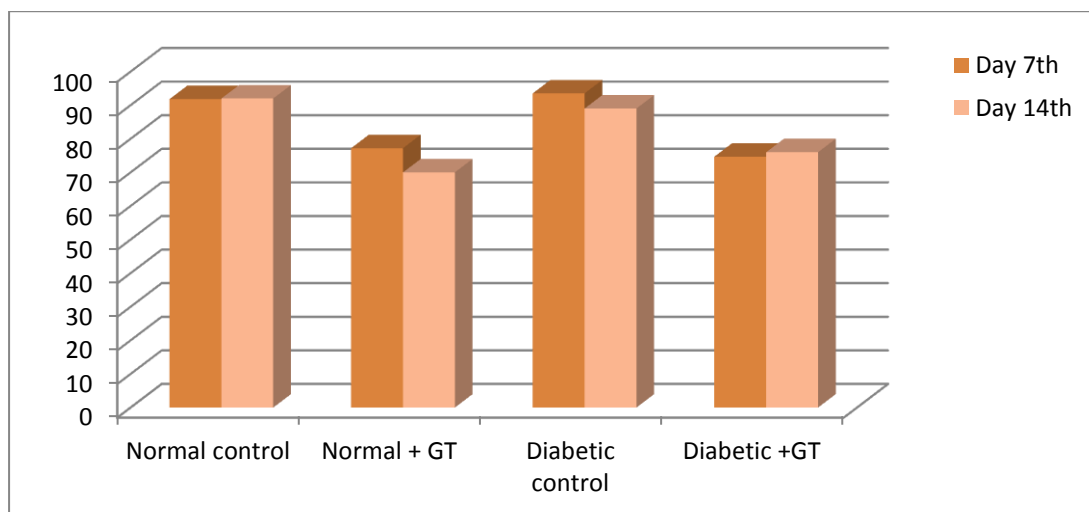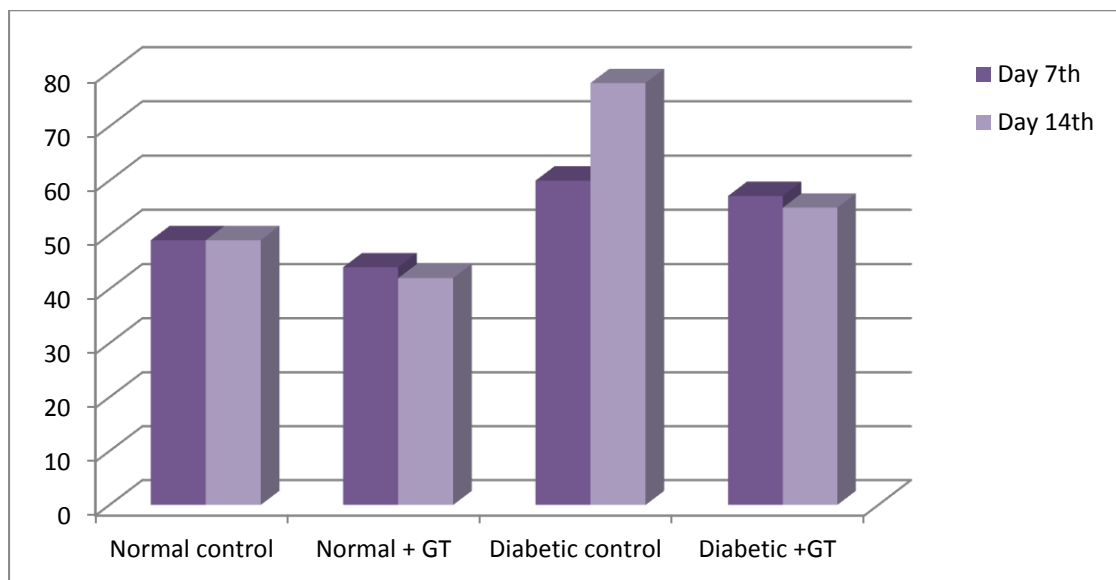
Mean ± SD

Fig no 6.7 (a): comparison of serum triglyceride data from 7th to 14th days b/w different 4 groups.

t-Test: Two-Sample Assuming Unequal Variances

	Diabetic control	Diabetic Green tea
Mean	78.83333	55.6969697
Variance	204.1667	597.6550964
Observations	6	6
Hypothesized Mean Difference	0	
Df	8	
t Stat	2.00139	
P(T<=t) one-tail	0.040171	
t Critical one-tail	1.859548	
P(T<=t) two-tail	0.080343	
t Critical two-tail	2.306004	

Table no 6.7.2: Triglycerides level is significant p vale (<0.05) when compare to diabetic control.

6.8 Effect of GTE on body weight

The means of initial, 7th and 14th day body weight in 4 groups at the beginning and at the end of study has been reported as mean ± SD are shown in table no. 6.8.1 Body weight of the Diabetic control were decreases as compare to normal control and after administration of green tea extract body weight improves in diabetic + GT rats compared to Diabetic control. Difference between body weight from 0 day to 14 day are graphical presented in fig no 6.8(a).

The results showed that the mean of 7th and 14th day body weight in both of diabetic control female rats and diabetic rats treated with 200 mg/kg green tea were significantly lower than those of normal control rats But at the 14th day of the study, the body weight of the diabetic rats treated with green tea increase the weight as compare to diabetic control.

Groups	0 day	7th days	14th days
Normal control	238±17.9	248±23.1	275±26.6
Normal green tea200mgkg⁻¹ B.W	255±28.1	270±32.1	299±39.6
Diabetic control	265±20.9	255±20.9	250±18.6
Diabetic green tea	274±15.9	257±13.8	226±16.6

Table No. 6.8.1 : Data has been reported as Mean ± SD in all four groups from 0 day, 7th day and 14th day.
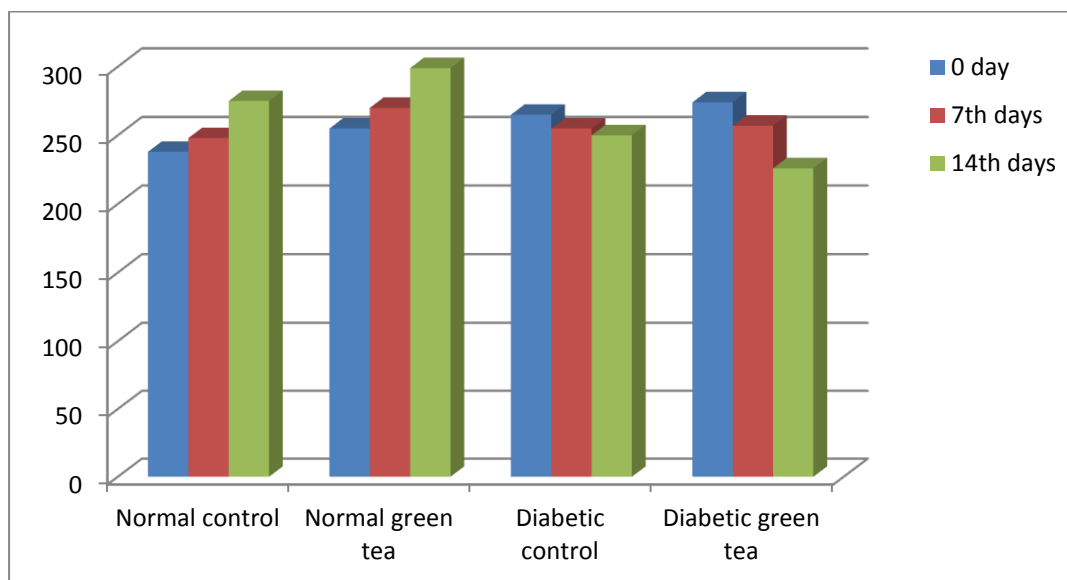
Mean ± SD

Fig no 6.8 (a): comparison of body weight data from initial day,7th to 14th days b/w different 4 groups.

6.9 Effect of GTE on total testosterone

Effect of GTE observed after 14th day it is shown graphically fig no 6.9 (a). Data of 14th day has been reported in mean are shown in table no 6.9.1

Groups	Day 14th
Normal control	45
Normal + GT	40
Diabetic control	7.75
Diabetic +GT	5.68

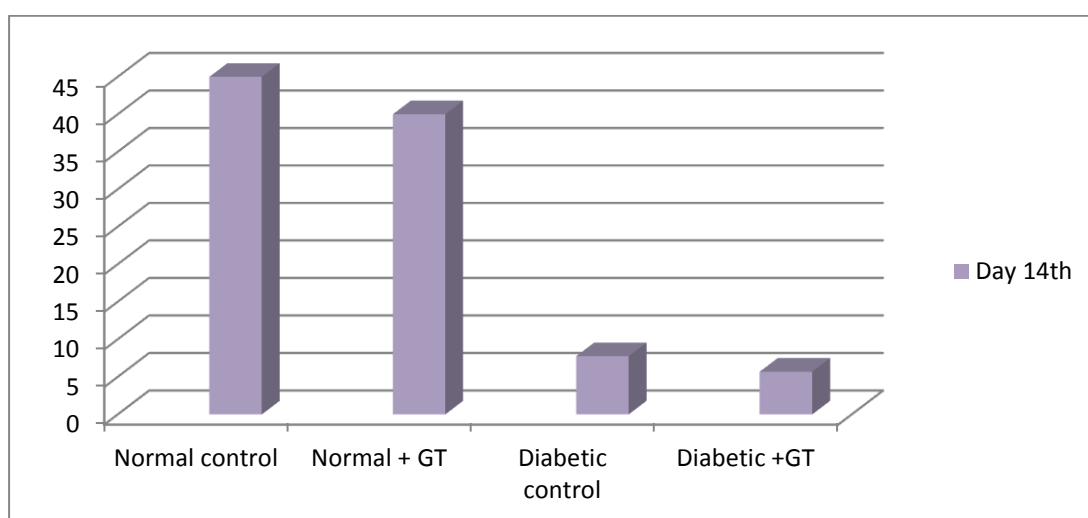**Table No. 4.9.1 :** Data has been reported as Mean all four groups from 14th day.

Mean

Fig no 6.9 (a): comparison of testosterone data from 14th days b/w different 4 groups.

6.10 Histopathology of testis

The overall histological appearance of biopsy tissue from the impotent diabetic was one of minimal to moderate pathology, with variation in the appearance of individual seminiferous tubules ranging from relatively normal to severely affected. There was apparent germ cell depletion of the seminiferous epithelium and malorientation of spermatids. Some tubules had greatly thickened walls (i.e., both cellular and noncellular components) and were nearly devoid of all germ cells.

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In testes from diabetic animals there were some degenerating pachytene nuclei and frequent sloughed pachytene spermatocytes in the tubule lumen, resulting in a thinned germinal epithelium. Alterations of intratubular structure were most apparent in the adluminal testicular compartment which presented as altered apical Sertoli cell cytoplasm and variably depleted germ cell populations. Seminiferous tubule walls were noticeably thickened in the testes of impotent diabetic men, as they are in the testes of diabetic rats. It is possible that the deleterious effects of diabetes on testicular structure and function may have resulted from accelerated tissue aging since thickening of the seminiferous tubule wall, peritubular and intertubular fibrosis, and tubular sclerosis are degenerative changes seen in testes from both impotent diabetics rats.
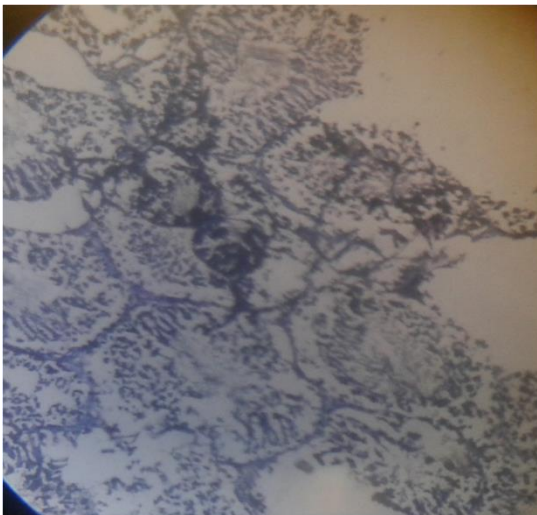

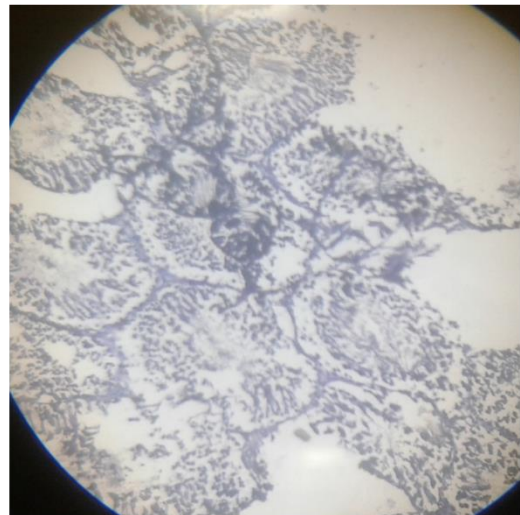Fig no 6.10.1 normal control testicular testicular
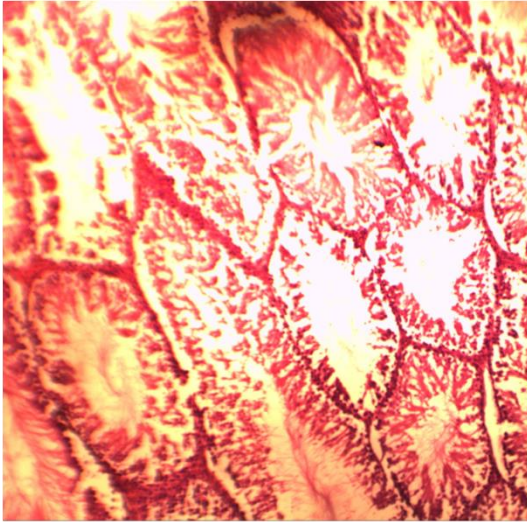

fig no 6.10.2 normal+green tea

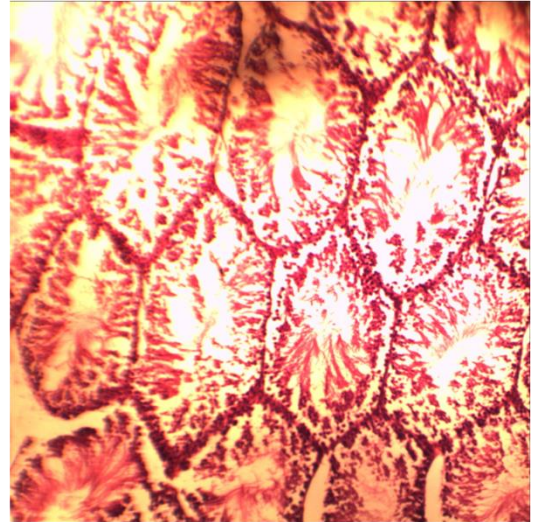Fig no 6.10.3 diabetic control testicular testicular

fig no 6.10.4 diabetic+green tea

CHAPTER 7

Discussion

Diabetes mellitus (DM) is primary or idiopathic disease that deals with the chronic metabolic disorder involving carbohydrate, lipid and protein by occurrence of insulin deficient or disorder. Other conditions like arteriosclerosis (hardening and loss of elasticity of the wall of arteries), nephropathy (kidney damage), neuropathy (peripheral nerves dysfunction) and micro-anginopathy (thickening and weakening of capillary walls) can also devoted through DM (Camilleri, 2008).

In modern societies, the DM is one of the most leading public health threats and in whole world. Its prevalence increased more rapidly day by days. According to WHO (World Health Organization in 2000), 171 million people were recorded with DM which showed that there were 60% elevation comparatively to 1995 (WHO, 2002).

DM is responsible for biochemical variation and other pathological changes that adapt male fertility. The sexual dysfunction such as spermatogenesis, retrograde ejaculation or erectile dysfunction occurs and it end up with decreased sexual appetite in diabetic individuals. DM affect on pathways by multiple molecular mechanisms with dramatic consequences to male reproductive functions. DM decreased the sperm quality and functioning competitively to normal. Alteration in testicular cells is concerned with glucose metabolism. Specific mechanisms hormonal control and glucose sensing machinery may also play critical role in sub fertility and fertility correlated to DM. many other reports also identify that the diabetes mellitus correlate with degradation of hormones particularly sex hormones. IDDM is correlated to decrease the motility and vitality of semen and decreased semen ejaculation without change in viscosity of sperm . Alteration in insulin can change the primary sexual glands functions and testicular functions. Which cause concentrate seminal insulin than that serum insulin.

Ejaculation alteration is a common problem in diabetes. The most probably disorder is retrograde ejaculation in which semen flows backward direction in to the bladder instead of flowing through anterior urethra. This usually result

in the patient which allow the pumping or pushing sensation correlated with ejaculation without semen come up from his penis. After ejaculation the urine which passes is appear cloudy. For conformation the diagnosis is based on finding of high number of spermatozoa in a orgasmic specimen of urine.

The experimental rats were divided into four groups each group contain 06 animals. These are Group-I (Control); Group-II (normal+green tea); Group-III (Diabetic control and Group-IV (diabetic+green tea). The total experimental protocol was maintained for 14 days after induction of diabetes. A normal mouse has a blood glucose level of around 100mg/dl (ranging from 60-130) after a 4-hour fast. This is close to humans, where 80-120mg/dl is considered normal. *Mus musculus* has tremendous reproductive potential.

Changes in body weight in different groups of rats were shown in Table: 6.8.1. There was a significant reduction in body weight at days 07 and days 14 in diabetic control (Group-II) when compared to control mice ($p<0.05$, $p<0.01$). The gradual reduction in body weight in Group- IV was diabetic + green tea when compare with normal control. The results obtained in present work clearly show the effects of *GTE* powder on the body weight in Streptozotocin–monohydrate induced diabetic mice. There was a slight decrease in body weight in Group-III, Group-IV and group- II when compared with Group-II ($p<0.05$, $p<0.01$).

The Fasting Blood Glucose Level (Table 6.3.1) of albino diabetic rats showed increasing trend with the increase of period (07 and 14). In this study, *GTE* powder at a dose of 200 mg kg^{-1} bw^{-1} was evaluated in normal and diabetic rats. The control animal showed 78 ± 17-80 ± 16 mg/100 ml of blood glucose in control animal during experiment which was found to be increased to 80 ± 16 mg/100ml at 14 days of Group-I. There was decreasing trend in the blood glucose level in Group-II when diabetic rats supplemented with *GTE* powder. It has been seen that treatment with *GTE* powder brought down FBGL from a higher value of 92 ± 11 mg/dl which is reduction from higher value on 14th day

upto 78±14 mg/dl. On other hand there were not seen any significant reduction in FBGL in Group III and Group IV after 14th day.

The Total Cholesterol of control rats (Group-I) ranged from 91±13 mg/dl to 92±23 mg/dl at day 7 to 14 day. There was a significant decrease in Total Cholesterol at all exposure periods ($p < 0.05$, $p < 0.01$) in diabetic control (Group- III) and diabetic + green tea (Group-IV) when compared to control rat.

The Serum Triglyceride of control rat showed 49±4 mg/dl to 49±6 mg/dl during all experimental periods. Not as much as Significant increase in Triglyceride was observed at days 7 and days 14 in Group- III and Group-IV ($p < 0.05$; $p < 0.01$) when compared with Group-I. Diabetic mice when treated with *GTE* showed Triglyceride decreased from 44±5.1 mg/dl to 42±4 mg/dl at day 14 but not at high decreased. Supplementation of *GTE* powder not significantly reduced but little bit, the Total Cholesterol and Triglycerides in serum as compared to the diabetic group.

The serum total protein of control rat showed 7.8±0.09 to 6.5±0.60 gm/dl at 14the day. The total protein in treated rats were 7.7±0.2 to 6.4±0.39 in normal green rea, 7.4±0.63 to 6.4±0.45in diabeteic control and 7.5±0.80 to 5.6±0.59 gm/dl. There were significant difference as compare with normal group ($p < 0.05$; $p < 0.01$)

The serum testosterone of control group show 45 and 40 ng/dl respectively and the treated group show very low value 7.75 in diabetic group and 5.68 ng/dl in diabetic + green tea group.it showed that diabetes decreased the reproductive hormones level, but still remaine to find out that weither it can treated with GTS or not? Because there were not seen any changed value in green tea and diabetic + green tea group.

To conclude it can be said confidently that *GTE* powder supplementation play a beneficial role in maintaining blood

glucose level and decreased the body weight in streptozotocin induced diabetic mice.

Histological study of testis of control rat (Group-I) and *GTE* powder supplemented rat (Group-IV) showed normal features. Results from this study demonstrate structural pathology in three areas of testes collected from impotent diabetic rat the seminiferous epithelium, 2) the seminiferous tubule wall, and 3) the interstitial compartment. Although the germ cell population of seminiferous epithelia clearly appeared affected quantitatively and in some tubules overt germ cell depletion was obvious, the overall impression of germ cell ultrastructure was one of relative normalcy.There was apparent germ cell depletion of the seminiferous epithelium and malorientation of spermatids. Some tubules had greatly thickened walls in normal group and same feature were seen in another group also. Seminiferous tubules were seen in all 4 groups there were not significantly changes in it.

Seminiferous Epithelium, Although altered germ cell morphology was not apparent, Sertoli cell pathology was. This was expressed as degeneration of apical Sertoli cell cytoplasm and structural alterations of those junctional specializations associated with acrosome phase spermatids. These types of specific structural alterations of Sertoli cells have been reported in the diabetic rat model. But there were no seen any changes in all group section.

Future study and conclusion

Following conclusion and future prospected can be drawn from the present investigation and results:

- ✓ Supplementation of *GTE* powder at fixed dose (200mg kg^{-1}bw^{-1}) orally two times a day in streptozotocin monohydrate induced diabetic mice caused hypoglycemic, hypotriglyceridemic and β-cell protective activities.

- ✓ There was a significant reduction in body weight in diabetic control, normal + green tea and diabetes+ green tea when compared to control rat. The gradual reduction in body weight in diabetic rat was ameliorated by supplementing fixed dose of *GTE* powder in drinking solution.

- ✓ The liqiud intake was found to be increased in diabetic control due to thirstily condition and this condition was treated by supplementing fixed dose of *GTE* powder in their food.

- ✓ It has been seen that treatment with *GTE* powder brought down FBGL from a higher

- ✓ Regarding the histological analysis (testis) of no significant pathological changes occurring in diabetic mice treated with *GTE* powder, it is noted discrete changes which confirms favourable development after three weeks of treatment and beneficial role of administration of these herbal remedies. We noted a correlation between changes in biochemical parameters determined and the degree of tissue deterioration.

- ✓ No hepatotoxicity, nephrotoxicity and reprotoxicity or other acute adverse effects were observed when *GTE* powder is treated in different groups of rat.

- ✓ Fertility rate (%) was 77.5% which was found minimum in diabetic control and this was increased to 82.5% in treated rat.

- ✓ Diabetes having the effects on male reproductive system and reproductive hormones.

- ✓ Diabetes having high effect on reproductive hormones cause the ED, RE, libido and other MSD and mostly seen to low level of testosterone.

- ✓ In future there will be need to more focus on the treatment of reproductive system with herbal medication.

- ✓ Future study can also done on Oral Glucose Tolerance Test and Glycated Hemoglobin (HbA1c) Test can be investigated.

- ✓ Hormone and enzymatic assay can be done to know the real biochemistry behind it.

- ✓ Genetically estimation can be done for the concerned organs

CHAPTER 9

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CENTRAL ANIMAL HOUSE FACILITY (CAHF)

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CERTIFICATE

This is to certify that the project titled *"Effect of green tea (Camellia sinensis) extract on testicular activity in   streptozotocin induced diabetic male rat model"* has been approved by the IAEC.

Name of Principal Investigator: Dr. Pranay Punj Pankaj

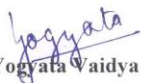**IAEC approval number:** LPU/LSPS/IAEC/CPCSEA/MEETING NO. 5/2014/2015 PROTOCOL NO.7

Date of Approval: 31/01/2015

Animals approved: 36 Rats

Remarks if any: - One animal from each group will be sacrificed for the study, 30 animals will be rehabilated , Protocol no 4 and 7 will be shared, No fresh animal will be given

Dr. Monica Gulati

Biological Scientist,
Chairperson IAEC

Mrs. Yogyata Vaidya

Scientist, COD Pharmacology

Mr. Bimlesh Kumar

Scientist In-Charge of Animal House