Effect of Green Tea (*Camellia Sinensis*) Extract on the Histoarchitecture of Reproductive Organs in Streptozotocin Induced Diabetic Female Rat Model

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Submitted by

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2015

DECLARATION

This is to submit that this written submission in my thesis entitled **"Effect of green tea (*Camellia sinensis*) extract on the Histoarchitecture of reproductive organs in Streptozotocin induced diabetic female 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
Place: Punjab, India

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CERTIFICATE

This is to certify that the present thesis entitled **"Effect of green tea (*Camellia sinensis*) extract on the Histoarchitecture of reproductive organs in Streptozotocin induced diabetic female rat model"** is the outcome of the original piece of work carried out by Miss. Savita Devi (Registration No: 11300727) herself under my guidance and the contents of her thesis did not form a basis of the award of any previous degree to her 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.

for Pankaj

(Dr. Pranay Punj Pankaj)

Supervisor

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Research is an arduous task, which despite of hard labour also requires patience and courage try - try and try again. This would only be made possible with the benediction and grace of almighty.

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Place: LPU, Punjab Savita

DEDICATION

I dedicate my dissertation work to my guide Dr. Prnay Punj Pankaj whose tremendous support and encouragement words has given me the strength.

I also dedicated this dissertation to my many friends who have supported me throughout the process.

Table of contents

List of abbreviations

DM	Diabetes Mellitus
T1DM	Type one diabetes mellitus
T2DM	Type two diabetes mellitus
GDM	Gestational Diabetes Mellitus
^{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	High Performance Liquid Chromatography
µl	Microlitre
C	Catechin
GC	Gallo Catechin
FSD	Female sexual dysfunction
ECG	Epicatechin gallate
ECGC	Epicatechin gallate catechin

List of Tables

18	T test data has been reported p value of glucose	5.5(2)
19	T test data has been reported p value of protein	5.6(1)
20	T test data has been reported p value of albumin	5.7(1)
21	T test data has been reported p value of TC	5.8(1)
22	T test data has been reported p value of TG	5.9(1)

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ABSTRACT

This study has been undertaken to investigate the Effect of green tea (*Camellia sinensis*) extract on the Histoarchitecture of reproductive organs in STZ induced diabetic female rat model. The aim of study is to assess Histo-architecture and biochemical effects of oral administration green tea extracts on reproductive function in female albino rats. Female rats were administered the green tea extract orally for 14 days, while the control group received only normal diet.

In this assay, twenty four (24) female albino rats of Wistar strain weight (150-200 g), age 3 months, were obtained from NIPER, Mohali. The rats (24) were divided into four groups of 6 each rats according to the design of experiment and treatment. Animals in group (I) are Non diabetic rats with Normal diet and consider as a normal control. Animals in group (II) Non- diabetic rats were treated with orally green tea extract 200mgkg^{-1} body weight. Animals in group (III), (IV) groups of 6 rats each were administered with dose of STZ. Group (III) animals were administered multiple dose of STZ (45mgKg^{-1}bw) i.e. dissolved in Normal saline. Group (IV) animals administered with Single dose of STZ (45mgKg^{-1}bw) i.e. dissolved in sodium citrate 200mgkg^{-1} body weight; green tea extract orally. Treatment design was selected to evaluate histo-architecture and biochemical effects of green tea extracts on reproductive function in female albino rat. Data were analyzed by ANOVA test and t test. At the end of experimental period, animals were sacrificed and their blood and ovary samples were collected for the analysis. Ovaries and uterus were removed for histopathology Based on the results, it is indicative that the supplementation of green tea extract has strong capability to decrease in serum glucose and total cholesterol levels and significantly improved the body weight loss in diabetic rats treated with 200 mg/kg green tea in comparison to diabetic control group because of the strong antioxidant effect of the phenols. And also effects on the hormones. No significant changes were observed in protein and albumin. To conclude that green tea extract has anti-hyperglycemic and hypocholesterolmic effect in diabetic rats, although further work is needed to effects and mechanism of the green tea on the histoarchetiture of the ovary and study of green tea on ovary cancer and breast cancer.

CHAPTER-I

GENERAL INTRODUCTION

1.1 Diabetes Mellitus

Diabetes mellitus (DM) is a group of metabolic disorders. It is characterized by chronic hyperglycemia in which the blood glucose is higher than the normal range .It may be due to the insulin deficiency or improper response of insulin cell receptors, resulting in disturbance of carbohydrates, proteins and lipid metabolism (Jayakar *et al*., 2003; Bastaki, 2005). Hyperglycemia or raised blood sugar is a cause of serious micro and macro vascular diseases which affects nearly or every system of the body especially the nerves and blood vessels. The resultant hyperglycemia produced the symptoms such as polyuria, polydipsia, and polyphagia and causes the complication (Scheen *et al*., 2013; Sandra et al., 2008).

Globally, 382 million people were detected with diabetes in 2013, and the number is expected to project to 592 million by 2035. The severity of diabetes is more in low and middle income countries with 80% of the population contributing to the global statistics (Bastaki, 2005; IDF, 2014). Close to four million deaths in the age group of 20-79 were recorded due to diabetes in 2010, accounting for 6.8% of global allcause mortality in this age group. There has been a 5.5% increase in diabetic deaths in 2010, as compared to the 2007 statistics (Scheen *et al*., 2013; IDF, 2014).

1.2 History

DM history is very old but modern civilization and its life style has become the prime cause of the epidemic of this disease in the present time. The occurrence of DM is mainly due to the high content of starchy diets, fast food, and intake of tinned or preserved food, preservatives used in food and beverages and the stressful life style are the contributors of DM (Brock, 1993). Diabetes name was first recorded in the second century AD by a Greek physician, Aretaevs. Thomas Willis (1621-1675) sugary taste of urine is reported by an English anatomist and physician. The French physiologist, Claude Bernard (1813-1878) studied sugar role in the body and firstly provided a rational basis for understanding DM. In 1889, Von Mering, a German doctor reported and proved that DM arose is mainly connected with a malfunction in the pancreas. Isolation of insulin from pancreatic tissue is done by Sir Frederick Banting and Charles Best in 1922. It was very important for the proper utilization of sugar were reported by John McLeod. DM is known to Indians from

Vedic period onwards by the name *Asrava* (*Prameha*). Age-old Unani system acknowledged diabetes and evolved for effective cure for diabetics. At that time Traditional Hakeems with their vast knowledge and good experience that evolved potent medicine for relief from DM. Now, modern medicine or allopathy has scaled great heights in research and treatment for DM, it has not provided a permanent cure to diabetes till date.

1.3 Diagnosis of Diabetes mellitus

Clinical diagnosis of DM is made by the presence of hyperglycemic symptoms such as polyuria polydipsia, polyphagia and glycosuria. The World Health Organization (WHO) established the criteria for defining diabetes when the levels will 126 mg/dl Fasting Plasma Glucose (FPG) or more or Post-Prandial (PP) 2-h Plasma Glucose (PG) level of 200 mg/dl or greater during an Oral Glucose Tolerance Test (OGTT) (WHO, 1985).

Casual plasma glucose $\geq$ 200 mg/dl (11.1mmol/l) It can define as level of Glucose any time of day without consider to time since last meal. Or
Fasting Plasma Glucose $\geq$ 126 mg/dl (7.0mmol/l) Fasting can defined as no intake of caloric for at least 8 hrs. Or
Post-Prandial (PP) 2-hour plasma glucose $\geq$ 200 mg/dl (11.1mmol/l) It is performed by using a loading glucose containing 75 g anhydrous glucose dissolved in water.

Table-1.3(1): WHO criteria for diabetes mellitus (WHO, 1985)

1.3 Incidence and epidemiology

Clinical manifestations and development of DM often differ significantly between countries and also between racial groups within a country. For example, estimated diabetics were 15.1 million people reported in North America, 18.5 million reported in Europe, 51.4 million in Asia and just under 1 million in Oceania in 1996 (Kuhlmann, 1996; Amos *et al*., 1997) later it increased to 151 million in the year 2000 (Castillo *et al*., 2012). It is projected to increase to 300 million by 2025 (King *et al*., 2011).

According to Centre for Disease Control and Prevention (CDCP, USA), the risk of death among individuals with diabetes is almost twice that of individuals who do not have diabetes of similar age (Wild *et al*., 2004). Diabetes is a huge burden on global healthcare system (Wild *et al*., 2004).

Abebe 1996, reported up to 60% Atherosclerosis accounts for all diabetes related deaths in the US and Europe. Although up to 33% of all cases of kidney dialysis and 50% of all amputations are reported in the United States and Europe which are the result of diabetes complication (Abebe, 1996). Diabetics have > 20 times more chance of CVD or stroke than the normal. Visual loss due to retinopathy is also common among diabetics. Glaucoma which leads blindness mostly developed about 6% in all diabetics. Neuropathy another complication of DM mainly occurs in half of all diabetics during the course of their disease. DM also tend to suffer from poor wound healing. Impotence complication of diabetes is a common in men (Yki-Jarvinen, 2004).

Today India leads the largest number of diabetic people with its world (Wild *et al*., 2004). Increase in prevalence of diabetes is rapid in urban areas from 2% in 1970s to 12% in 2000 and in rural areas; it is also increasing (Cheng and Fantus, 2005). As per the estimation of WHO, the number of diabetics is likely to increase to 57.2 million by 2025 (WHO, 2003). In our country every fifth patient is a diabetic, hence India has now been declared as the "Diabetes capital of the world". Diabetes management is one of the most important subjects in clinical practice. In the Indian context, increasing urbanization, industrialization and changing lifestyles seem to be contributing to increasing prevalence of diabetes

1.5 Diabetes mellitus classification

According to WHO (WHO, 2015), Diabetes Mellitus classified into four clinical classes, T1D, T2D, gestational diabetes and other types of diabetes due to other causes.

1.5.1 Type 1 Diabetes Mellitus

It is caused due to b-cell destruction, which usually leads insulin deficiency. Most commonly T1DM is an immune mediated disorder which deals with the deficiencies or production of the insulin from the destruction of beta cells of the pancreas. Although in some occurrence this cannot be confirm either immune mediated or not. Some cases or conditions of T1DM are considered idiopathic. It is also called as insulin dependent diabetes mellitus (IDDM) and was often thought of as juvenile onset diabetes **(Ian, 1999).**

1.5.2 Type 2 Diabetes Mellitus

It is caused due to a progressive secretory defect of insulin on the background of insulin resistance. T2DM is a result of insulin resistance due to the action a cellular/receptor, which may be Intensify by deficient compensation in insulin secretion to decreased insulin action. About 90 % of all patients with T2DM and before diagnosis it may be present at an asymptomatic level for long periods. It is also known as non-insulin dependent diabetes mellitus (NIDDM) or adult onset diabetes (Ian, 1999).

1.5.3 Gestational Diabetes Mellitus (GDM)

GDM is another type of DM which can describe the development of glucose intolerance and recognition is done in the second or third trimester of pregnancy .This glucose intolerance condition subsequently boldness in the great majority of cases after delivery of pregnancy. About 4 % of all pregnancies patients having GDM complications are reported in the USA (Engelgau MM, 1995). Women having GD have higher risk of later developing diabetes (Harris MI, 1988).

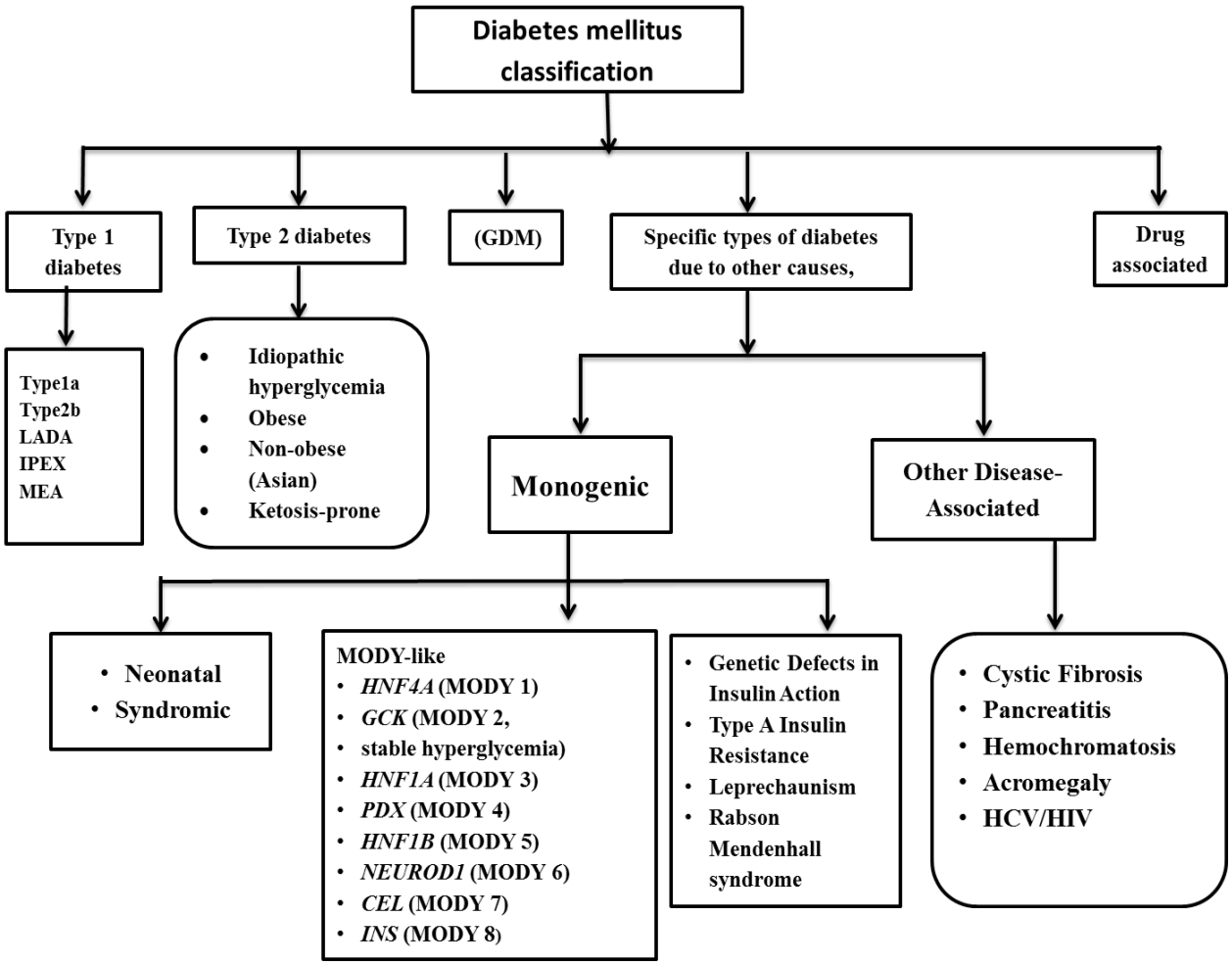

Fig. no 1.5 (a): Classification of DM (WHO, 2015).

1.5.4 Specific types of diabetes due to other causes

DM is caused number of detriment for examples monogenic diabetes syndromes, neonatal diabetes and maturity-onset diabetes of the adult [MODY]), which are the main diseases exocrine pancreas like cystic fibrosis, and drug induced diabetes (such as DM develop in the treatment immune-compromised diseases for e.g. HIV/AIDS or after transplantation of organs).

1.6 Diabetes Mellitus complication

Retinopathy, Nephropathy, and Neuropathy both peripheral and autonomic are the major complication of the DM. Diabetic person have a more risk of atherosclerotic vascular disease (CVD) which mainly associated with microvascular and neuropathic complications. Both are related duration of DM and the severity of hyperglycemia (raised blood glucose); the increased risk for vascular disease actually antedates the onset of hyperglycemia to the degree associated with diabetes mellitus.

Diabetic retinopathy is one of the complications of DM which leads visual loss or blindness in the world wide and it is also leading cause of blindness in young people as well as old people.

Diabetic nephropathy is a major complication which mainly leads kidney problem and need for renal replacement therapy (dialysis or transplantation).

Diabetic neuropathy complication or lower extremity vascular diseases which combine to make DM the leading cause of nontraumatic lower extremity amputations. Finally, diabetic person increases risk of atherosclerotic vascular disease by 2-5 folds. So, in light of these observations, strategies for detection, prevention and management of complications are all important in dealing with patients who have DM.

Problem associated with diabetes is that the increase in blood glucose level may lead to severe complications that affect various systems of the body. These complications are divided into two types. A Microvascular complication includes retinopathy nephropathy and neuropathy. The major macro vascular complication includes accelerated cardiovascular disease (CVD) resulting in myocardial infarction (MI) and cerebrovascular disease manifesting as strokes (Forbes, 2013).

1.7 Female sexual dysfunction (FSD)

Sexual dysfunction is one of the chronic complications in women with DM (Altman A, 2006). Women sexual functions impaired in both type 1 and T2DM (Enzlin P Enzlin P et al., 2003, Enzlin P et al.,1998, Schreiner et al.,1987,Arshag et al.,1990). Female sexual dysfunctions (FSD) are more common in diabetic subjects (Altman A, 2006). Vascular and nerve disorders is cause by hyperglycemia which impaired the sexual response and also affects the structural and functional changes. Diabetic vasculopathy impaired the blood flow in the vessels so

due to low blood flow inhibition of clitoris engorgement occurred and also effects the vaginal lubrication which results dyspareunia (Park K et al., 2002).

Endocrine disorders may also accompany DM, such as thyroid disorders, hypothalamic-pituitary disorders, and polycystic ovary syndrome, and may also contribute to sexual problems in diabetic women. In diabetic women Depression is an established risk factor for sexual dysfunction (Schram et al., 2009).

Epidemiologic studies shown that the prevalence of sexual dysfunctions (SD) in type and type 2 diabetic groups are 71% and 42% respectively worldwide (Alexandra et al., 2011). It exhibits disorder of libido, arousal, orgasm, and sexual pain leads to interpersonal difficulties and personal distress (Macfarlane et al., 1997). Characterized due to disturbances in sexual desire and associated change in sexual response cycle. (APA.,1994). It is indicated that type 1 diabetic women are mostly affected by SD as compared to type 2 diabetics and healthy women (Doruk, 2005). Research related to etiology and risk factors for sexuality of females with DM patients is still a controversial issue (Ziaei-Rad et al., 2010). Sexual desire, arousal, lubrication, orgasm disorder, dyspareunia, and sexual function complications were reported lower in the T1D and also suggested that sexual function varies during the menstrual cycle in Type 1 diabetic women (Salonia et al., 2006). This is comparable in luteal phase of the menstrual cycle it cannot observe in the follicular phase. (Salonia et al., 2006) but low level of 17 beta-estradiol were reported in DM during both the follicular and luteal phases of the menstrual cycle (Salonia et al., 2006). Zarzycki *et al.*, (2005) were reported in Type 1 diabetic women usually have an early onset of menopause and delayed menarche in their comparative with non-diabetic women. Type 1 diabetic woman having a high risk of menstrual disturbances such as, amenorrhea and oligomenorrhea. Which lead to risk of sexual and gestational problems is higher in type 1 diabetic women than in the general population (Zarzycki and Zieniewicz, 2005). Overall studies suggested women with DM can intensify SD compared to their counterparts (Enzlin P et al., 2002). The aim of present review is to discuss diabetic effect on female reproductive functions, and advice risk factors and pathways etiologies as well as offer their evidence-based strategies for FSD management.

1.8 Prevalence of Sexual Dysfunction in diabetic women

246 million people in world currently affected with DM and are expected to be 380 million by 2025 (IDF, 2013). A number of clinical studies in men and women SD with DM have been reported. That the prevalence of SD is higher in diabetic men and estimated to be 20‑85% which is less in diabetic women (Fatemi and Taghavi, 2009, Abu et al., 2008). Ziaei-Rad *et al.*, (2010) reported that out of 200 patients (100 male and 100 female) SD prevalence was high in diabetic patients were 165 (82.5%) patients experienced at least one SD in their study. There are uncertainties in the prevalence of FSD. It was reported in epidemiological studies of sexual attitudes and behaviors in female showed sexual difficulties in female common in middle-aged group's worldwide (Aumann et al., 2005) and ranged between 40-60% (Aumann et al., 1999, L ewis at al., 2004, Simons JS and Carey MP, 2001, Nazareth 2003). Aumann *et al*., reported that 43% of women, aged18 to 59, years, had sexual concerns in the United States. Additionally, Dennerstein *et al*., showed that the prevalence of FSD which are increased from 42% - 88% from the early to late menopause period (Dennerstein at al., 2003).

Although data are scant and there is a great deal of uncertainty, lack of subjectively Arousal (17%) of women, (Cain VS et al., 2003) insufficient vaginal lubrication (5-28%) (Aumann et al., 1999, Cain VS et al., 2003, Fugl-Meyer et al., 2006) Orgasmic disorder (5%) (Ventegodt et al., 1998) and dyspareunia (3-12%) have been observed (L aumann et al., 1999 Cain VS et al., 2003, Fugl-Meyer et al., 2006 Ventegodt S et al.,1998, Harlow and Stewart, 2003).

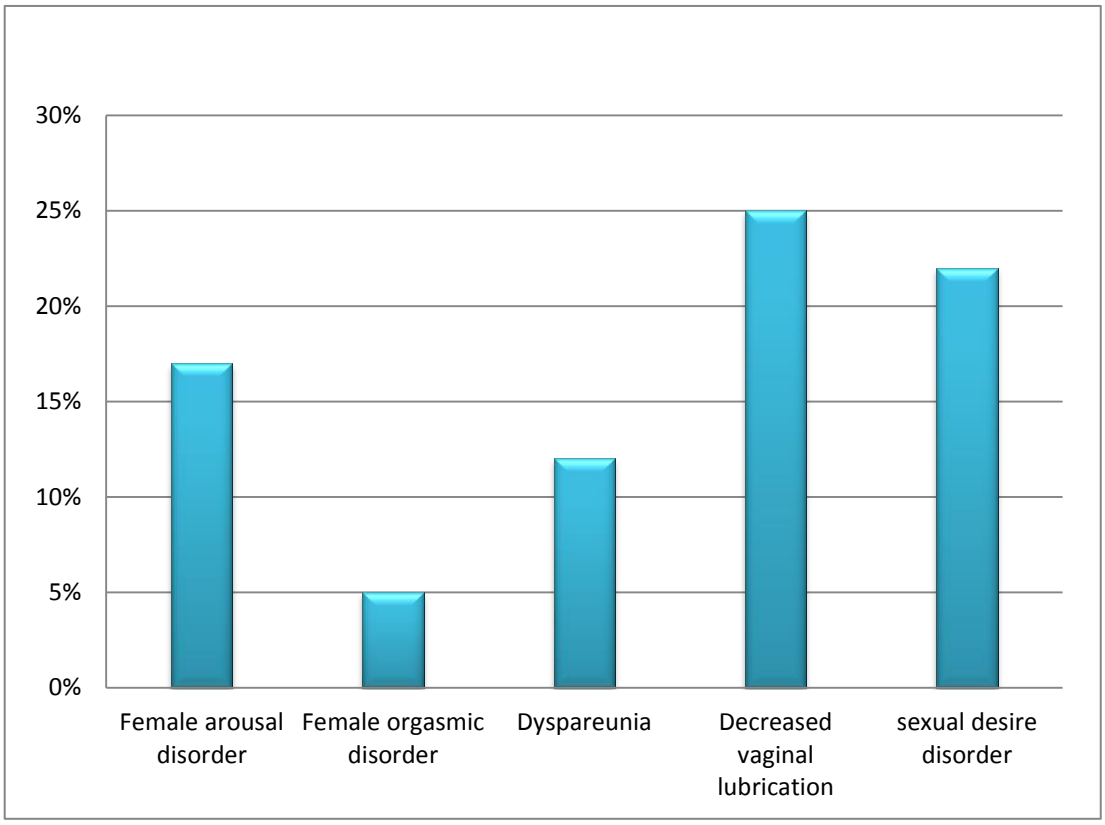

Fig No. 1.8(a): According to data available in the literature, epidemiological study on the prevalence and complication of FSD 20-25% difficulties in the desire phase, 15-20% difficulties in the arousal phase, 25% referred to changes in lubrications; 5% had disorders in the orgasm phase; and 10-15% mentioned dyspareunia are showing in this graph.

1.9 Causes and risk factors of SD in diabetic women

FSD etiologies in diabetics individual are still not clear, but physiological and psychological factor contribute to this problem (Schiavi and Hogan, 1979). A link between DM and FSD has been recognized since 10th century "collapse of sexual function" when Avicenna mentioned this it as specific complications of DM (Lindau, 2007).

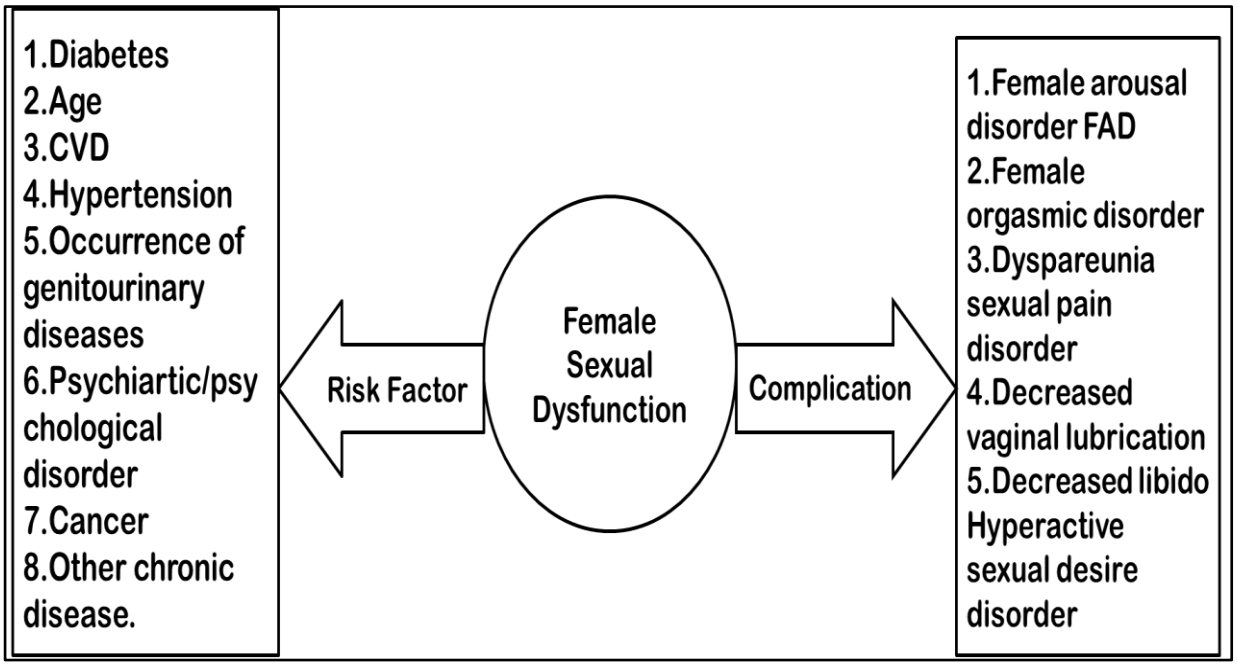

Fig. No. 1.9(a): causes and risk factors of FSD.

1.10 Current diagnostic approach to diabetic females with SD

An accurate diagnosis of FSD requires medical history including sexual life history, physical examination, lab testing and other tests. Female Sexual Dysfunctions is more complex so, it is very difficult to categorize due to a ladies perception about sex and sexual life particular in case DM. It is very challenging for the assessing FSD and their complications such as decrease in sexual desire, inability to achieve an orgasm and the painful sexual intercourse due to women perception about sex. For the evaluation of FSD need a free talk about sex and its problems is essential for both doctor and the patient. In diagnosis of FSD most important step is to gather information about all sexual life and its possible sexual difficulties or disorders. To identify whether a diabetic woman has SD, begin with a complete medical history that includes a sexual can follow the gynecological history like symptoms dyspareunia, the genital pain associated with sexual intercourse, veganism's, noncoital sexual pain disorder and the intercourse, veganism's, noncoital sexual pain disorder and the persistent involuntary spasm of the outer third of the vagina which interferes with vaginal penetration, and genital pain induced by noncoital sexual stimulation, However, for the investigation comorbid illness Comorbid factors, surgical history, medication, and status of menopausal, life style like habits such as smoking, alcohol consumption, and exercise type as well as her

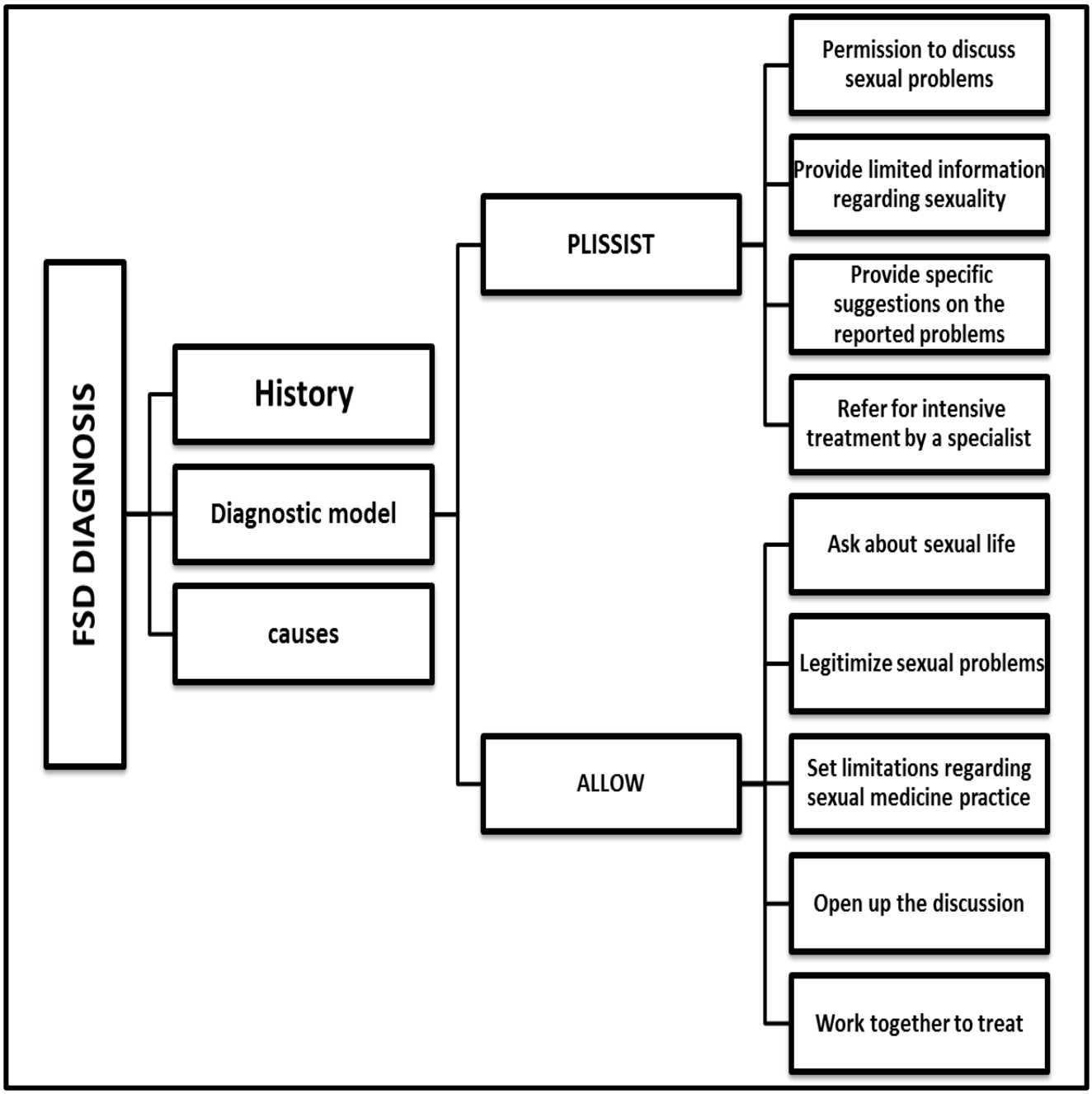

Fig no 1.10 (a): Current diagnostic approached of FSD.

Medication should be examined and also examined those factors which contribute to SD such as the female current interpersonal and psychosocial status (Fugl-Meyer and Fugl-Meyer K, 2006, Basson ,2005). Many the causes can contribute to FSD and evaluation of the presence of chronic medical condition such as CVD, neurological disorder, diabetes and physical disability is necessary, because this condition may also affect sexual functioning (Feldhaus et al.,2009,Frank et al.,2008,). Diabetic complications may cause depression and it can affect the woman's sexual desire and thus SD in women with DM. There are several Diagnostic models that help in the estimation of FSD especially in DM in order to facilitate medical practice mainly two

models have been proposed i.e. ALLOW and PLISSIT for the assessment by the doctor in the initial approach of diagnosis of sexual (Frank et al., 2008, Whitehouse et al., 2009).

ALLOW and PLISSIT model are the most commonly used methods for the diagnosis of FSD. To be more detailed and extensive evaluation of FSD, self-reported validated questionnaires and structured interviews are offered for the diagnosis of FSD. Structured interviews have a more personal character as they provide the opportunity for clarifying possible details, answering questions, and explaining terms. Validated questionnaires are also important which on the other hand are categorize by confidentiality privacy, and mainly adjusted to the female population for analyzing the measurable data in population (Hatzichristou et al., 2010).

No specific guidelines for the treatment of SD in diabetic women. Because large number of factors presents that can lead to SD in women with DM, and effective treatment may entail psychological as well as pharmacological treatment, includes lifestyle changes, optimal diabetic control, psychotherapy, and pharmacotherapy. Depression Treatment is also crucial in diabetic women with SD. Sexual dysfunction is one of the reason which put a high psychological pressure on patients with diabetes, and marital relations get impacted by diabetes by the presence of a chronic illness. For the recognition of sexual problems in women with diabetes should be an invitation for clinicians to address this problem during their consultations (Navneet Magon, 2011).

CHAPTER-II

LITERATURE REVIEW

2.1 Diabetes in Female Abnormalities

Uncontrolled blood glucose value and DM complications play has with female reproductive performance. Chronic high blood sugar promotes opportunistic infection which mainly cause by yeast and leads to vaginal irritation or itching. Vascular damage caused by poorly controlled diabetes restricts the blood flow to the vagina which causes vaginal dryness and interferes with arousal. Females have neuropathy to the genital area. The reproductive organs may have difficulty in achieving orgasm and dissatisfaction appears in female and also causes Urinary Tract Infections (UTIs) (Barlett and Garris, 1987).

Incompetence of the ovarian atrophy and reproductive tract is recognized by the consequences of the progressive expression of the Diabetes Obesity Syndrome (DOS) in mice (Garris, 1987). Reproductive tract dysfunction is a recognized consequence of the diabetes-obesity-syndrome in both humans and experimental animal models (Garris, 1987). Female reproductive performance altered in response to the progressive hyperglycemic-hyperinsulinemic systemic conditions that characterize non-insulin dependent (Type II). Suppression of cyclic ovarian follicular recruitment patterns, ovulation, depressed ovarian steroid hormone synthesis and release hypovascularization and tissue ischemia, enhanced follicular atresia, premature tissue atrophy and involution have been recognized to occur in association with DOS expression (Rajkhowa *et al*., 1994).

PCOS (Polycystic Ovary Syndrome) occurs frequently as a precursor to diabetes and defined as oligomenorrhea associated with hyper-androgenism (Kitziner and Willmott, 2002). Women with PCOS have a higher degree of hyperinsulinemia, insulin resistant, dyslipedmia, elevated LH: FSH ratio, infertility (Conway *et al*., 1990). Treatment of diabetes with common medicine may complex the situation pregnancy concern (Harborne *et al*., 2003).

2.2 FSD Pathogenesis

In order to respond to erotic stimuli in female sexual response needs the integrity of sensory and autonomic nervous systems as well as offer the

blood supply to the external genitalia and vagina area. FSD with DM appears more quiet and complex, which involves vascular, neurological, hormonal and psychosocial aspects. There are number of pathogenic factors in FSD.

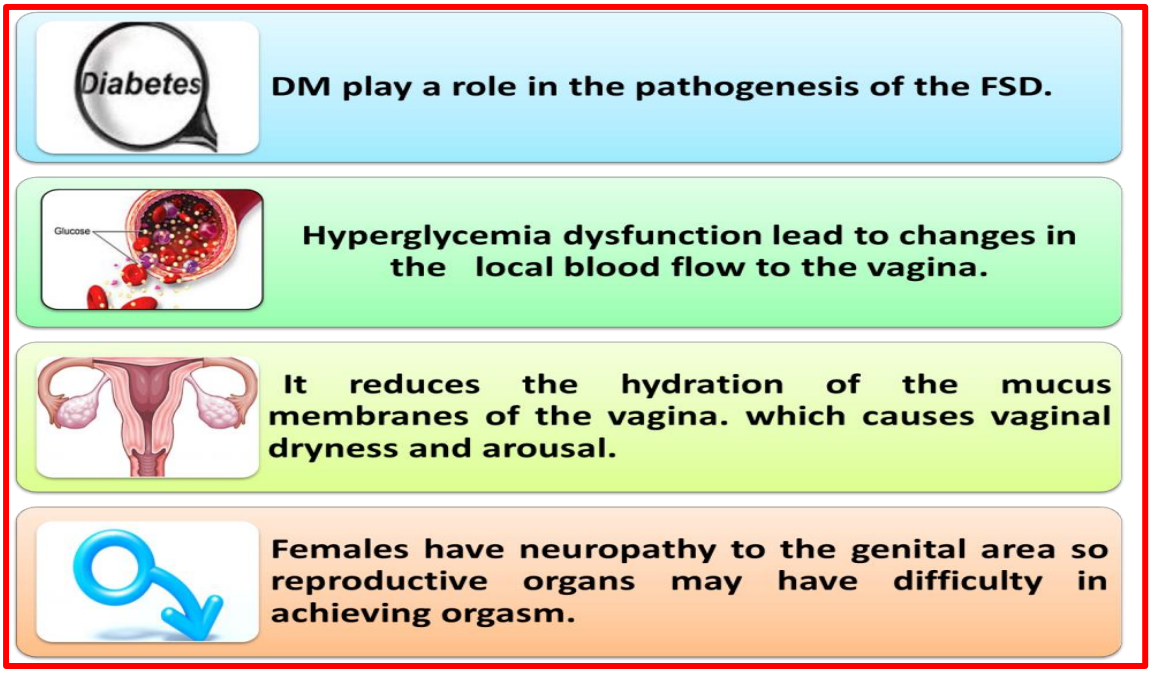

Fig no 2.2(a): Pathogenesis of FSD in DM.

The mechanisms involved include hyperglycemia, vascular and neurological damage, hormonal disorders and infections (Bargiota et al., 2011). Due to the action of nonadrenergic/noncholinergic neurotransmitters, such as vasoactive intestinal polypeptide (VIP) and NO results relaxation of female genitalia erectile and also enhance blood flow. NO/cGMP pathway is responsible in women for the blood flow regulation and clitoral erectile function. NO and PDE5 can identified in human on clitoral smooth muscle (Berman et al.,2003, Min et al.,2002) its presence indicating a key role of NO in FSD. But hormones level should be normal and also required for physiologic sexual activity. DM may affect all of these endocrine integrated systems, leading to SD (Bargiota et al., 2011).

Animals studies indicated structural and functional changes observed in the female genital tract, may result in impairment in sexual function such as arousal and orgasmic sexual response in DM (Park et al., 2002, Park et al., 2001). Hyperglycemic vascular dysfunction leads to changes in the local blood flow and also cause the

inhibition of the engorgement of the clitoris and lubrication of the vagina during arousal, resulting in dyspareunia or decreased arousal during sexual activity (Par et al .,2002 Kim et al., 2006). It has been hypothesized that hyperglycemia reduced hydration of mucus membranes in the vaginal tissue which results in poor vaginal lubrication and dyspareunia (Rockliffe and Kiemle G, 2003)

Hyperglycemia leads to dyspareunia due to its association with genitourinary infections an increased incidence which, may lead to discomfort of vagina and dyspareunia symptoms like (burning in genitalia, itching and dryness or discharge of the vagina, pain of genitalia area, general discomfort in the pelvic floor) can observed. It is required to treat, and the sexual abstinence recommended as part of some therapies (Alexandra et al., 2011). Animals study showed that DM may affect arousal and orgasmic sexual responses by inducing impaired relaxation responses of the vaginal tissue to almost all transmitter systems (Giraldi et al., 2001). Due to the decrease stimulation nerve clitoral and decreased vaginal blood flow, produced diffuse fibrosis of the clitoris and vaginal tissues, and also reduce the muscular and epithelial thickness in vagina (Park et al., 2002, Park et al., 2001). It is hypothesized that hormonal imbalance that accompanies DM play a role in the FSD pathogenesis. Correlation has been shown between the observed changes in the levels of hormones androgens, estrogens and sex hormone binding globulin (SHBG) in diabetic female (Feldhaus et al.,2009).

Hyperglycemia is main determinant of vascular diabetic complications, may participate in the pathogenetic mechanisms of SD in DM still not cleared. On the other side, present a number of risk factors for SD in both sexes so, diabetic person may present with several clinical conditions, including hypertension, overweight and obesity, metabolic syndrome, cigarette smoking, or atherogenic dyslipidemia, which are themselves risk factors for SD (Seftel et al., 2004, Esposito et al., 2005, Lewis et al.,2010, Miner et al 2012, Esposito et al.,2009, Esposito et al.,2005).

It is very complex to conclude FSD pathogenesis in DM and no evidence of current studies have yet clarified or not? Pathological pathways involved; but these studies are limited by small sample sizes, lack of standardized definitions of sexual dysfunction, and inadequate characterization of DM with regard to glycemic control, the presence of complications and depression. In contrast to description in men,

Female sexual function appears that is releated to social and phychological aomponents than to the physiological consequence of DM.

2.3 Potential mechanism of the diabetogenic action streptozotocin

Streptozotocin (STZ, 2-deoxy-2-(3-(methyl-3- nitrosoureido)-D-glucopyranose has a molecular formula of C8H15N3O7, 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.(Donal 1997). 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 (Junod et 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 STZ 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: Due to the formation of carbonium ion (CH3 +) resulting in the activation of the nuclear enzyme poly ADP-ribose synthetase as part of the cell repair mechanism and consequently, NAD+ depletion;

(ii) Nitric oxide (NO) production

(iii) Generation of free radicals as hydrogen peroxide

STZ has an intracellular action that results in changes of DNA in pancreatic B cells comprising its fragmentation (Yamamoto et al. 1981, Morgan et al. 1994). Main reason for the B cell death by STZ-induced is alkylation of DNA recent experiments have proved this (Delaney et al., 1995, Elsner et al., 2000). STZ alkylating activity is related to its nitrosourea moiety, at the O6 position of guanine leading to DNA damage with resultant necrosis of the pancreatic beta cells, through the depletion of cellular energy stores, is one explanation for the cell death that results from STZ induction. After the induction of STZ to rats, different methylated purines were found in tissues of animals (Bennett and Pegg 1981). The resultant activation of polyADP-ribose polymerase (PARP), in an attempt to repair the damaged DNA, depletes the cellular NAD+ and consequently, ATP stores as a result of overstimulation of DNA repair mechanisms STZ also methylates proteins; this DNA methylation is most responsible for beta cell death, though STZ methylation of proteins could also contribute to its toxicity to the beta pancreatic cells.

Nitric oxide (NO) production

Another mechanism of the diabetogenic action of STZ that results in cell death has been attributed to its ability to act as NO donor in pancreatic cells. Since STZ is a nitric oxide (NO) donor but not a spontaneous and NO was found to bring about the destruction of pancreatic islet cells because NO inhibits aconitase activity, leading to DNA alkylation and damage, it was proposed that this molecule contributes to STZ-induced DNA damage (Kröncke et al., 1995,Morgan et al. 1994). Due to the participation of NO in the cytotoxic effect of STZ was confirmed in several experiments (Turk et al., 1993, Kröncke et al. 1995). B cells of Pancreas exposed to STZ manifested changes characteristic for NO action, i.e. STZ increased activity of guanylyl cyclase and enhanced formation of cGMP which are characteristic actions of NO (Turk et al., 1993). When STZ is metabolized inside of the cells NO will liberate, but it is not required for this effect (Kröncke et al., 1995). β-cell are particularly sensitive to damage by nitric oxide and free radicals because of their low levels of free radical scavenging enzymes.

Generation of free radicals

STZ was found to generate reactive oxygen species, which also contribute to DNA fragmentation and evoke other deleterious changes in the cells (Takasu et al. 1991b, Bedoya et al. 1996). The formation of superoxide anions results from both STZ action on mitochondria and increased activity of xanthine oxidase (EC 1.1.3.22). It was demonstrated that STZ inhibits the Krebs cycle (Turk et al. 1993) and substantially decreases oxygen consumption by mitochondria (Nukatsuka et al. 1990). These effects strongly limit mitochondrial ATP production and cause depletion of this nucleotide in B cells (Nukatsuka et al. 1990, Sofue et al., 1991). Restriction of mitochondrial ATP generation is partially mediated by NO. This molecule was found to bind to the iron-containing aconitase inhibiting enzyme activity (Welsh and Sandler 1994). Augmented ATP dephosphorylation increases the supply of substrate for xanthine oxidase (B cells possess high activity of this enzyme) and enhances the production of uric acid – the final product of ATP.

2.4 Effects of green tea

2.4.1 General information

Green tea is the most consuming beverage after water worldwide. It is obtain from plant of Theacea Family, *Camellia sinensis*. Green tea is obtained by non-fermenting process of plant leaves while black tea is fermented part of same pant. Tea is the infusion of plant leaves cultivation of plants take place approximately at 30 countries.

2.3.2 Scientific classification

Kingdom: Plantea

Order: Ericales

Family: Theacea

Genus: *Camellia*

Species: *Camellia sinensis*

Since ancient time green tea is popular beverage worldwide and a major source of dietary flavonoids (RIJKEN et al., 2000). It is produced from the plant *Camellia*

Since ancient time green tea is popular beverage worldwide and a major source of dietary flavonoids (RIJKEN et al., 2000). It is produced from the plant *Camellia sinensis* (Gupta et al., 2004). Now a day's green tea have a health benefits (McKay et al., 2002) which includes the anticarcinogenic (Kavanagh et al.,2001) and cardiovascular diseases (Sueoka et al., 2001)() the anti-inflammatory Dona et al., 2003) antiarthritic, (Haqqi et al.,1999 antibacterial(Sudano 2004), antiangiogenic, (Sartippour et al., 2002) antioxidative, (Osada et al.,2001) antiviral, (Weber et al.,2003), neuroprotective (Weinreb et al., 2004) and cholesterol-lowering effects (Raederstorff et al., 2003).

Green Tea (*Camellia sinensis*) medically considered important healthy beverage since ancient times but recently, a great attention has been given among scientific community because of its antioxidant properties (Rijken et al., 2001, Anderson et al., 2001, Cabrera et al., 2003, Nakagawa et al., 2002, Zhang et al., 2004, Zhu., 1999). Some epidemiological studies suggested that tea consumption lowers the risk of several types of cancer (Ang-Lee et al., 2001, Sato et al., 2000, duToit et al., 2001). Its polyphenols have an effective chemo preventive agent (Cabrera et al., 2003, Gosslau et al., 2004). It have many other health benefits properties such as enhancing the activity of insulin [Cabrera et al., 2003, Anderson et al., 2002), antimicrobial (Sato et al., 2000, Hamilton-Miller et al.,1995, Stapleton et al.,2004), imunostimulatory (Sato et al.,2000, Matsunaga et al.,2002), anti-inflammatory protective effect against cardiovascular diseases (Sato et al.,2000, Sano et al.,2004).and cerebral ischemic damage (Suzuki et al., 2004). Recently, scientists reported that Epigallocatechin gallate (EGCG), a green tea catechin, have anti-HIV specifically when bound to CD4 (Kawai et al., 2003).

Different factors can influence the tea composition for e.g. species of the tea, season and climate of the tea, age of the tea leaf and horticultural area (Fernández et al., 2002, Lin et al., 2003). Its contains different chemical components such as some proteins, carbohydrate, caffeine, alkaloids, saponins, tannins, catechin and some poly phenol compounds like Epigallocatechin gallate (EGCG), Epicatechin gallate (ECG), Epigallocatechin (EGC),Epicatechin (EC), Myricetin, Querection, K aempferol. Some of the chemicals have already been proved for antioxidant activity as well as antimicrobial activity (Lin et al., 2003, Plazonić et al., 2009).

2.4.3 Anti-diabetic effect of Green tea

Haidari F et al. (2012) studied the effects of green tea on body weight, serum glucose, and lipids in STZ induced diabetic rats. The result suggest that the administration of the green tea extract effects on glucose level caused the significantly decrease the serum glucose and total cholesterol and also body weight changes observed overall study suggest green tea extract had both anti-hypocholstermic and antihyperglycemic effects in rats.

Hiroshi Tsuneki et al (2004) analyzed the effects of green tea on blood sugar level and serum protomic patterns in diabetic mice model and on glucose metabolism in healthy humans. Result suggests the green tea has an antidiabetic effect which lowered the glucose levels in the bloodstreams of diabetic mice experimental animal model without affecting insulin levels.

A study by Haidari F et al. (2012) reported that clearly showed that the oral administration of green tea extract improved the glycemic control and even reached the serum glucose levels to the normal values.

Ramadan *et al*. (2009) reported that green tea aqueous extract significantly alleviated hyperglycemia (resulting from type 1 and 2 diabetes) induced by STZ or cholesterol-rich diet in rats.

Babu *et al*.(2006) showed that the green tea have effects on the stimulation of insulin glucose uptake that will increase and inhibition of GLUT system in the intestine and also effects on the gene expression that control gluconeogenesis. However, Wu *et al*. (2004) indicated that the supplementation of green tea catechins does not change the blood glucose concentration in normal rats, which is consistent with our finding in normal group.

CHAPTER-III

EXPERIMENTAL PLAN WORK

3.1 Rationale

The animals are required for the evaluation of biochemical and histological changes in diabetic animal after administration of green tea extract orally and Large availability of data, responses of other drugs and pathways on these animals. Rats share 90% of the genome with humans. Rat more accurately reflects human physiology than mice. It is hypothesized that green tea have an anti-hyperglycemic and antioxidant effects so supplementation of green tea will prevent alteration in sexual patient in diabetic women

3.2 Aim and Objectives

- To evaluate the effect of green tea extract on gonads of female albino rat.
- To study Histo-architecture and biochemical effects of green tea extracts on reproductive function in female albino rat.

3.3 Plan work

Month	**(Months)**			
	0-1	**1-2**	**2-3**	**3-4**
Research designing				
Induction of diabetes and glucose estimation				
Determination of blood parameters of experimental groups				
Female reproductive tissue ovary sampling for histopathological analysis				
Analysis of data and application of statistical tools and report writing.				

Table no 3.3(1): Plan work

CHAPTER-IV

MATERIALS AND METHODS

4.1. Requirements

S. No.	Glassware
1	Test tube plane vial
2	Beaker
3	Syringes
4	Surgical kits
3	Conical flask
4	Measuring cylinder
5	Dropper
6	Slide and cover slip
7	Micropipette
8	Test tube stand

Table no. 4.1(1): Requirements used in research

S. No.	Instruments
1.	Incubator
2.	Colorimeter
3.	Spectroscope
4.	Microtome
5.	Weighing balance
6.	Hot plate
7	Water bath
8	Microscope
9	Micropipette

10	Refrigerator
11	Centrifuge
12	Slide warmer
13	Hot air oven
14	Cold centrifuge
15	Elisa reader
16	Glucometer
17	Semi-auto analyzer

Table no 4.1(2): Instruments used for performing blood parameters and histopathology of ovary**.**

4.2 Plant Material Green Tea Extract

The extract material was collected from A.M LABS New Delhi, India in the month of April 2011.General specification of Green Tea Extract Powder (98% polyphenols / 40% EGCG). Compositions of green tea are mention below 4.2(a).

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

Fig no.4.2 (a): Composition of green tea extracts *camellia sinesis* **(A.M Lab, 2015).**

Manufacturing Process of Green Tea Extract Powder (98% polyphenols / 40% EGCG)

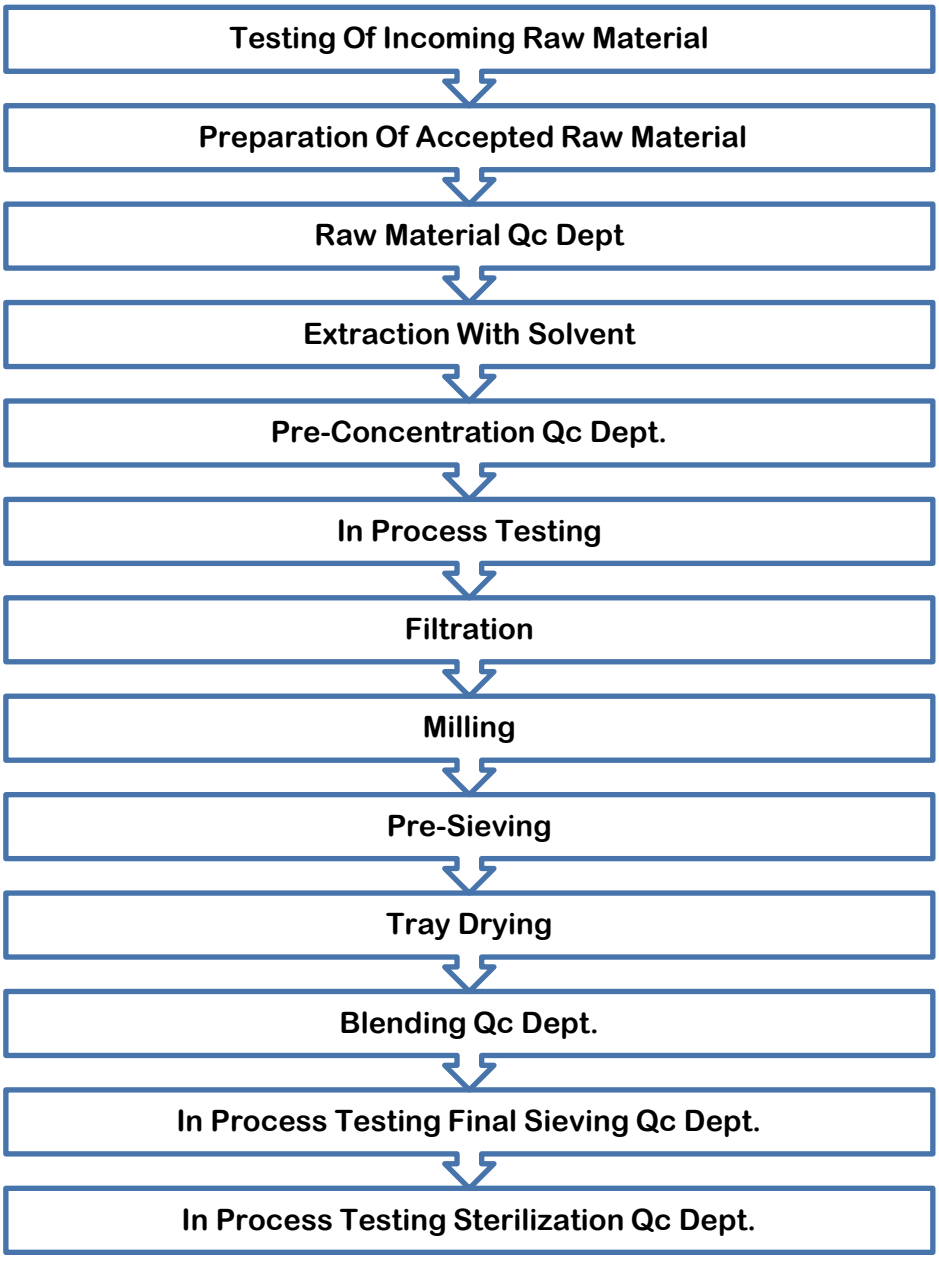

Fig no.4.2 (b): Manufacturing processing of green tea extracts **(A.M Lab, 2015).**

4.3 Chemical

4.3.1 Preparation of Drug

STZ was obtained from lovely professional university. It is prepared by dissolving in normal saline at RT and always prepared freshly for immediate use within 5 min and injected by intraperitoneal routes, in overnight fasted rats and the doses were determined according to the body weight of animals weight of STZ (150mg/kg) required in mg. volume of STZ (5%W: V) required in ml. Healthy adult albino female rats (150-200 g) were used in the study.

Programming for dose calculation in mg and ml =

$$\frac{\text{B.W of animal (grams)} \times \text{weight of STZ (mg)}}{1000}$$

4.4. Animals

In this assay, thirty six (36) female albino rats of wistar strain weight (190-300 g), age 3 months, were obtained from Nipher, Mohali. Due to the high mortality this study was carried out 24 rats. The 24 rats were divided into four 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 which are shown in fig 4.4 The study was conducted at the Lovely professional University, Punjab after obtaining Institutional Animals Ethical Committee clearance approved by CPCSEA APPROVAL NI. 954/PO/06/CPCSEA bearing the number LPU/LSPS/IAEC/CPCSEA/Meeting No 5,2014/ 2015 Protocol No.6.

Fig no4.4(a)

Fig no 4.4(b)

Fig no4.4(c)

Fig no4.4(d)

Fig no 4.4(a): Animal house animals are in steel cage.
Fig no 4.4 (b): Air conditioner room for maintaining temp (22 ± 3°C).
Fig no4.4(c): Animals were housed in the steel cages each cage consist 3 rats.
Fig no 4.4(d): Hydrometer 55 ± 5% humidity checker.

4.5.1 Experimental Grouping and Treatment

Animal female rats were randomly allocated to four groups (Groups I -IV). Each group (six animals per group) was housed in separate steel cages; each cage consists of not more than 3 rats and treated as follows:

I. **Animals in group (**I**)** are Non-diabetic rats with Normal diet and consider as a normal control.

II. **Animals in group (**II) are Non-diabetic rats were treated with orally green tea extract 200mgkg^{-1} body weight Total dose 1ml.

III. **Animals in group (**III) 6 rats each were administered with Single dose of STZ 45mgkg $^{-1}$ body weight single dose.

IV. **Animals in group (**IV**)** Diabetic single dose STZ induced rats each were treated with orally green tea extract 200mgkg^{-1} body weight Total dose 1ml.

Treatment design was selected to evaluate Histoarchitecture and biochemical effects of green tea extracts on reproductive function in female albino rat. The animals were distributed in to six experimental groups. Each group consisted of 6 rats in the beginning of the study. Animals in group 3, 4 were intraperitoneally administered single injection of 45 mg/kg of STZ. Food and 5% dextrose were presented to the animal only 30 min after the drug administration. After 3 days to the drug administration the animals show the following sign polydipsia, polyuria, weight loss (weighed after 3 days to the drug administration and weakness (asthenia). The blood glucose concentration was measured after three days from the day of STZ injection. The blood samples were collected from the tail vein determination of blood glucose is done by glucometer.

The total experimental protocol was maintained for 14 days after induction of diabetes. The following characteristic were measured before of diabetes and then on 7th, 14th ^{t} day of induction of diabetes. Behavior of animal Motility and responses, Weight Body temperature, Blood biochemical parameters

4.5.2 Induction of Diabetes

STZ monohydrate was obtained from 8B (503) Biochemistry lab, lovely professional university. It is prepared by dissolving in normal saline at RT and always prepared freshly for immediate use within 5 min was injected by intraperitoneal routes, in overnight fasted rats 18 hour prior and the doses were determined according to the body weight of animals weight of STZ (45mg/kg) required in mg. Volume of STZ (5%W/V) required in ml. Healthy adult albino rats (150-200 g) were used in the study. STZ was obtained from and always prepared freshly for immediate use within 5 min.

The Experimental animals were kept on fast for 18h prior to induction of diabetes. Diabetes was induced by intra-peritoneal (i.p) administration of STZ injections and the doses were determined according to the body weight of animals are shown fig no. (4.5.2).

Programming for dose calculation in mg and ml =

$$\frac{\text{B.W of Animal (grams)} \times \text{weight of STZ (mg)}}{1000}$$

The blood glucose concentration was measured after three days of STZ injection. The blood samples were collected from the tail vein and the blood was used immediately for the determination of blood glucose by glucometer. STZ induced a dose-dependent mortality in 60%, 50% and 20% of rats receiving 150 mg/kg, 45 mg/kg, and 90 mg/ kg respectively of STZ within 15 days of injection, respectively. STZ induced diabetes in 60% of rats receiving either 45 mg/kg or 150 mg/kg of STZ and 60% of rats receiving 45 mg/kg dose in diabetic rats random fluctuations in FBG with blood sugar returning to the non-diabetic range at various time periods during the study duration was observed. These fluctuations had no discernible pattern. These were noted with both low and high doses of STZ in 1000 rats. Stable diabetes for 10 days was produced in 60% of rats receiving 45 mg/kg of STZ. The rats that were hyperglycemic after initial dose 150mg/kg of STZ showed. High mortality (30-60%) of experimental animals with STZ has been reported Mortality very high observed. These results indicate that the toxic effect of STZ differs among animals of the same species and female very sensitive. This mortality is attributed due to initial hyperglycemic shock.

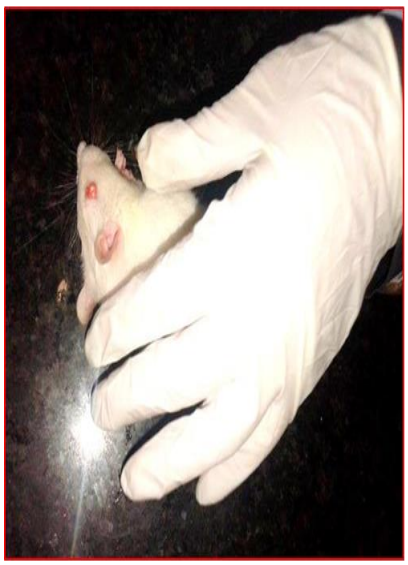

Fig no4.5.2(a)

Fig no4.5.2(b)

Fig no4.5(c)

Fig no4.5.2(d)

Fig no4.5.2(e)

Fig no4.5.2(f)

Fig no 4.5.2(a, b, c, d): Shown the handling of animal for i. p induction of STZ.

Fig no 4.5.2(e, f): Shown the interaperitoneal injecting of STZ.

4.5.3 Administration of Orally Green Tea Extract

Green tea extract dissolved in water 200mgkg^{-1} BW; Orally. Green tea prepared daily in morning and feeds with drinking bottles.

4.6 Assay kits

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

Assay Kits	Company
Blood Glucose	ERBA diagnostic Mannheim GmbH.
Lipid profile ➢ Total cholesterol ➢ Triglyceride	ERBA diagnostic Mannheim GmbH.
Serum protein ➢ Total protein ➢ Albumin ➢ Globulin	ERBA diagnostic Mannheim GmbH.
Hormonal analysis ➢ Luteinizing hormone ➢ Follicle stimulating hormone ➢ Prolactin	ELISA kits

Table no 4.6(1): Biochemical estimation kits.

4.7 Sample collection and Preparation

4.7.1 Blood collection

Blood collection is done by three methods the blood was collected from lateral tail vein and retro-orbital route and cardiac puncture. As per general guidelines for collecting of blood sample from rat, for maintaining of optimal

health condition the volume of blood should be at a lower normal range only for healthy animal it can be at maximum level.

1. **Intra Venous (ivy)**

   For (ivy) puncturing in tail vein the following steps were followed and showed in fig no 4.7.1(d). Rats were anesthetized by light ether. Tail of rat was warmed by applying compress of warm water. The veins were prominent at both sides of the tail and the needle was inserted at lower portion of the tail approx.1/3 part of tail from the top. Before inserting, the needle was kept parallel to the tail and the bell of needle was up side.

2. **Retro-orbital route**

   However retro-orbital route is considered as a category 2 procedures but as per study design we needed high volume of blood from a live animal so we too took blood by this route are shown in fig no 4.7.1(e). Rats were anesthetized by light ether. A sterile glass capillary tube was penetrated through retro-orbital plexus. About 7.5% of total blood volume was collected either in Eppendrof's tube or gel containing vacationer. From each eye the blood was collected only once. The animal was under observation for 24 h for the presence of any complication. The blood was transferred to the yellow top colored vacationer and allowed to clot.

3. **Cardiac puncture**

   At the end of the experimental period, the rats were anesthetized by light ether and dissected before disectioning fasting blood samples were drawn directly from cardiac ventricles region are presented in fig no 4.7.1(f). Cardiac puncture had done only after 14^{th} day for removing ovary for histopathological examination. About <5ml blood were collected from cardiac puncture.

Fig No. 4.7.1(a) Fig No. 4.7.1(b) Fig No. 4.7.1(c)

Fig No. 4.7.1(d) Fig No. 4.7.1(e) Fig No. 4.7.1(f)

Fig no 4.7.1(a, b, c): Anesthesia procedure of animals by ether.

Fig no 4.7.1(d, e, f): ivy, retro-orbital and cardiac puncture blood collection procedure are presented.

4.7.2 Sample preparation

Then blood samples were centrifuged at 3000 rpm for 15 minutes and. After the last treatment, blood samples (2ml) were taken from each animal by cardiac puncture. The samples were immediately transferred into plain vial tubes.

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.
4. Serum was not need to be diluted before assaying then serum was separated and used for the biochemical analysis.

Serum glucose, protein, albumin, triglycerides (TG), and total cholesterol (TC) levels were determined enzymatically using the standard methods. The hormonal assays (prolactin, follicle stimulating hormone and luteinizing hormone) were determined by ELISA method.

4.8 Biochemical Analysis

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, protein, albumin, triglycerides (TG) and total cholesterol (TC) and levels were determined enzymatically using the standard methods and analyzer and colorimeter shown in fig no 4.8(a, b).

4.8.1 Determination of blood glucose by the glucose assay kit

Fasting blood glucose level was determined on the 7^{th}, 14^{th} 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)

α-D-glucose Mutarotase → β-D-glucose

β-D-glucose +H2O+O2 Glucose oxidase → D-gluconic acid+H2O2

H2O2+ 4-aminophenazone+phenol Peroxidase → Quinonemine +4 H_2O

The intensity of the color formed is proportional to the glucose concentration in the sample. The intensity of the colour produced is directly proportional to the glucose concentration in the sample .The serum glucose concentration was expressed as mg/dl.

Apparatus Plane vial, Micropipette.

Chemical required

Glucose kit which has glucose reagent (kept at 2-8^{0}C)

Glucose reagent

Glucose standard 100mg/dl(5.55mmol/L)

Procedure

Three test tubes (1st as Blank B, 2nd as standard S and 3rd as test T) were taken.

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

Table no 4.8.1(1): Glucose estimation procedure by GOD-POD.

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

Calculation $\frac{\text{Abs T}}{\text{Abs S}} \times 100$

4.8.2 Estimation of Total Protein

Principle (Biuret method)

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 stabilizer 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). **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

Procedure

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

Table no 4.8.2: Protein estimation procedure by biuret method.

Mix and incubate for 10 minutes incubation in the dark. Absorbance of the sample and the standard (calibrator) against reagent blank is read in interval 30 minutes.

Calculation: $\frac{\text{Abs of T}}{\text{Abs of S}} = 6$

4.8.3 Estimation of albumin

Principle (BCG Method)

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.

Procedure

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

Table no 4.8.3: Albumin estimation procedure by BCG method.

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.

Calculation

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

4.8.4 Biological assays of lipid profile

The serum was used for the estimation of lipid profile. Total Cholesterol (TC) and TG were obtained by standard procedure.

Total Cholesterol assays (CHOD/POD Method)

Principle

Cholesterol esterase hydrolyses esterified 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.

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

Three test tubes (1st as Blank B, 2nd as standard S, 3rd as test T) were taken.

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

Table no 4.8.4(1): cholesterol procedure by enzymatic method.

These are shaken and incubated at 37 ^{0}C for 5 minute. Optical density was determined by spectrophotometry at 505 nm.

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

4.8.5 Estimation of Serum Triglyceride

GPO/POD method

Apparatus – Test tube, micropipette & pipette.

Chemical – Kit contains

Reagent 1 Triglyceride enzyme reagent 1

Reagent 2 standard 200mg/dl

Procedure

Reagents	Test	Standard	Blank
Reagent 1	1000 µl	10 µl	10 µl
Standard		10µl	
Serum	10 µl		
Distilled water			10 µl

Table no 4.8. 5(1): TG procedure.

These test tubes are incubated at 37^0C for 5 minute. Optical density was determined by spectrophotometry at 520 nm. Mix well and incubate for 10 minutes at 37 ºc. 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

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

4.8.6 Hormonal assessment

The sera obtained from the animal's blood sample were labeled and analyzed. Serum prolactin, luteinizing hormone and follicle stimulating hormone were assayed by the CMIA method which is termed carbonylmetalloimmunoasassy.it is a new non radio isotopic immunoassay procedure. Hormone assessment is done from metro polis lab Jalandhar.

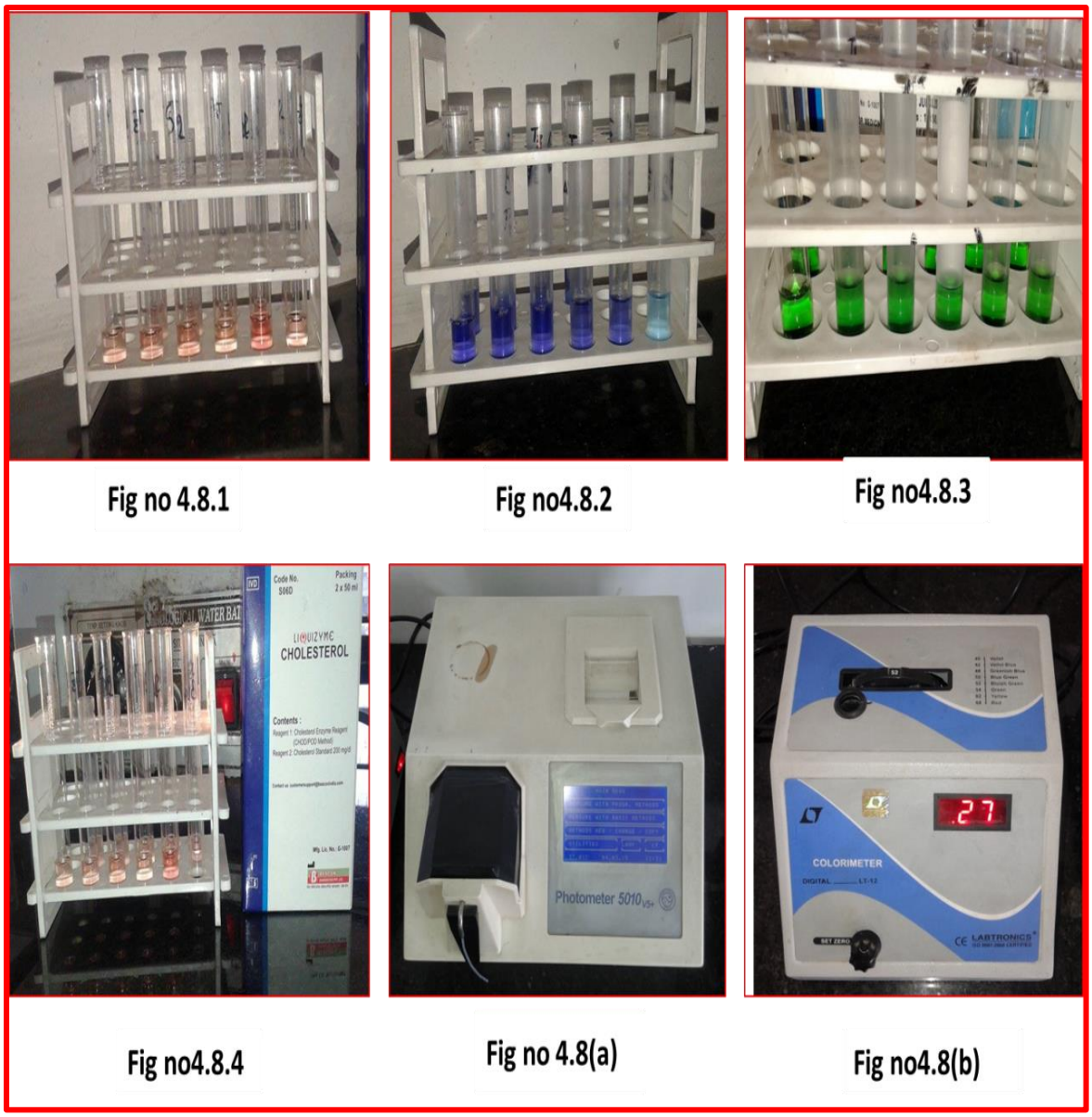

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Fig no 4.8.1: Glucose estimation by GOD-POD.

Fig no 4.8.2: Biuret estimation of protein.

Fig no 4.8.3: Albumin estimation by BCG method.

Fig no 4.8(a, b): Analyzer and colorimeter.

4.9 Histological procedures

At the end of the experiment, animals were sacrificed and their organs were removed .After the extraction of the ovaries 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 ovary 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 58º-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 a graded alcohol, cleared in xylene and mounted with DPX for histological assessment under the light microscope stained with heamatoxylin and eosin. The slides were viewed under light microscope with high power magnification. Light photograph of histological slides of ovaries of both diabetic and diabetic green tea rats were taken by a Nikon camera attached to light microscope Gargi diagnostic lab Jalandhar.

Fig No. 4.9 (a)

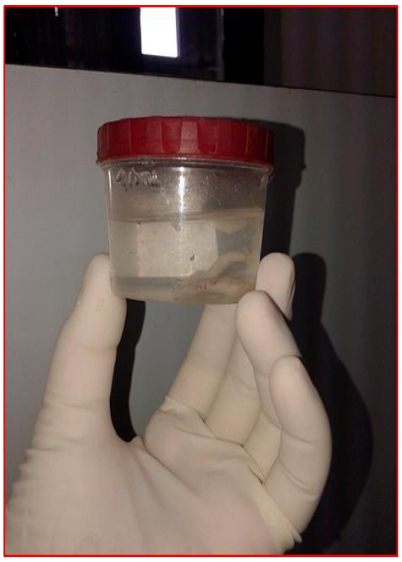


Fig No. 4.9(b)

Fig No. 4.9(c)

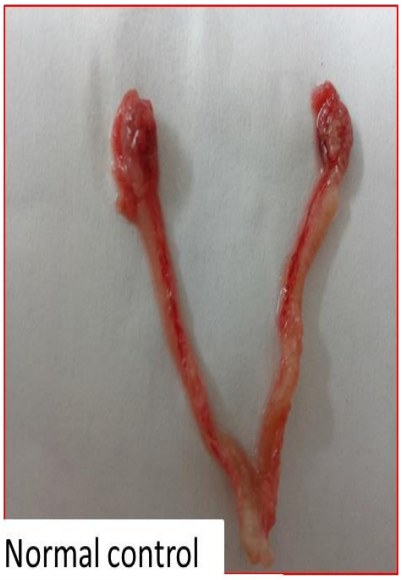


Fig No. 4.9(d)

Fig No. 4.9(e)

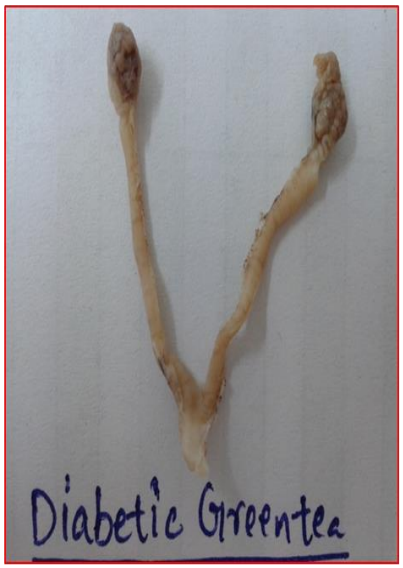


Fig No. 4.9(f)

Fig no 4.9(a, b): Tissue sample obtained from animal after disectioning.

Fig no 4.9(c): Ovary in 10% formalin fixation.

Fig no 4.9(d, e, f): Ovary of normal control diabetic control and diabetic + green tea

4.10 Vaginal smear (PAP)

To see the morphological changes in the reproductive organs of the female albino rats vaginal smear prepare to observe the reproductive cycle of the female. The estrous cycle can be subdivided into four stages: proestrous, estrous, metestrous and diestrous.

Stage	Smear	Cell type	Uterus	Ovary and Oviduct
Proestrous	ECL to EC	Many cell layers, Outer nucleated, Active mitoses Few L	Hyperemia and Distension, Active mitosis, Few L	Follicles large, Few mitosis in germinal E
Estrous	EC to C+	Superficial layer lost C layer, superficial Mitosis decreasing, L absent	Mitotic activity reach the peak then decrease, No L	Ovulation occurs followed by distension of the upper end of oviduct, Active mitosis
Metestrous I	C++	C layer delaminated, L start to appear	Distension decreased L in E	Early CL present Eggs in oviduct Many atretic follicles
Metestrous II	C++EL++	4-7 E cell layers, Many L in outer layer	Walls collapsed Rare mitosis Many L	Growing CL Eggs in oviduct Few mitosis
Diestrous	EL Mucus	4-7 E cell layers with L, Growth begins towards end of diestrous	Walls collapsed, E with many L, Secretion of uterine glands	Follicles start rapid growth towards end of period

Table no 4.10(1): Estrous cycle and the characteristic changes in the reproductive organs of mice (adapted from Bronson *et al*., 1966).

Preparation of Vaginal Smear

Hafez, 1970 method used for taking vaginal smear. The animals were held with ventral side up. A drop of 0.9% w/v normal saline was inserted carefully in to the vagina with a dropper, without damaging the vagina to avoid false positive smears. The drop of normal saline was aspirated and introduced twice, before

withdrawing from vagina the withdrawn fluid was transferred on to a microscopic glass slide .A cover slip was placed carefully on the smear avoiding the entry of air bubbles. The slide was then observed under an optical microscope are shown in fig no 4.10(a, b, c, d).

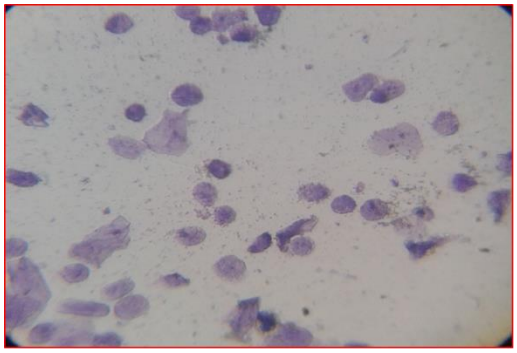


Fig no 4.10(a)

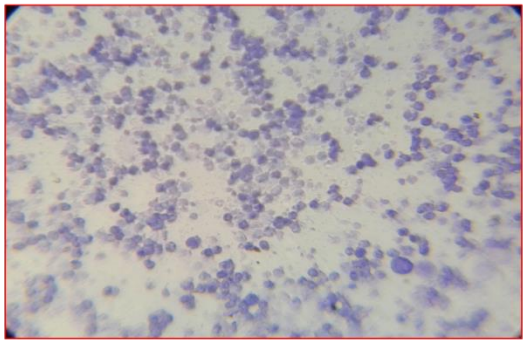


Fig no4.10(b)

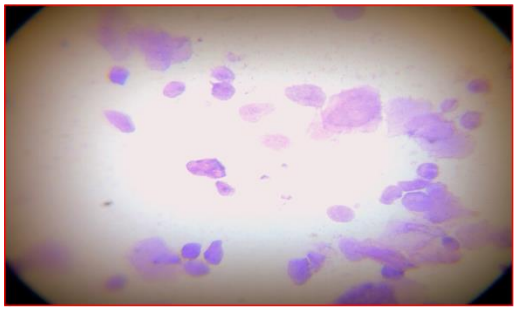


Fig no 4.10(c)

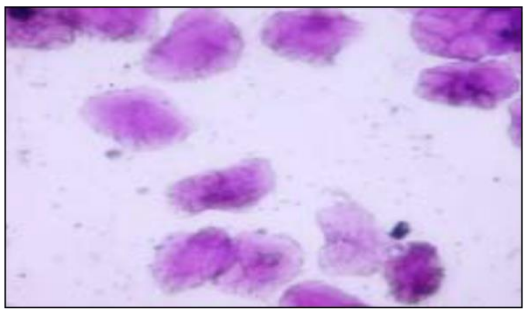
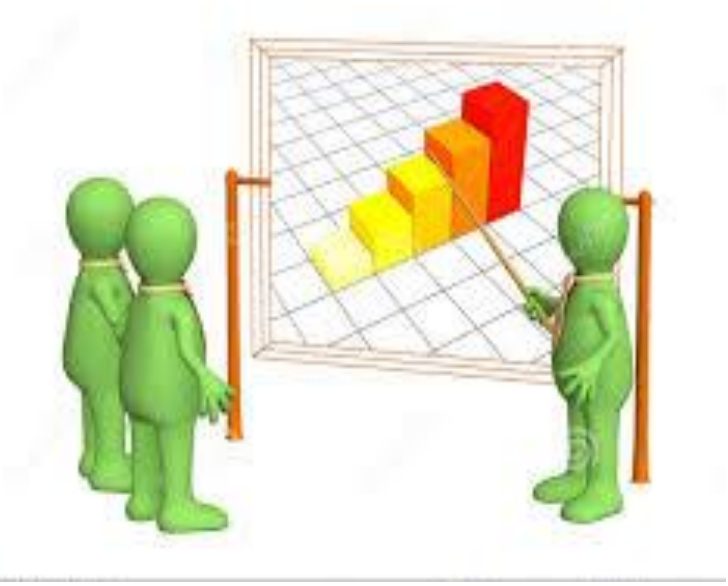


Fig no 4.10(d)

Fig no 4.10(a, b, c, d): Microscopic view of vaginal smear in estrous cycle Diestrous phase**,** proestrous phase and metaesterous phase of cycle observed lower magnification (PAP) stain.

CHAPTER-V

RESULT AND DISCUSSION



5.1 Statistical analysis

Result of effect of green tea extract (200mg/kg B.W) on body weight, different biochemical parameter and Histoarchitecture are presented below in table and fig form. Results are presented mean ± SD. Data was analyzed by using unpaired, one tailed Student t-test and two-way analysis of variance (ANOVA). MS Excel software was used for the application of these tools. p value of <0.05 were considered as significantly important for comparison. Statistical analysis were carried out by Microsoft excel 2010. All data of 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.

5.2 Effect of green tea extract on different biochemical parameter reported at the 7th day

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 7th day are presented in table No 4.2 (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 5.2 (2). Comparison of different biochemical parameters of different 4 groups are presented in graphical form fig no 5.2(a).

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. 5.2(1): Data has been reported as Mean ± SD in all four groups at the 7th day.

Anova: Two-Factor Without Replication

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. 5.2 (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.

5.3. Effect of green tea extract on different biochemical parameter reported at the 14th day

Effects of green tea extract on different parameters are shown in Table 5.3 (1). The mean of blood sugar, Total protein, albumin, globulin, TG and cholesterol in all 4 groups at the 14th day are presented in table no. 5.3(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 5.3(2). Comparison of different biochemical parameters of different 4 all groups are presented in graphical form fig no 5.3(a).

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. 5.3 (1): Data has been reported as Mean ± SD in all four groups after 14th day.

Anova: Two-Factor Without Replication

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. 5.3 (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.

5.4 Effect of green tea extract on body weight

The means of initial, 7^{th} and 14^{th} 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. 4.4(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 bodies weights from 0 day to 14 day are graphical presented in fig no 5.4(a). The results showed that the mean of 7^{th} and 14^{th} 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 the14th 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**	**7^{th} days**	**14^{th} days**
Normal control	236±10	243±17	249±11
Normal green tea200mgkg^{-1} B.W	247±18	246±67	248±33
Diabetic control	233±19	190±8	187±7.58
Diabetic green tea	230±15	208±30	210±29.735

Table No. 5.4 (1): Body weight Data has been reported as Mean ± SD in all four groups from 0 day, 7^{th} day and 14^{th} day.

5.5 Effect of orally administered green tea extract on serum glucose value

Effect of green tea extract observed after 7^{th} and 14 day it is shown graphically fig no 5.5 (a). Serum glucose level is significant ($p>0.05$) when compared to normal as it is shown in table no 5.5(2). Data of 7^{th} and 14^{th} day has been reported in mean ±SD are shown in table no 5.5(1).

Groups	Day 7th	Day 14th
Normal control	78±17	79.7849 ±16
Normal + GT	92±11	77.650 ±14
Diabetic control	151±15	154±12
Diabetic +GT	137±5	122±5.7

Table no. 5.5(1): Data has been reported in mean ±SD day 7th and 14th.

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 5.5(2): Blood glucose level is significant p vale (<0.05) when compare to diabetic control.

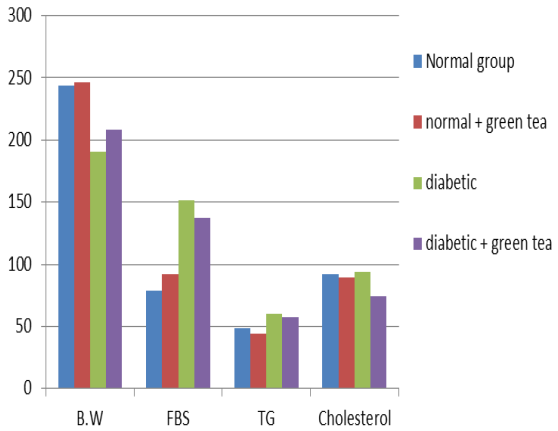


Fig No. 5.2(a)

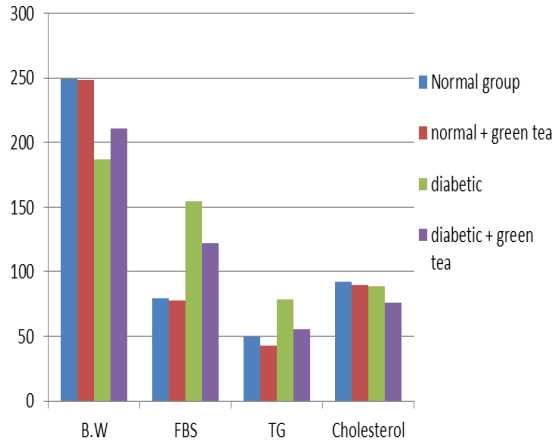


Fig No. 5.3(a)

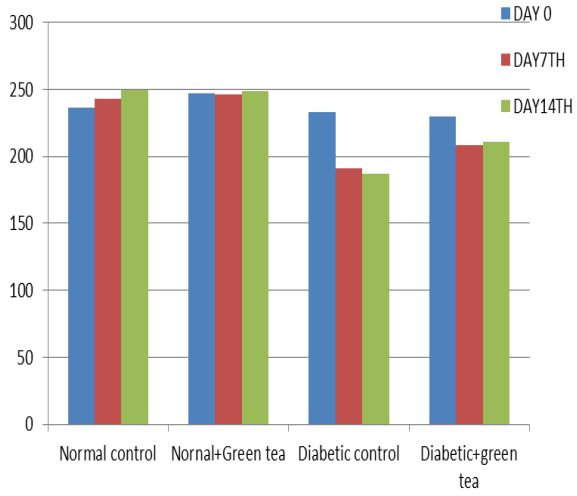


Fig No. 5.4(a)

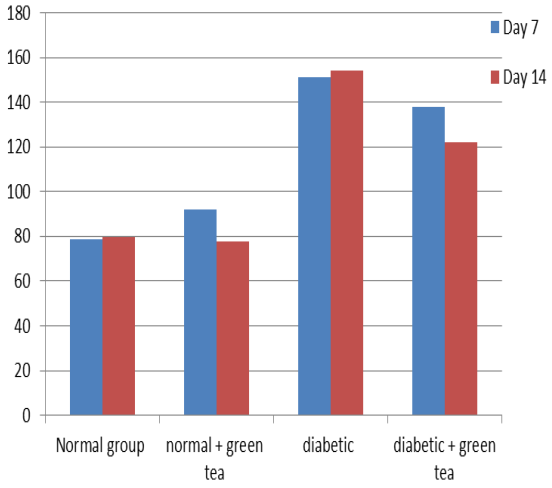


Fig No. 5.5(a)

Fig no 5.2(a): Comparison of different biochemical parameters B.W, FBS, TG, and TC after 7th day of different groups is presented.

Fig no 5.3(a): Comparison of different biochemical parameters B.W, FBS, TG, TC after 14th day of different groups are presented

Fig no 5.4(a): Comparison of body weight from initial, 7th and 14th day of different groups.

Fig no 5.5(a): Comparison of serum glucose from 7th and 14th day between different groups.

5.6 Effects of green tea extract on total protein

Green tea supplementation effects on protein as well data has been reported student t test between 7^{th} and 14^{th} day of the treatment are presented table no 5.6(1). Total protein level is significant p vale (<0.05) when compare to normal. Comparison between 7^{th} day and 14^{th} day in all group were presented in fig no 5.6(a)

t-Test: Two-Sample Assuming Unequal Variances

	Diabetic control	*Diabetic +GT*
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 5.6 (1): Total protein level is significant p vale (<0.05) when compare to normal.

5.7 Effects of green tea extract on albumin

Green tea effects on albumin were also reported after 14th day p-vale <0.05 significant when it compared to normal. Data were calculated in student t test to find the differences between different groups from initial day to 14th day are presented in table no 5.7(1).

t-Test: Two-Sample Assuming Unequal Variances

	Diabetic control	*Diabetic + GT*
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 5.7(1): student t test for calculate p value <0.05 were significant compare to normal.

5.8 Effect of green tea extract on lipid profiles

The effects of oral administration of green tea extract on serum lipid profiles are shown in Table 5.8(1). As it is shown, serum TC p value was significantly <0.05 when compare to those of normal control group. Diabetic control group had also significant lower levels of TC when compare to diabetic +GT after 14th day. The present data also indicated that only the administration of 200 mg/kg green tea extract in diabetic rats caused a significant decrease in TC levels compared to the diabetic control are presented in fig no 4.8(a). Serum concentrations of TC of normal, normal + GT did not change significantly.

t-Test: Two-Sample Assuming Unequal Variances	*Diabetic control*	*Diabetic + GT*
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.5.8 (1): student t test were calculated for obtaining p value which are <0.05.

5.9 Effects of green tea extract on Triglyceride

The effects of oral administration of green tea extract on serum lipid profiles are shown in Table 5.9(1). As it is shown, serum TG p value was significantly <0.05 when compare to those of normal control group. Diabetic control group had also significant lower levels of TG when compare to diabetic +GT after 14^{th} day. The present data also indicated that only the administration of 200 mg/kg green tea extract in diabetic rats caused a significant decrease in TG levels compared to the diabetic control are presented in fig no 4.8(a). Serum concentrations of TG of normal, normal + GT did not change significantly.

t-Test: Two-Sample Assuming Unequal Variances

	Diabetic control	*Diabetic + GT*
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 5.9(1): Triglycerides level is significant p vale (<0.05) when compare to diabetic control.

5.10 Effects of green tea extract on reproductive hormone

The studies were conducted on adult female rats (150-250g) for 14 days. To study the effects of green tea on hormones they are divided into four groups. Reproductive hormones follicle stimulating hormone, luteinizing hormone and prolactin levels were assayed by CMIA method using assay kits. From the chart 5.10(a) the value of FSH, LH and prolactin testosterone concentration level in all groups measured and showed a non-significant changes in normal control and normal +GT whereas decrease value of LH, FSH and Prolactin observed 14^{th} day when it compare to normal it was very low in diabetic control but due to supplementation of green tea it little bit improves in diabetic + GT group after 14^{th} day.

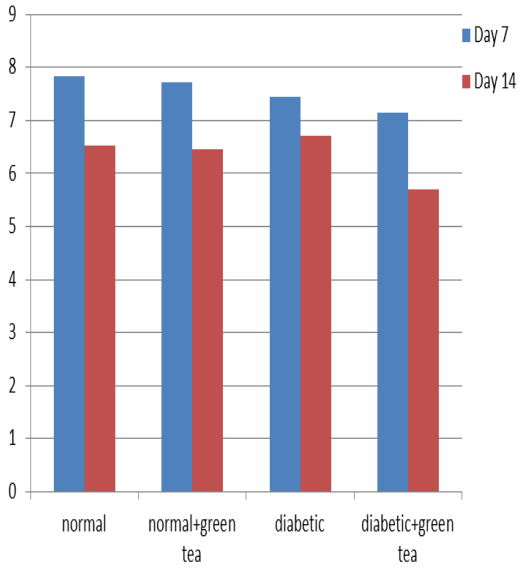


Fig No. 5.6 (a)

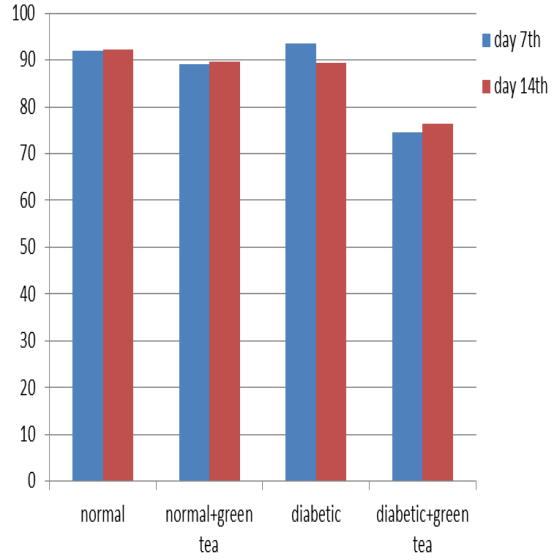


Fig No. 5.8 (a)

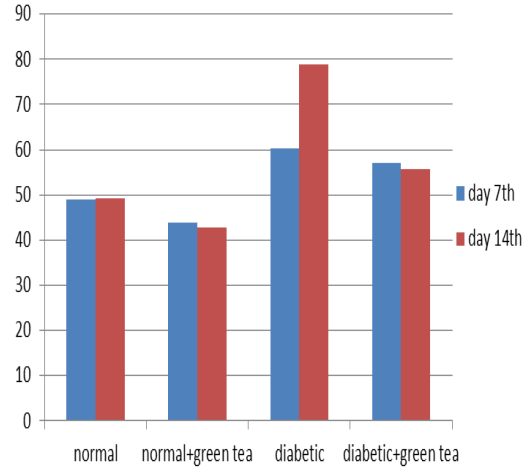


Fig No. 5.9 (a)

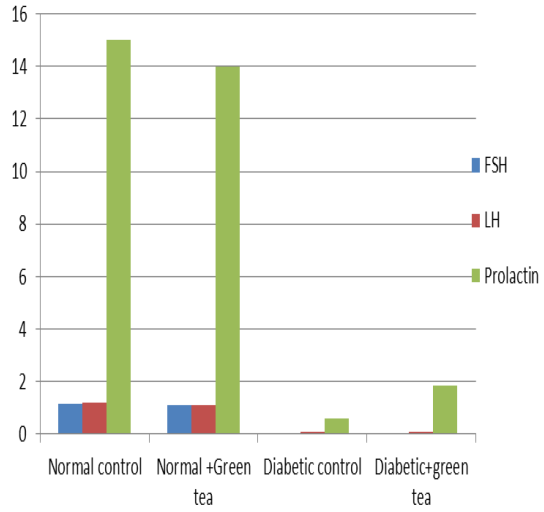


Fig No. 5.10 (a)

Fig no 5.6 (a): Comparative study of proteins in all groups between 7th to 14th day.

Fig no 5.8(a): Graphs presented the significant difference between TC in all groups.

Fig no 5.9(a): Shown the TG level 7th and 14th day.

Fig no 5.10(a): Shown the decreased value of LH, FSH and prolactin which was much decreased in diabetic control whereas it improve little bit in Diabetic +GT.

5.11 Histopathological finding

Histological examination of sections in the ovaries of female albino rat treated with green tea extract for 14 successive days were normal when compared to normal. And diabetic and diabetic +GT no significant changes observed in the follicle numbering of the follicles are normal and the appearance are also normal. Green tea extract thus, induces recovery of serum insulin levels and this would be one of the mechanisms involved in the improvement of ovary structure and function (Ambali *et al*., 2010). After treatment with Green tea extract, at 14th day the numbers of normal follicles were normal in both diabetic and diabetic + Green tea group no significant difference were observed because of the acute diabetes as well short duration of treatment time. It was shown in fig no 5.11(a, b, c, d). Follicles appear under microscope and no significant difference observed. Scientist have shown that herbal plant one of them green tea could have beneficial and supporting effects on ovarian tissue and folliculogenesis, if used as a hypoglycemic agent in diabetes. It is hypothesized that green tea has an effect on ovarian function.NO significant difference observed in normal and normal+ Green tea .

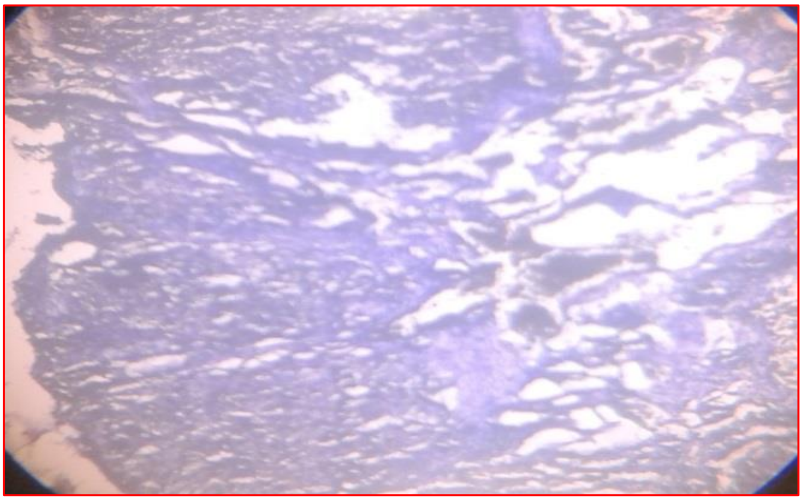


Fig no 5.11(e): microscopic view of normal control ovary low power

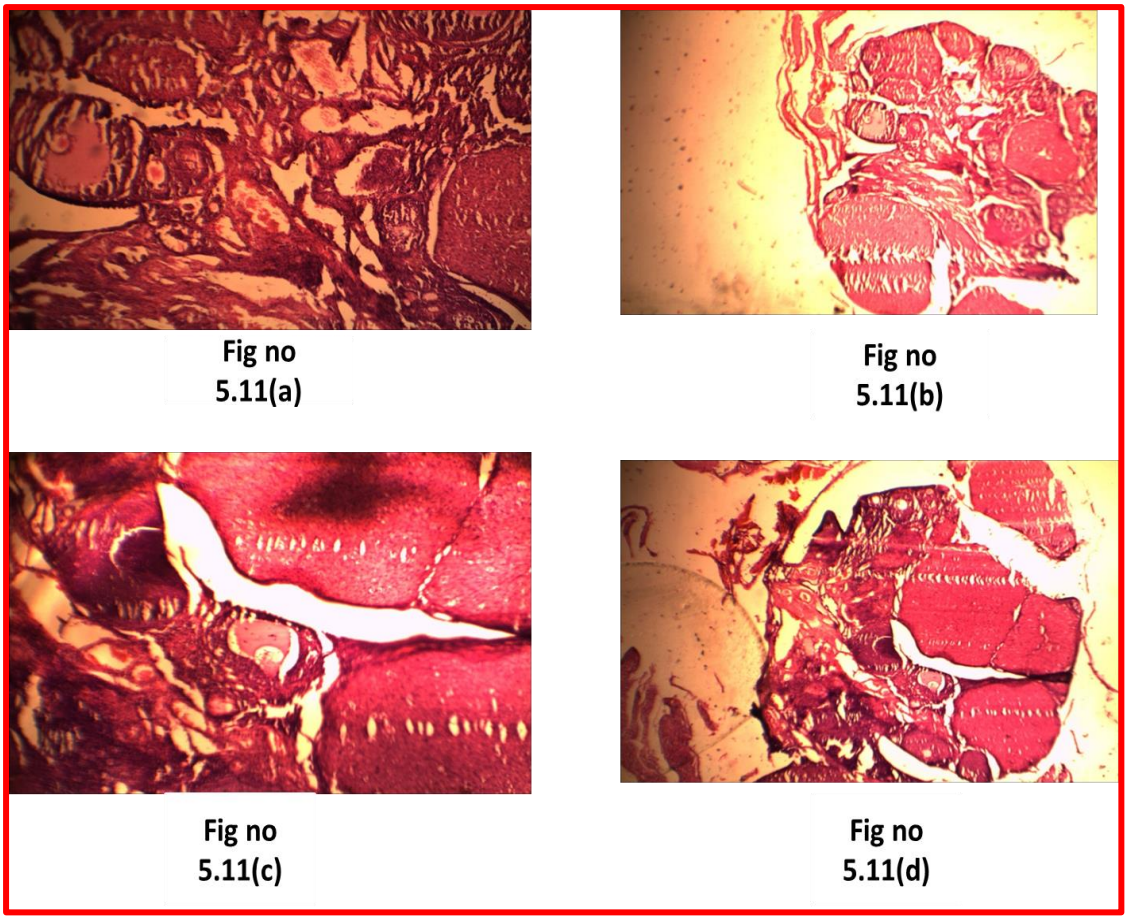


Fig no 5.11(a): High magnification examination of Diabetic control showing follicle.

Fig no 5.11(b) Low magnification microscopic view of diabetic ovary also showing follicle.

Fig no 5.11(c): High magnification microscopic view of Diabetic +Green tea ovaery showing number of follicles

Fig no 5.12(d): Low magnification examination of diabetic +Green tea ovary.

Discussion

Diabetes mellitus is primarily a metabolic disorder. It disorients carbohydrate metabolism with multiple etiological happenings. There is absolute or relative insulin deficiency or insulin resistance or both in this case. Whatever the cause may be, diabetes ultimately leads to hyperglycemia (Den et al., 2012; Spotti et al., 1999). Different synthetic drugs, plant remedies and dietary modification play an effective role in the reduction of the suffering that it causes. The potential roles of medicinal plants as hypoglycemic agents have been reviewed by several authors (Grover et al., 2002).

The experimental rats were divided into four groups of 04 animals. These are Group-I (Control); Group-II (Normal + Green tea); Group-III (Diabetic control and Group-IV rats fed with green tea. The total experimental protocol was maintained for 21 days after induction of diabetes.

Changes in body weight in different groups of rat were shown in Table: 5.4(1). There was a significant reduction in body weight at days 07, days 14 and days 21 in diabetic control (Group- III) when compared to control rats ($p<0.05$). The gradual reduction in body weight in Group-II was ameliorated in diabetic control by induction of STZ. It was found that at days 14, body weight rises from 208±30 to 210±29.735 in Group-III when compared with diabetic rat ($p<0.05$). The results obtained in present work clearly show the effects of green tea extract on the body weight in STZ–monohydrate induced diabetic rats. There was a slight increase in body weight in Group-IV when compared with Group- III ($p<0.05$).

The Fasting Blood Glucose Level (Table no 5.5(1) of albino diabetic rats showed increasing trend with the increase of period (07, 14 and 21). In this study, green tea extract at a dose of 200 mg kg^{-1} bw^{-1} was evaluated in normal and diabetic rat. The control animal showed 78±17-80.000±16 mg/100 ml of blood glucose in control animal during experiment which was found to be increased to 92±11-78±14mg/100ml at 14 days of Group- III. There was decreasing trend in the blood glucose level when diabetic rat supplemented with green tea extract. It has been seen that treatment with Green tea extract brought down FBGL from a higher value of 137±5mg/dl to 122±5.7 mg/dl which is one times reduction from higher value on 14^{th} day. Control rats when fed with green tea extract do not show any significant increase in FBGL. But little bit

changes observed 7th day but that may be an error in procedure. In the present study, green tea extract supplementation to the test subjects for the 14 days resulted in significant lowering of fasting blood glucose level ($p<0.05$). Hiroshi Tsuneki et al (2004) analyzed the effects of green tea on blood sugar level and serum protomic patterns in diabetic rat model and on glucose metabolism in healthy humans. Result suggests the green tea has an anti-diabetic effect which lowered the glucose levels in the bloodstreams of diabetic rat experimental animal model without affecting insulin levels. The possible mechanism of anti-hyperglycemic effect could be due to improvement of insulin action at the cellular level or due to insulin like effect of the active principle present in the extract.

In the present investigation STZ monohydrate induced rats for different time duration (7 days, 14 days showed decreased protein values. The cause of protein depletion may be due to elevation in the activity of proteases and subsequent elevation in the free amino acid content and activity of aminotransferases (Suhasini et al., 2006). The depletion in protein value may be due to the defect in protein synthesis, altered relationship between ribosomes and membranes of endoplasmic reticulum and may be due to diversification of energy to meet the energy demands as the animal was under pathological stress (Laurman, 1980).

The Total Cholesterol of control rat (Group-I) ranged from 91.8±13.97mg/dl to 92±23mg/dl at day 1 to 14. There was a significant increase in Total Cholesterol at all exposure periods ($p<0.05$) in diabetic control (Group-III) when compared to control rat. It was found that at days 14, Total Cholesterol of diabetic control rises from 74.66±13.4mg/dl to 76.66±13.5mg/dl in Group-III whereas Diabetic +GT treated animals show decreases at 14th day.The Serum Triglyceride of control rat showed 49±4 mg/dl to49 ±6mg/dl during all experimental periods. Significant increase in Triglyceride was observed at days 14 in Group-III ($p<0.05$) when compared with Group-I. Diabetic rat when treated with green tea extract showed Triglyceride decreased from 57.16667±12 mg/dl to 55.69697±24 mg/dl at day 14. Control rat when fed with green tea showed insignificant change in Triglyceride. The maximum value of Triglyceride was recorded 78.83333±14 mg/dl at 21 days of diabetic rat in Group-III.

Hormone assessment of normal control was normal under normal range. There was no significant change in normal control and normal + GT. It is found that at days

14, LH, FSH and Prolactin decrease significant changes observed in diabetic control and diabetic + GT. Both Group III and IV have decreased value of LH, FSH but prolactin of group IV at 14^{th} day little bit increase means supplementation of green tea effects on the hormones. Due to lack of time significant change didn't observed but little bit outcome of the result at the 14^{th} day also observed.

Histological study of ovary of control Rat (Group-I) and green tea extract supplemented rat (Group-IV) showed normal oogenesis process. The total numbers of growing follicles, secondary follicles, primordial follicles and tertiary follicles were normal in diabetic rat (Group-II) Were normal

STZ monohydrate alters blood glucose level and lipid profile in diabetic rat that interfere with ovarian function. Decreased insulin acts directly on the ovary or indirectly at the hypothalamic and pituitary gland level (Smith, 1983). Increased blood glucose level and decreased insulin causes alterations in various metabolic functions of vital organs that lead to toxicological changes in histoarchitecture of ovary. It was identified by comparing the histological sections of test animals under compound microscope. Marked reduction in growing follicles in diabetic rat is due to suppression of secretion or release of gonadotropins or desensitilization of the ovary to gonadotropins as suggested by Raphael et al (2002).

Ovarian dysfunction in diabetes mellitus may be associated with imbalanced glucose utilization, follicular atrophy and impaired steroidogenesis (Garris et al., 1987). Tatewaki et al (1989) observed the percentages of the primary follicles decreased in diabetic rat (Tatewakil et al., 1989).

Deficiency of insulin is one of the determinant factors that influence ovary structure. Green tea extract thus, induces recovery of serum insulin levels and this would be one of the mechanisms involved in the improvement of ovary structure and function (Ambali et al., 2010). After treatment with Green tea extract, at 14^{th} day the numbers of normal follicles were normal in both diabetic and diabetic + Green tea group no significant difference were observed because of the acute diabetes as well short duration of treatment time. Scientist have shown that herbal plant one of them green tea could have beneficial and supporting effects on ovarian tissue and folliculogenesis, if used as a hypoglycemic agent in diabetes. It is hypothesized that green tea has an effect on ovarian function.

Ovaries in female albino rat are paired structure lying in the body cavity near the kidneys. Each ovary is normally enclosed in a fibrous capsule. Two compartments are distinguished, the cortex with many follicles in various stages of growth and medulla with an important blood network. The ovaries contain several distinct types of cell and tissues.

Ovarian bursa consists of a poorly defined central medulla and outer cortex. It consists of primordial and maturing follicles and corpora lutea separated by loose fibrous stroma and groups of ovarian interstitial cells. Primordial follicles contain oocytes, clear round cells surrounded by a single layer of fat cells. The majority of primordial follicles eventually undergo atresia.

Oocyte is surrounded by the acellular zona pellucida. The maturing follicles cause the germinal epithelium to bulge which ruptures at ovulation, releasing the oocyte with its cluster of cells from the Graafian follicle. Granulosa and theca cells change the follicle to a corpus luteum which consists of lutein cells. Large cells with a clear eosinophilic cytoplasm and a large nucleus are organized in cords around sinusoids. To conclude in the present study it has been shown that treatment with STZ-monohydrate to induce diabetes showed little bit changes because of short time treatment with green tea but it may hypothesized that green tea have capability to improve ovarian functions in female as well biochemical parameters.

CHAPTER-VI

SUMMARY AND CONCLUSION

Summary

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion and/or insulin action. In uncontrolled diabetes, glucose and lipids (fats) remain in the bloodstream and with time damage the body's vital organs.

The rapid increase in population with urbanization and changes from traditional lifestyles most likely trigger a diabetes epidemic. With every fifth patient visiting a consulting physician and every sixth patient visiting a family physician is a diabetic; India has now been declared as the **Diabetes capital of the world**.

Green tea is a beverage with a long history of use for its medicinal properties. The strong antioxidant effect of the phenols in green tea has been confirmed with implications for the use of this beverage against the increasing es of conditions such as cardiovascular disease, some cancers, diabetes and obesity.

This study has been undertaken to investigate the Effect of green tea (*Camellia sinensis*) extract on the Histoarchitecture of reproductive organs in STZ induced diabetic female model. The aim of study is to assess Histo-architecture and biochemical effects of oral administration of green tea extracts on reproductive function in female albino s. Female s were administered the green tea extract orally for 14 days, while the control group received only water.

In this assay, thirty six (24) female albino s of Wistar strain weight (150-200 g), age 3 months, were obtained from NIPER, Mohali. The s (24) were divided into four groups of 6 each s according to the design of experiment and treatment. Animals in group (I) are Non-diabetic rats with Normal diet and consider as a normal control. Animals in group (II) Non- diabetic s were treated with orally green tea extract 200mgkg^{-1} body weight. Animals in group (III), (IV), groups of 6 rats each were administered with dose of STZ. Group (3) animals were administered single dose of STZ (45mgKg^{-1}bw) i.e. dissolved in Normal saline. Group (IV) animals administered with Single dose of STZ (45mgKg^{-1}bw) i.e. dissolved in NS 200mgkg^{-1} body weight; green tea extract orally. Treatment design was selected to evaluate Histo-architecture

and biochemical effects of green tea extracts on reproductive function in female albino. Data were analyzed by mean ± SD, ANOVA test and student t test.

At the end of experimental period, animals were sacrificed and their blood and ovary samples were collected for the analysis. Ovaries and uterus were removed for histopathology. Based on the results, it is indicative that the supplementation of green tea extract has strong capability to decrease in serum glucose and total cholesterol levels and significantly improved the body weight loss in diabetic s treated with 200 mg/kg green tea in comparison to diabetic control group because of the strong antioxidant effect of the phenols. And also effects on the hormones. No significant changes were observed in protein and albumin. To conclude that green tea extract had antihyperglycemic and hypocholesterolmic effect and also helps in body weight in diabetic s, although further work is needed to effects and mechanism of the green tea on the histoarchetiture of the ovary and study of green tea on ovary cancer and breast cancer.

Histological study of ovary of control rat (Group-I) and green tea extract fed rat (Group- II) showed normal oogenesis. The total numbers of growing follicles, secondary follicles, primordial follicles and tertiary follicles decreased in diabetic rat (Group-III) and diabetic +Green tea have the normal follicles present no significant change observed because of the acute diabetes

Conclusion

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

- Supplementation of green tea extract $200kg^{-1}bw^{-1}$ in orally for 14^{th} days in STZ monohydrate induced diabetic s caused hypoglycemic, hypotriglyceridemic and β-cell protective activities.
- There was a significant reduction in body weight in diabetic control when compared to normal control. The gradual reduction in body weight in diabetic was amelioed by supplementing green tea.
- The food and water intake was found to be increased in diabetic control due to polyphagia and polydipsia condition and this condition was treated by orally administion of green tea.

- ❖ It has been seen that treatment with green tea extract little bit brought down FBGL from a higher value of 14st day diabetic s.
- ❖ Regarding the histological analysis Ovary of pathological changes occurring in diabetic treated with green tea, it is noted discrete changes which confirms favorable 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 detrition.
- ❖ High Mortality e (0-4 days) % was also found in female s after the induction of STZ diabetes where as it low in male s.
- ❖ Control female when fed with green tea showed insignificant change in body weight, biochemical parameter as well in histopathological.
- ❖ The female animal showed STZ monohydrate is very toxic and produced toxicity as well female was very sensitive to STZ monohydrate.
- ❖ **Future studies**

I. The anti-diabetic activity of green tea can be tested in genetically diabetic animal models.

II. Biomarker of the endometrium and ovary can be investigated.

III. Oral Glucose Tolerance Test and Glycated Hemoglobin (HbA1c) Test can be investigated.

IV. Antioxidant activities can be investigated.

V. Effect of green tea extract on the breast cancer and Cervical dysplasias, PCOS Cervical atypical Ovarian cancer.

VI. Native PAGE and SDS- PAGE can be done of the blood serum, different organs (Liver, Kidney, Ovary, Uterus and Pancreas) extract and uterine fluid for the proper investigations of key biomolecule(s) involved in diabetic as well as treated model in rat.

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

VIII. DNA and RNA estimation can be done for the concerned organs (Liver, Kidney, Brain, Ovary, Uterus and Pancreas).

IX. SEM and TEM of the concerned organs (Liver, Kidney, Brain, Ovary, Uterus and Pancreas) can be done for further investigation.

X. Reproductive organs study can be done for further investigation

XI. Male diabetic rat can also be taken in consideion during reproductive study.

Recommendation

Based on the present study the following studies are recommended to be undertaken prior to subject it to clinical trial.

- Multiple dose of STZ can use for induction of diabetes.
- STZ toxicity in female albino rats
- Oral Glucose Tolerance Test
- Hb1Ac Test
- Mechanism of anti-diabetic action.
- Mechanism of Green tea action.
- Biomarker of the Ovary.
- Effects of Progressive Hyperglycemia on Ovarian Structure:
- Insulin action in the female reproductive system

CHAPTER-VII

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CERTIFICATE

This is to certify that the project titled *"Effect of green tea (Camellia sinensis) extract on histoarchitecture of reproductive organs in streptozotocin induced diabetic female 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.6

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 5 and 6 will be shared, No fresh animal will be given

Dr. Monica Gulati	**Mrs. Yoggata Vaidya**	**Mr. Bimlesh Kumar**
Biological Scientist, Chairperson IAEC	Scientist, COD Pharmacology	Scientist In-Charge of Animal House