STUDY OF STEROID INDUCED HYPERGLYCEMIA AGAINST TRADITIONAL HYPOGLYCEMIC PLANT (*Gynura procumbens*) EXTRACT IN RAT MODEL

A Thesis

By

RUPSANA PERVEN BORSHA

Registration No. 1805420

Session: 2018-2019

Semester: January-June, 2020

MASTER OF SCIENCE (M.S.)
IN
PHYSIOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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JUNE, 2020

DEDICATED

TO MY

BELOVED PARENTS

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ABSTRACT

Now a days corticosteroids are used in various therapeutic purposes and longtime use of the drug elevates the blood glucose level results in steroid induced hyperglycemia. Therefore, treatment of hyperglycemia by synthetic drug may develop health risks along with adverse effects. Moreover, present study was undertaken to examine the efficacy of *Gynura procumbens* plant on steroid induced hyperglycemia in rats. Fifteen Long evan male rats were randomly divided into three different groups (n=5 in each group) for 21 days. Group T_0 served as negative control, Group T_1 served as positive control was treated with dexamethasone intramuscularly @ 5 mg/kg body wt. Group T_2 served as treatment group injected with dexamethasone and *Gynura procumbens* ethanolic extract @ 150 mg/kg body wt. orally for 21 consecutive days. During and at the end of the experiment blood glucose, lipid profile, HbA1c, Plasma insulin and live body weights were recorded. Histopathology of rat pancreases was also observed. Blood glucose level were significantly increased after steroid treatment compared to negative control group. *Gynura procumbens* treatment for 21 days does not have any significant effects on blood glucose level. Steroid treatment significantly reduces body weight. However, *Gynura procumbens* does not have any significant effects on body weight. Similarly *Gynura procumbens* does not have any effects on steroid induced elevated HbA1c, lipid profile and insulin level. Histopathological evaluation indicates hyperplastic growth over the Langerhans cells in pancreas of steroid treatment rat which were not altered by *Gynura procumbens* treatment. Finally this study shows that chronic steroid therapy induced hyperglycemia in rat is irresponsive to *Gynura procumbens* treatment.

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CHAPTER I

INTRODUCTION

Hyperglycemia is a fast growing health problem throughout the world. High levels of sugar in the blood occur when the body does not produce or use enough insulin, which is a hormone that absorbs glucose into cells for energy. High blood sugar is a leading indicator of diabetes. Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by persistent hyperglycemia (Neelesh *et al.,* 2010). During hyperglycemia polyuria, polydipsia, polyphagia and weight loss abnormalities occurs due to deficiency of β-cells of the endocrine pancreascausing insulin deficiency and/or from subsensitivity to insulin in peripheral cells (Rajasekaran *et al.,* 2005), primary changes occursin carbohydrate metabolism and secondarily of lipids and proteins (Al-Attar and Zari, 2010).Recent data from the International Diabetes Federation (IDF) indicates that Diabetes mellitus affects over 366 million people worldwide and this is likely to increase to 552 million or even more by the year 2030 (Whiting *et al.,* 2011). Steroid-induced diabetes mellitus (SIDM) has been recognized as a complication of glucocorticoid use for over 50 years. (Hwangand Weiss, 2014).

There are a large number of factors responsible for hyperglycemia. Corticosteroid is one of these which iswidely used as medicine and frequently prescribed as anti-inflammatory, immunosuppressant and replacement therapy (Van-Raalte *et al.,* 2013). Steroid therapy can promote chronic obstructive pulmonary diseases, acute gout, chemotherapy protocols, bacterial meningitis and in pregnant women for fetal lung maturation. Chronic glucocorticoid therapy can cure pulmonary diseases such as idiopathic interstitial pneumonia, hypersensitivity pneumonitis, sarcoidosis, autoimmune diseases, neurological disorders such as myasthenia gravis and multiple sclerosis and inflammatory bowel diseases. More recently, chronic glucocorticoid therapy plays an important role in modulating the immune system following solid organ transplantation. Although widely prescribed for their anti-inflammatory and immunosuppressive properties, glucocorticoids have various common metabolic side effects including hypertension, osteoporosis and hyperglycemia.However, this corticosteroid can have detrimental side effects on blood glucose it increases “insulin resistance” making the insulin less effective. Steroid elevates blood glucose level by increasing hepatic glucose production (gluconeogenesis) and inhibiting glucose uptake into muscles. It also has a

complex effect on beta cell function (Chen *et al.,* 2017). Phosphoenylpyruvate carboxykinase (PEPCK) is reciprocally upregulated in liver and downregulated in adipose by glucocorticoids (Cadoudal *et al.,* 2005). This results in a buildup of free fatty acids in the blood, which in turn result in insulin resistance and increase gluconeogenesis.The development of hyperglycemia in human beings during steroid therapy is well known (Bookman *et al.,* 1953).

There is a variety of glucose-lowering agents available for reducing blood sugar level which are expensive for the large diabetic population of developing countries. Thus, new strategies like herbal remedies are needed for the prevention and treatment of diabetes. Recently *Gynura procumbens* plant commonly used as hypoglycemic agent. The bioactive compounds of *Gynura procumbens* is flavonoids and glycosides (Akowuah *et al.,* 2001, 2002). Most importantly, small molecule kaempferol (3,4′,5,7-tetrahydroxyflavone) is a yellow crystalline solid, a type of flavonoid, found in a variety of plants and plant-derived foods which promotes insulin sensitivity and preserved pancreatic *β*-Cell mass in mice is slightly soluble in water and highly soluble in hot ethanol, ethers, and DMSO. Whereas, insulin resistance and a progressive decline in functional *β*-cell mass are hallmarks of developing steroid induced hyperglycemia. Kaempferol a naturally occurring anti-diabetic agent may improve peripheral insulin sensitivity and protecting against pancreatic β-cell dysfunction (AlAlgaririy *et al.,* 2015).

Reviewing the previous research work, there is little knowledge about this plant to treat corticosteroid induced hyperglycemia on rat. Therefore, present study will be undertaken to evaluate the possible antihyperglycemic activity of the ethanolic extract of *G. procumbens* during corticosteroid therapy on hyperglycemic rats. So, the general objective of this study is to determine the antihyperglycemic effect of *G. procumbens* leaf extract with the following specific objectives:

- To determine the blood glucose level, plasma insulin concentration, lipid profile and HbA1c level on corticosteroid induced hyperglycemic rat treated with extract.
- To know the body weight on corticosteroid induced hyperglycemic rats treated with *Gynura procumbens* leaf extract.
- To observe the histopathological changes of pancreas in steroid induced hyperglycemic rat.

CHAPTER II

REVIEW OF LITERATURE

The purpose of this chapter is to provide a selective review of the research works performed in relation to the present study. Literature on consequences of steroid induced hyperglycemia against traditional hypoglycemic plant in rat model which is related to this study has been reviewed under the following heading.

2.1 Hyperglycemia

Hyperglycemia refers to high levels of sugar, or glucose in the blood. The criteria for diagnosing hyperglycemia by the American Diabetes Association is an 8 h fasting blood glucose ≥ 7.0 mmol/L (126 mg/dL), 2 h post 75 g oral glucose tolerance test (OGTT) ≥ 11.1 mmol/L (200 mg/dL) and a random plasma glucose of ≥ 11.1 mmol/L (200 mg/dL) (Hwang and Weiss, 2014). Hyperglycemia results from insulin deficiency, impairment of insulin's action in peripheral tissues (decreased glucose use), increased hepatic gluconeogenesis and glycogenolysis, or a combination of these (Nelson, 2012). High blood sugar is a leading indicator of diabetes. If a person with diabetes does not manage the sugar levels in their blood, they can develop a severe complication called diabetic ketoacidosis, is a dangerous complication of diabetes (WebMD, 2019). Patients with diabetic ketoacidosis may present with nausea, vomiting and abdominal pain (Mouri and Badireddy, 2019).

2.2 Present Status of Hyperglycemia

Recently compiled data shows that approximately 150 million people have diabetes mellitus worldwide and this number may well double by the year 2025. IDF and WHO predict that the number of women in the world with diabetes will double in less than 20 years. In Bangladesh, the number of women with diabetes will grow from the current 2 million to 4 million by 2025. The global diabetes prevalence in 2019 is estimated to be 9.3% (463 million people), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045. The prevalence is higher in urban (10.8%) than rural (7.2%) areas and in high-income (10.4%) than low-income countries (4.0%) (World Health Organization 2019). About 10 million people in Bangladesh are affected with diabetes (Chaity, 2017). Another study gives an idea about more alarming facts that is almost one in ten adults in Bangladesh was found to have diabetes (Akter*et al.,* 2014). According to IDF,

approximately USD $41 was spent on per person with diabetes in Bangladesh. The low-level of diabetes-related health expenditure has caused unwanted growth in diabetes-related death. WHO estimates that diabetes was the seventh leading cause of death in 2016. According to IDF 2019 Diabetes caused 4.2 million deaths. Diabetes in Bangladesh is affecting young people and causing disability, loss of income and early death. About 12% of households either borrow money or sell household assets to pay for diabetes treatment, said a report published in WHO Bulletin in 2013. The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014 (Akter *et al.,* 2014). It is more prevalent in older populations (Warram *et al.,* 2005) but now more and more younger populations are being diagnosed with type *2* diabetes. Around 438 million people (7.8%) at the age of 20-79 year will be diabetic by 2030 worldwide (Saeedi *et al.,* 2020). The global economic cost of diabetes in 2014 was estimated to be USD 612 billion dollars (International Diabetes Federation 2013). Diabetes caused at least USD 760 billion dollars in health expenditure in 2019-10% of total spending on adults (Top *et al.,* 2020).

2.3 Risk Factors for Glucocorticoid-Induced Hyperglycemia

Suh and Park (2017) mentioned the following risk factors for glucocorticoid induced hyperglycemia.

- Higher dose of glucocorticoid treatment (prednisolone >20 mg, hydro-cortisone >50 mg, dexamethasone >4 mg)
- Longer duration of glucocorticoid treatment
- Advanced age
- High body mass index
- Previous glucose intolerance or impaired glucose tolerance
- Personal history of gestational diabetes or previous glucocorticoid-induced hyperglycemia
- Family history of diabetes mellitus

2.4 Pathophysiology

Insulin is secreted after a meal or carbohydrate load and it suppresses endogenous glucose production (EGP). On the other hand contra regulatory hormones cortisones and glucagon increases the EGP under fasting conditions (Barthel *et al.,* 2003). Cortisol, the active principal of glucocorticoid in humans, is secreted by the adrenal gland and is converted to cortisone, the inert glucocorticoid, primarily in kidney (Wang 2005).After inducing glucocorticoid drugs it increases the endogenous glucose production (EGP) by accruing gluconeogenesis rather than glycogenolysis (Bollen *et al.,* 1998). Glucocorticoids stimulate gluconeogenesis by enhancing the activity of phosphoenolpyruvate carboxykinase (PEPCK) (Jin *et al.,* 2004) and glucose-6-phosphatase (Vander Kooi *et al.,* 2005) are key enzymes of gluconeogenesis. This PEPCK gene contains a glucocorticoid response element (GRE) in it's promoter region and act as a key player in glucocorticoid induced gyperglycemia (Vegiopoulos *et al.,* 2007).

The effect of glucocorticoids on glucose metabolism is likely the result of impairment ofmultiple pathways including beta cell dysfunction (sensitivity to glucose and ability to release insulin) and insulin resistance in other tissue (Hwang and Weiss, 2014). The insulin-mediated pathways of glycogen synthesis, protein degradation and synthesis are directly influenced by glucocorticoids. Skeletal muscle is responsible for the majority of insulin-mediated glucose uptake. Insulin recovers GLUT4 glucose transporters to the cell surface enabling glucose uptake into the cell. Glucocorticoids impair insulin-mediated glucose uptake by directly intervening with components of the insulin signaling cascade, such as glycogen synthase kinase-3, glycogen synthase and GLUT4 translocation. An increase in protein degradation and decrease in protein synthesis is due to glucocorticoid inhibition of post-insulin receptor cascades involving PKB/Akt and mTOR pathways.

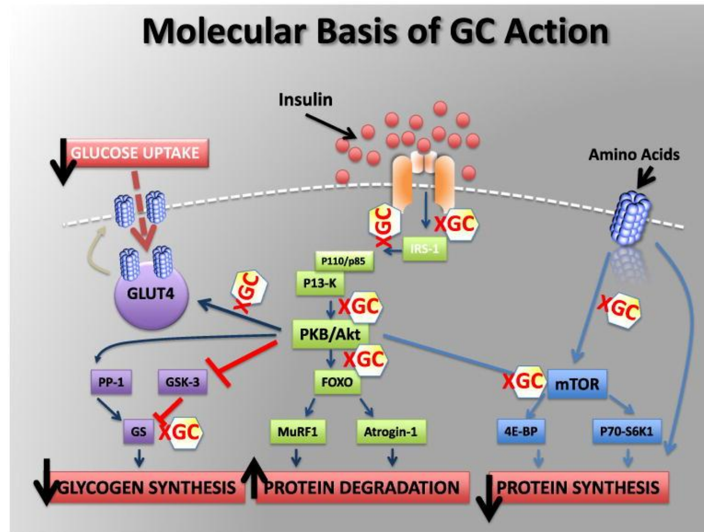


Figure 2.1: Molecular Basis of Glucocorticoid (GC) Action (Van-Raalte *et al.,* 2013)

Glucocorticoids (GCs) induce their diabetogenic effects both by inducing insulin resistance at the level of liver, skeletal muscle and adipose tissue and by impairing pancreatic islet-cell dysfunction (Van-Raalte *et al.,* 2013).

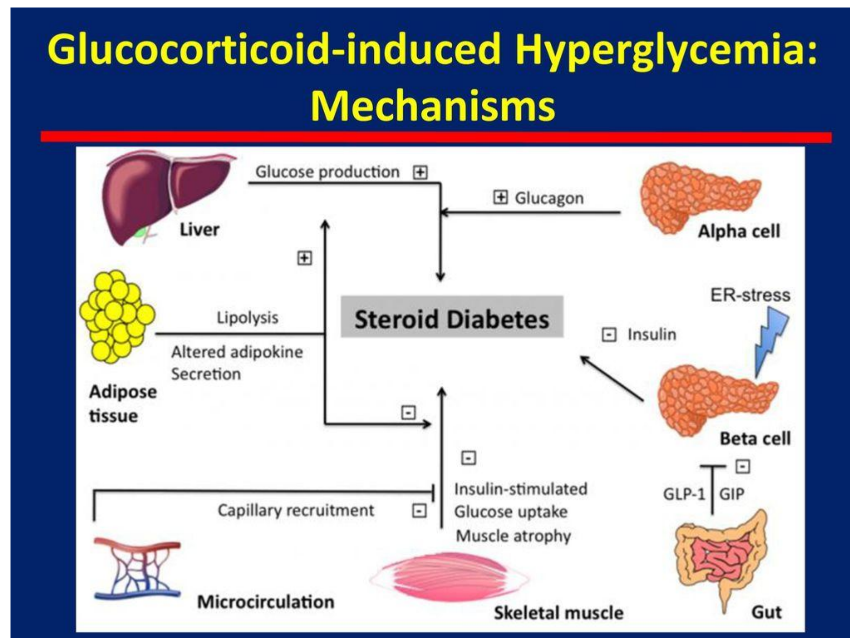


Figure 2.2: Mechanisms of Glucocorticoid Induced Hyperglycemia (https://slideplayer.com/slide/12331921/)

Glucocorticoid reduces peripheral insulin sensitivity and/or promotes weight gain. Glucose production enhances through accretion of hepatic gluconeogenesis. Thus, pancreatic cells debacles, leading to β-cell injury (inflammation) and release of insulin

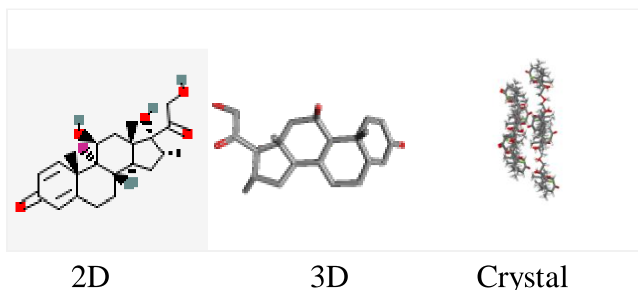
hampered by β-Cell dysfunction. Finally glyceroneogenesis inhibited and results in increased fatty acids.

In rats, dexamethasone was shown to impair the insulin-signaling cascade, leading to reduced activation of insulin target proteins and genes in liver cells (Saad *et al.,* 1993). Thus, the liver is an important player in the diabetogenic effects induced by glucocorticoid treatment.

2.5 Management

Hyperglycemia is a chronic disease and without proper management it can not be cured. Affected people can live long and remain healthy if early detection is followed by well management. Management practice helps to keep the blood sugar level as close to normal by maintaining exercise, The American Diabetes Association (ADA) recommends getting 30 minutes of moderate to vigorous exercise on at least 5 days of the week, diet (vegetables, fruits, grains, proteins, low-fat non-dairy or non-fat cheese, milk, and yogurt) and having appropriate medications. Foods like fried, salty such as potato chips, sugary candy, ice-cream, cakes, energy drinks etc. should avoid. Furthermore, blood glucose control should be monitored through regular measurement. Glycated haemoglobin (HbA1c) is the method of choice for monitoring glycaemic control in case of hyperglycemia.Adequate knowledge about the disease and following the treatment is vital because if blood sugar levels are properly managed complications are less severe (Nathan *et al.,* 2009). Attention should pay on other health problems i.e: high blood pressure, obesity, high cholesterol level because these may ascent the pernicious effect of hyperglycemia.

2.6 Hyperglycemic Agent Dexamethasone

Trade Name	:	Dextason
Generic Name	:	Dexamethasone sodium phosphate USP 5 mg/ml.
Structure	:	
Molecular Formula	:	$C_{22}H_{29}FO_5$
Synonyms	:	Dexamethasone, Decadron, Dexamethazone.
Molecular Weight	:	392.5 g/mol
Elimination half life	:	190 minutes (3.2 hours)
Plasma half life	:	Ranges from 80-270 minutes depending on the type of Glucocorticoid used

2.6.1 Synthesis

To synthesize dexamethasone, 16β-methylprednisolone acetate is dehydrated to the 9,11-dehydro derivative (Arth *et al.*,1958). This is then reacted with a source of hypobromite, such as basic *N*-bromosuccinimide, to form the 9α-bromo-11β-hydrin derivative, which is then ring-closed to an epoxide. A ring-opening reaction with hydrogen fluoride in tetrahydrofuran gives dexamethasone.

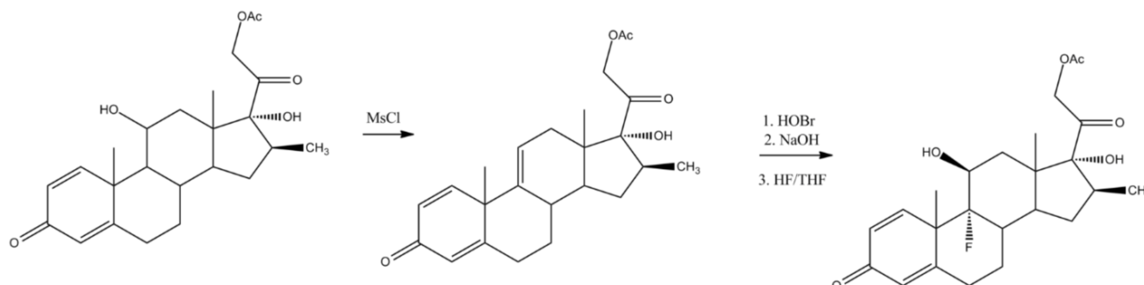


Figure 2.3: Dexamethasone synthesis (Wikipedia, 2019)

2.6.2 Corticosteroid Induced Hyperglycemia

Corticosteroid used to reduce inflammation canelevate the blood glucose concentration, which is called steroid hyperglycemia. Glucocorticoids stand against insulin action and stimulate gluconeogenesis, especially in the liver, resulting in a net increase in hepatic glucose output and induce insulin resistance, hyperglycemia, hyperlipidemia (Heather *et al.,* 2012). The development of insulin resistance is mainly postprandial and varies on the type of steroid used i.e: intermediate-acting and long-acting GCs.The most common glucocorticoids which cause steroid hyperglycemia are prednisolone and dexamethasone.Prednisone and methylprednisolone are classified as intermediate-acting GCs which shows peak action after 4-6 h of administration. Their effect on glucose levels is mainly during the afternoon and night. When they are administered in a single dose no effect on fasting glucose. On the other hand, they cause persistent hyperglycemia when administered in divided doses. Dexamethasone is a long-acting GCs, with a steroid hyperglycemia that lasts for more than 24 h, with a slight decline during an overnight fast (Perez A *et al.,* 2014). Corticosteroids increase endogenous glucose production, accelerate gluconeogenesis and antagonizing the metabolic actions of insulin. It enhances the effect of other counter regulatory hormones, such as glucagon and epinephrine which increases the endogenous synthesis of glucose. Corticosteroids reduce peripheral glucose uptake at the level of the muscle and adipose tissue. Corticosteroids also inhibit the production and secretion of insulin from pancreatic β-cells and induce β-cell failure indirectly by lipotoxicity.

2.6.3 Effect of Glucocorticoids on Pancreatic Beta and Alpha-cells

Glucose metabolism is directly influenced by the pancreatic beta-cells. Beta-cell dysfunction develops the metabolic defect during hyperglycemia (Kahn, 2003). when workload on the beta cell increases (by factors such as obesity, insulin resistance or low-grade inflammation) healthy beta cells can adapt by rising insulin secretion to meet this increased demand, thus maintaining euglycaemia the beta cells fails to secrete insulin sufficiently to meet insulin demands (Raalte *et al.,* 2011). Finally results in glucose intolerance and hyperglycemia. In addition to diminishing insulin secretion, GCs impaired insulin biosynthesis and induced beta-cell apoptosis following more prolonged incubation (Ranta *et al.,* 2006). GCs induce 'ER stress' (Endoplasmic Reticulum (ER), a cell organelle responsible for the synthesis of all secreted proteins, most importantly

insulin). ER stress is characterised by an accumulation of misfolded proteins inside the organelle. This ER stress may result in reduced insulin production, may also trigger beta-cell apoptosis (Linssen *et al.,* 2011).

The pancreatic alpha cell has an important role in glucose metabolism because it secrets glucagon hormone (Gromada *et al.,* 2007) which stimulates hepatic glucose production by promoting glycogenolysis and gluconeogenesis. The effects of GCs on glucagon levels are dose-dependent: only high-dose (30 mg prednisolone daily), but not low-dose (7.5 mg prednisolone daily) GC treatment increased fasting and postprandial glucagon levels following a two-week treatment in healthy men (Raalte *et al.,* 2013). The effect of high-dose GCs on glucagon levels was already evident. So in GC-induced hyperglycaemia both fasting and postprandial hyperglucagonaemia are present.

2.7 Plant with Hypoglycemic Agent (Insulin)

- **Description of the Medicinal Plant (*Gynura procumbens*):**

  Vernacular Names

  Thailand: Paetumpung,

  Malaysia: Mollucan spinach, Sambung Nyawa,

  Indonesia: Daun Dewa, Sambung Nyawa,

  Chinese: Akar Sebiak, Kelemai Mearh, Nan fei Ye, Bai Bing Ca,

  United States: Longevity spinach

  Bangladesh: Insulin Plant, Diabetes Plant

Gynura procumbens family (asteraceae) locally known as Insulin plant, Sambung Nyawa, Longevity spinach, Anti-cancer Spinach, Anti-cholesterol Spinach, Anti-diabetes spinach, Anti-hypertension spinach, Healing herb, Miraculous herb, Wonder plant, Leaves of the gods is available in south east Asia especially in Malaysia, Thailand, Indonesia. It is a small plant of 1-3 m long with fleshy stem and leaves are ovatelliptic or lanceolate (Rahman *et al.,* 2013) 3.5 to 8 cm long, 0.8 to 3.5 cm wide. These leaves have high medicinal values and used to treat illnesses such as eruptive fevers, rash, kidney diseases, migraines, constipation, hypertension, cancer and diabetes mellitus (Perry and Metzger, 1980). The plant leaf is commonly consumed and scientifically has been shown to be safe for consumption (Yam *et al.,* 2008). It has anti-hyperglycemic, anti-cancer, anti-microbial, anti-inflammatory, anti-oxidant, anti-hypertensive and fertility enhancement effects.

Lee *et al.,* 2011, described that *Gynura procumbens* contains considerable medicinal values. They investigate the antidiabetic properties on aqueous and ethanolic extracts. Again another research justified that *G. procumbens* as an anti-hyperglycemic agent may be beneficial for male diabetic patients that suffer from sexual dysfunction as a side effect of prolonged hyperglycemia (Pusparanee *et al.,* 2015). Algariri *et al.,* 2013, established that *G. procumbens* contains antidiabetic principles, most extracted in 25% ethanol. Another research denotes that *G. procumbens* only significantly increased glucose uptake by muscle tissues and it did not show a significant effect on insulin level either in the in vivo test or in vitro RIN-5F cell culture study. *G. procumbens* also showed minimal effects on β-cells of the islets of Langerhans in the pancreas (Zurina *et al.,* 2010). Zhang *et al.,* 2000 found that the leaves of *G. procumbens* may have biguanide-like activity and the extract significantly reduced serum cholesterol and triglyceride levels in rats. Algariri *et al.,* 2014, stated *Gynura procumbens* leaves a promising source of new antidiabetic natural products. It contains high level of phenols and flavonoids that shows anti-hyperglycemic activity. Hamid *et al.,* 2004, suggested that *G. procumbens* leaves extract possess hypoglycemic activity in normal rats and stimulate insulin release in insulin secreting cell line without any cytotoxic effects. Choi *et al.,* 2016, demonstrated that *Gynura procumbens* extracts might improve insulin sensitivity and inhibit gluconeogenesis in the liver because it decreases the expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase in the liver.

G. procumbens extract may help to alleviate postprandial hyperglycemia by inhibiting carbohydrate digesting enzymes (Choi *et al.,* 2016). Hassan and Ahmad 2004, explained that the aqueous extract of *G. procumbens* leaves shows hypoglycemic activity due to extra pancreatic action rather than it's insulinotropic activity.

2.7.1 Biochemical Constituents

Gynura procumbens leaves contain several active chemical constituents such as flavonoids, saponins, tannins, terpenoids and steroil glycosides (Akowuah *et al.,* 2002). The leave extract have potent anti-hyperglycemic effect. Ethanolic extract of GP leaves have anti-hyperglycaemic and anti-hyperlipidemic activities in diabetic rats (Zhang and Tan, 2000) and the n-butanol fraction from the GP leaves also has hypoglycemic effects. In addition hexane and ethyl acetate fractions have potential in stimulating glucose uptake in T3-F44 adipocytes (Bohari *et al.,* 2000). Astragalin the phytochemical present

in *Gynura procumbens* leaves exerts the anti-hyperglycemic activity (Kanzil and Pritesh, 2010).

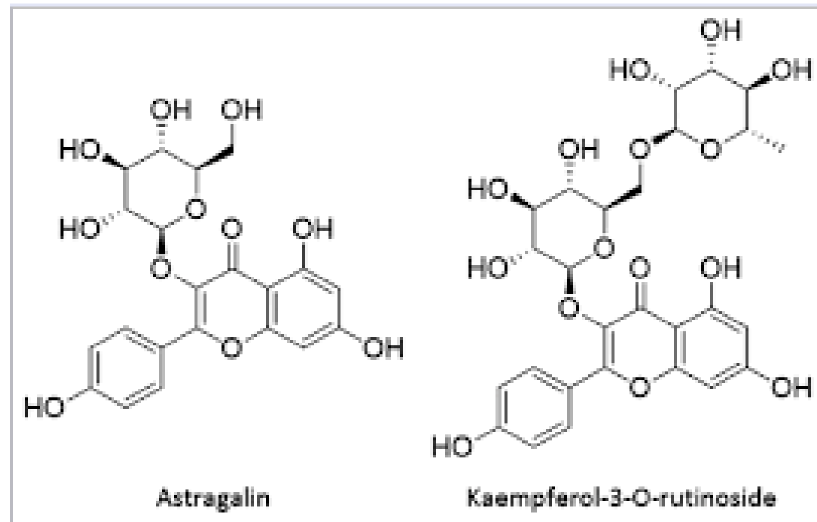


Figure 2.4: Phytochemicals: Astragalin and kaempherol-3-O-rutinoside (http://www.phcog.com/viewimage.asp)

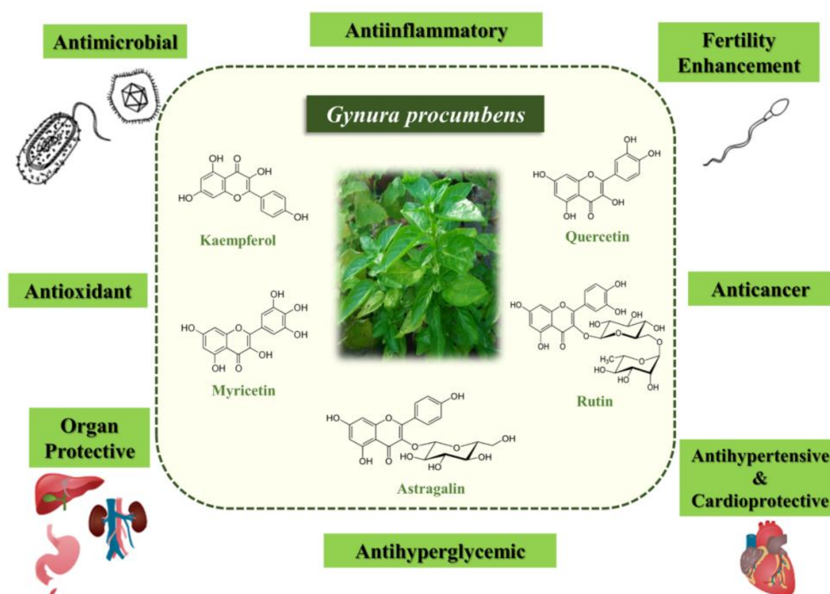


Figure 2.5: Bioactive Compounds of Insulin Plant (Pharmacol, 2016)

2.7.2 Antihyperglycemic Activity of *G. procumbens*

In traditional medicine the leaf is commonly used for diabetes treatment. In vivo studies reported thatit has hypoglycemic effect (Hamid *et al.,* 2004; Algariri *et al.,* 2014). It significantly decreases fasting blood glucose levels and curbs glucose elevation during glucose tolerance test in diabetic rats but not normal rats (Zhang and Tan, 2000; Algariri *et al.,* 2013). *G. procumbens* treatment exhibits it's effect on insulin level. Insulin secreting cell lines are stimulated by *G. procumbens* extract (Hamid *et al.,* 2004). But due to different response of different cell lines clonal pancreatic cells with the extract exposure did not stimulate insulin secretion (Hassan *et al.,* 2008). However, it shows hypoglycemic activity due to its extra-pancreatic effect instead of insulinotropic activity (Hassan *et al.,* 2008; Lee *et al.,* 2011).

Glucose uptake activity influences the hypoglycemic effect. *G. procumbens* treatment stimulated glucose uptake on 3T3 adipocytes. Insulin enhances the glucose uptake activity (Bohari *et al.,* 2006). *G. procumbens* extract indicates a direct effect on glucose uptake and utilization at the peripheral levels because Hassan *et al.,* 2010 reported that glucose uptake enhanced by the muscle tissue of diabetes rat. *G. procumbens* exert an effect on glucose metabolism in liver. Phosphorylation occurs in diabetic rats liver and glycogen synthase kinase 3 (GSK3) become inactivated. However, either direct or indirect effects on the upstream components activity in the insulin signaling pathway *Gynura procumbens* performs the hypoglycemic activity (Gansau *et al.,* 2012). In addition it increases the glucokinase activity, pyruvate dehydrogenase and phosphorylation of ATP-citrate are known to play roles in glucose metabolism (Kang *et al.,* 2015). Lee *et al.,* 2011 mentioned that after the GP treatment specific activity of liver hexokinase, phosphofructokinase and fructose-1,6-bisphosphatase enhances. Thus indicates *G. procumbens* increased hepatic glucose utilization and decreased endogenous glucose production. *G. procumbens* in combination with other herbal therapies also develops hypoglycemic effects.

CHAPTER III

MATERIALS AND METHODS

This research work was conducted from 13th October 2019 to 4th November 2019 at animal laboratory under the department of Physiology and Pharmacology in Hajee Mohammad Danesh Science and Technology University, Dinajpur to evaluate the hypoglycemic effect of *Gynura procumbens* ethanolic extract on corticosteroid induced hyperglycemic rats.

3.1 Management of Experimental Rats

The weight 50 to75gm of Long Evans (out bred) male rats of 35 days oldwere obtained from Animal Resource Facility, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr, b). Experimental procedures, guidelines for care and use of experimental animals were approved by the department of Physiology and Pharmacology. Animals were acclimatized in the laboratory for 15 days before initiating the experimental works. The rats were housed in wire cages measuring 30×13×15 cm at room temperature (28±5)°C under a light/dark cycle of 12 hours. Rats were maintained on a standard commercial rat pellet diet supplied by icddr, b with water ad-libitum throughout the experimental period.

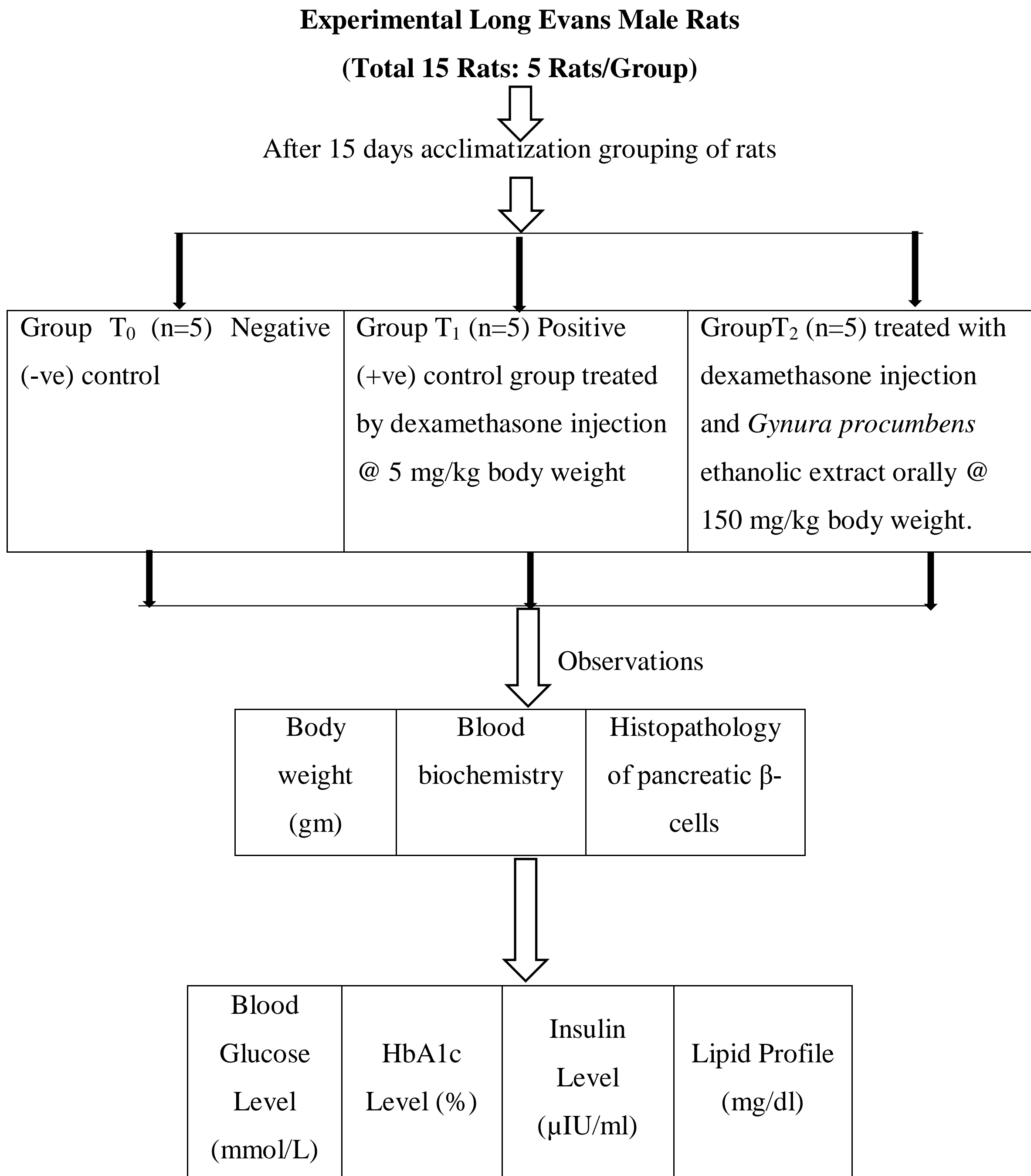
Figure 3.1: Management of Experimental Rats.

3.2 Experimental Design

Fifteen rats were used to execute this experiment. These 15 rats were divided into three groups named T_0, T_1, T_2 containing 5 rats in each group. T_0 was the negative control group without any induction whereas T_1 was the positive control (treated with dexamethasone) and the T_2 group was treated with both of dexamethasone and *Gynura procumbens* leave extract. Dexamethasone (Dextason) was injected intramuscularly at

the dose rate of 5mg/kg body weight into the rats of both T_1 and T_2 group. In the T_2 (treatment group) hyperglycemia was treated with *Gynura procumbens* ethanolic extract at the dose rate of 150 mg/kg body weight orally for 21 consecutive days.

3.3 Experimental Layout



3.4 Collection of Drug and Equipments

Dxamethasone sodium trade name: Dextason injection was collected from Ziska pharmaceuticals Ltd. 1ml syringe were used for intamuscular injection. Sugar check machine was collected for blood sugar monitoring with blood sugar monitoring test strips.

Figure 3.2: Dexamethasone Drug and Equipments.

3.5 Induction and Determination of Hyperglycemia in Rats

Corticosteroid induced hyperglycemia was introduced by giving higher dose (5mg/kgbody weight) dexamethasone injection (Trade name: Dextason, each ampule contains 5mg in 1 ml) via intamuscular route. After 3 days of injection the fasting blood sugar was determined onstrip method by Sugar Check machine.Blood was obtained from tail vein of overnight fasting rats and rats with blood glucose levels 12.8 mmol/L were considered as hyperglycemic. The reference values of glucose in plasma or serum is about 4.5 to 6.1 mmol / L (Tietz, 1995). Fasting blood glucose was measured on day 1 (before the experiment starts) and then 4th , 7th , 14th and the last 21st day for continuous monitoring.

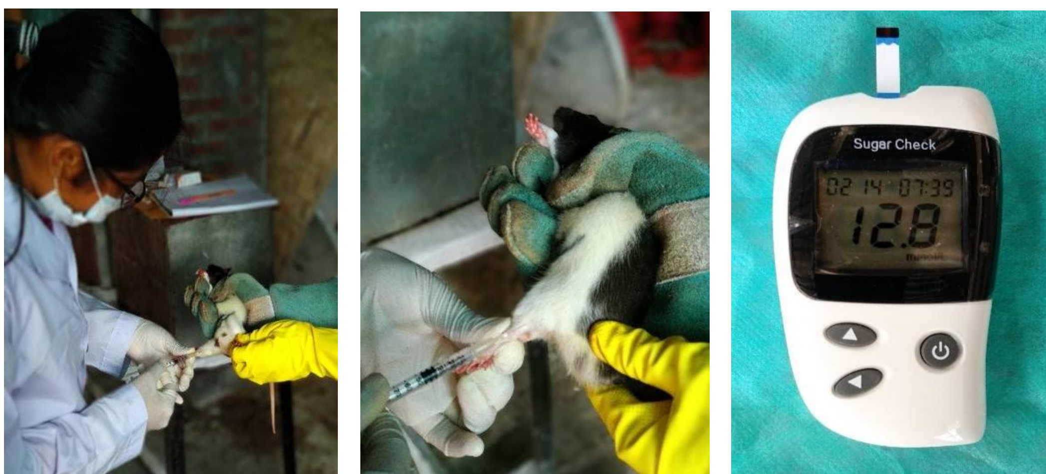


Figure 3.3: Induction and Determination of Hyperglycemia in Rats

3.6 Plant Materials

Leaves of *G. procumbens* were collected from the faculty of Agriculture, HSTU and then it was cultivated under proper conditions.

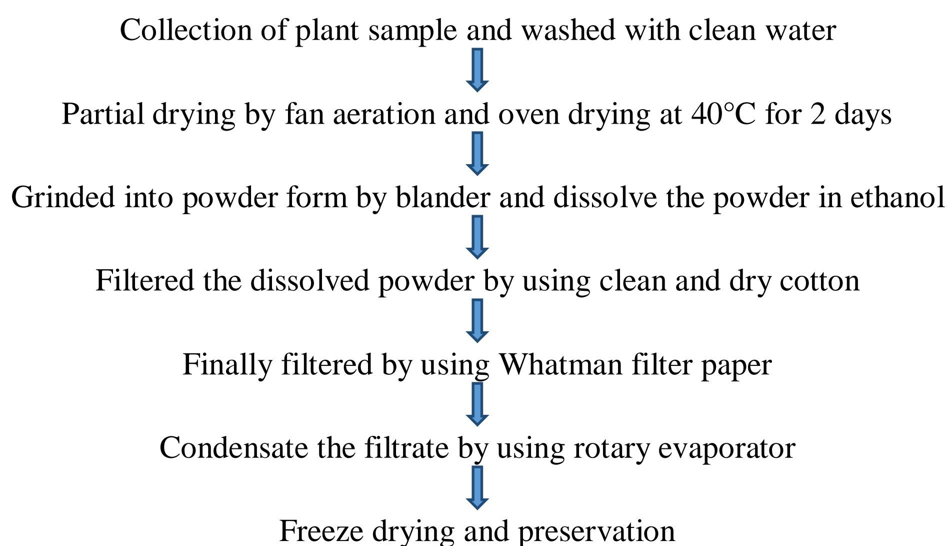
Figure 3.4: Traditional Hypoglycemic Agent Insulin Plant

3.6.1 Collection and Preparation of Extract

Firstly the *Gynura procumbens* leaves were collected. Fresh leaves were washed, weighed and dried in oven (Sheldon Manufacturing, Inc., USA) at 45°C for 3 days. After drying, dried leaves were blended into powder form. Ethanolic extract of *G. procumbens* leaves were prepared according to the method of Zhang and Tan (2000). Fresh leaves of *G. procumbens* (30 gm) were washed, blended and mixed with 95% ethanol (200 ml) for 7 days at room temperature. The extract was then filtered, obtained supernatant was concentrated by using rotary evaporator at 40°C. To yield yellowish dark green powder of *G. procumbens* crude ethanolic extract were stored at normal freezing temperature (4°C).

Figure 3.5: Preparation of Ethanolic Extracts

3.6.2 Extraction Procedure



3.6.3 Administration of *Gynura procumbens* Extract

The prepared extracts were fed orally at a dose rate of 150 mg/kg body weight to the treatment group with the help of a dropper. Dropper with leveling marks ensured the adequate quantity which was fixed on the basis of body weight of each individual rat. Mortality and general behaviors of the animals were closely obseved for the first 4 hours and intermittently for the next 6 hours. Again after 24 hours and 48 hours of following dose administration grooming, alertness, sedation, light reflex, respiratory rate were observed.

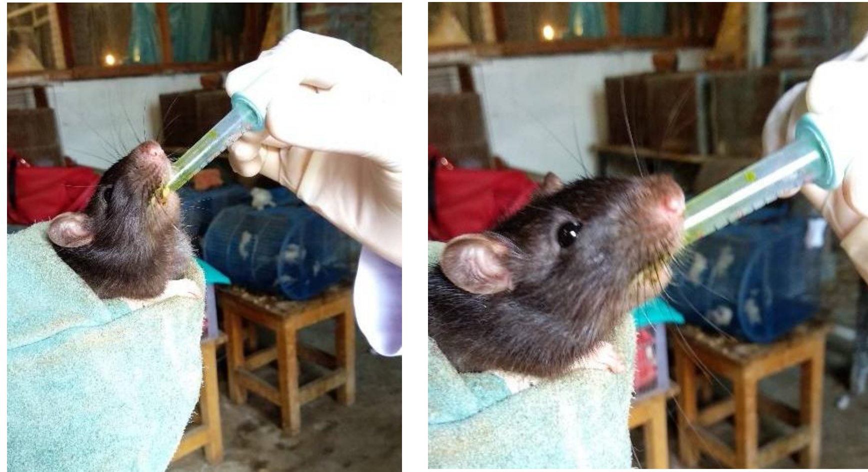


Figure 3.6: Administration of *Gynura procumbens* extract

3.7 Recording of Different Parameters

3.7.1 Determination of Body Weight

Body weight was taken on 1st day (pretreatment) and 7th , 14th, 21st day (during treatment).

3.7.1.1 Materials Required

- Leather gloves
- Electric balance
- Plastic basket

3.7.1.2 Procedure

Initial body weight of all groups were recorded before the treatment on 1st day, during the treatment period on 7th , 14th and 21st day with the help of an electric balance.

Figure 3.7: Recording of Rat Body Weight

3.7.2 Recording of Blood Glucose Level

3.7.2.1 Collection of Blood

Firstly the tail was rubbed for a few minutes and then fresh blood was collected from the tail vein.

3.7.2.2 Materials Required

- Leather gloves
- Lancet
- Cotton
- Sugar check machine
- Sugar check test strips
- Ethanol
- Active monitor

3.7.2.3 Procedure

Blood samples were collected from the tail vein by piercing with a lancet. Then the drop of blood was immediately placed on the strip of the sugar check machine. Sugar check machines active monitor quickly find the glucose level and expressed it in mmol/L.

3.7.2.4 Determination of Blood Glucose Level

Blood samples were collected from tail vein at 1st, 7th, 14th, 21st day for estimation of blood glucose level by the Sugar check machine active monitor system (strip method). A drop of blood was poured on the test zone of the strip at the same time Sugar check

machines active monitor was started. Before using the test strip new choding chip was inserted by the side of the monitor. Final values were expressed in mmol/L.

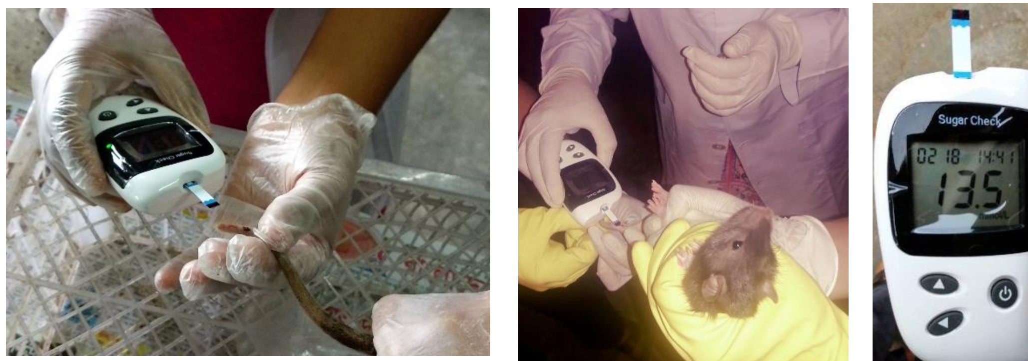


Figure 3.8: Determination of Blood Glucose Level

3.7.3 Determination of Plasma Insulin Concentration

At the end of the experiment period, all groups of rats were euthanized by diethyl ether anesthetic agent and the blood samples were collected from the heart by direct puncture. After that blood samples were centrifuged for 10 minutes at 3000 rpm by the centrifuge machine and the serum samples seperated into microtubes stored at -20°C until analysis. Final analysis was done by auto analyzer.

3.7.4 Determination of Glycated Hemoglobin HbA1c Level

At the end of the experiment period, all groups of rats were euthanized by diethyl ether anesthetic agent and the blood samples were collected from the heart by direct puncture. The amount of 1 ml of blood was collected into Vacuette EDTA tubes used for determination of HbA1c concentration and samples were immediately transferred to the laboratory for the estimation of HbA1c level in %. Estimations were carried out by Hemocue machine. Then the reports were delivered for further analysis. The reference values of HbA1c in non-diabetics patients are about 4 to 6.2% (Goldstein*et al.,* 1995).

3.7.5 Determination of Lipid Profile

At the end of the research period blood samples were collected by euthanizing the animal. Blood was taken directly from the heart. About 1 ml of blood sample was taken into Vacuette EDTA tubes used for determination of lipid profile. After that samples were immediately transferred to the laboratory for the estimation of lipid profile in

mg/dl. The value were estimated by using Dimension EXL 200 is a fully biochemistry analyzer.

3.7.6 Histopathological Examination

Histopathology was performed to find the pancreatic cells in normal and hyperglycemic rats by the following ways. Firstly the tissue sections of pancreas were prepared from the preserved samples. Then kept under running tape water drop by drop overnight . Washed at (50%, 70%, 80%, 95%) alcohol for 1 hours respectively. Three times washing in 100% alcohol within 1 hour. Again two times chloroform washing within 1.5 hours were done then kept at paraffin bath (56°C) for 3 hours. Then paraffin block were prepared and then tissue sectioning were done by using Microtome machine and cut at 6 µtome. Afterthat staining was performed by using xyline, alcohol (100%, 95%, 80%, 70%) for 2 minutes respectively. Afterthat slides were kept under distilled water and Haematoxylin dye for 10 minutes individually. Few drops lithium carbonate were also applied and kept under Eosin dye for 30 seconds. Finally DPX solution were applied and made the slides permanent by putting cover slips over there and observed under microscope at 10x objective.

Figure 3.9: Histopathological Examination

3.8 Stastical Analysis

The results of various parameters were expressed as ±SEM. Stastical data analysis were done by using SPSS version 22 and Microsoft Excel. Statistically significant differences between group means were determined by Analysis of Variance (ANOVA).

CHAPTER IV

RESULTS

Table 4.1: Effect of *Gynura procumbens* plant extracts on blood glucose level (mmol/L, mean±SE) in steroid induced hyperglycemic rats (n=5) at seven days interval

Group	Day 0 (Mean ± SE)	Day 7 (Mean ± SE)	Day 14 (Mean ± SE)	Day 21 (Mean ± SE)
T_0	$5.76^a \pm 0.06$	$6.08^c \pm 0.07$	$5.67^c \pm 0.04$	$4.15^c \pm 0.07$
T_1	$5.43^a \pm 0.06$	$9.90^a \pm 0.07$	$10.49^a \pm 0.04$	$7.14^b \pm 0.07$
T_2	$5.26^a \pm 0.06$	$8.70^b \pm 0.07$	$7.02^b \pm 0.04$	$8.35^a \pm 0.07$
P Value	0.010**	0.016**	0.005**	0.014**

Values with the different superscripts in the same column are statistically significant at ($p<0.01$) Here ** denotes 1% level of significance. Figures indicate the Mean ± SE (standard Error).

N.B: T_0 = Negative Control (Normal animals), T_1 = Positive Control (Steroid induced hyperglycemia), T_2 = Hyperglycemia with ethanolic extract of insulin plant (150 mg/kg body weight i.e. 2.5 ml/kg).

In the table 4.1 result showed that the blood glucose levels were similar at the beginning in all groups whereas on 21st day blood glucose level were significantly increased in both positive control group (Steroid induced hyperglycemia) T_1 (7.14±0.07) and the treatment group T_2 (8.35±0.07) which were statistically significant. However little or no significant response was observed on blood glucose level in treatment group (T_2) treated with the ethanolic extract of insulin plant.

Table 4.2: Effect of *Gynura procumbens* plant extracts on live body weight (gm) in steroid induced hyperglycemic rats (n=5) at seven days interval

Group	Day 0 (Mean ± SE)	Day 7 (Mean ± SE)	Day 14 (Mean ± SE)	Day 21 (Mean ± SE)
T_0	$222.4^{a} \pm 0.19$	$226.2^{a} \pm 0.13$	$210.5^{a} \pm 0.14$	$204.6^{a} \pm 0.13$
T_1	$224.0^{a} \pm 0.19$	$175.4^{c} \pm 0.13$	$164.0^{c} \pm 0.14$	$156.0^{c} \pm 0.13$
T_2	$219.2^{a} \pm 0.19$	$176.5^{b} \pm 0.13$	$171.3^{b} \pm 0.14$	$163.0^{b} \pm 0.13$
P value	0.110**	0.049**	0.056**	0.048**

Values with the different superscripts in the same column are statistically significant at ($p < 0.01$) Here ** denotes 1% level of significance. Figures indicate the Mean ± SE (standard Error).

N.B: T_0 = Negative Control (Normal animals), T_1 = Positive Control (Steroid induced hyperglycemia), T_2 = Hyperglycemia with ethanolic extract of insulin plant (150 mg/kg body weight i.e. 2.5 ml/kg).

The table 4.2 showed that the body weight of different groups were almost similiar at the initial day. But the body weights were varied significantly with the advancement of ages. Here body weight of positive control group (Steroid induced hyperglycemia) T_1(156.0±0.13) was significantly decreased from the negative control T_0 (204.6±0.13) at the last 21st day. After the treatment it showed a slight weight gain at T_2 (163.0±0.13) comparing to the positive control group but the body weights of different groupswere not statistically significant.

Table 4.3: Effect of *Gynura procumbens* plant extracts on lipid profile (mg/dl) in steroid induced hyperglycemic rats (n=5) at the end of experiment

Group	Cholesterol (Mean ± SE)	HDL (Mean ± SE)	LDL (Mean ± SE)	Triglyceride (Mean ± SE)
T_0	$54.42^{c} \pm 0.20$	$53.23^{a} \pm 0.14$	$10.37^{c} \pm 0.14$	$167.2^{b} \pm 0.15$
T_1	$120.5^{b} \pm 0.20$	$50.23^{b} \pm 0.14$	$13.38^{b} \pm 0.14$	$147.2^{c} \pm 0.15$
T_2	$140.5^{a} \pm 0.20$	$13.60^{c} \pm 0.14$	$18.42^{a} \pm 0.14$	$292.3^{a} \pm 0.15$
P Value	0.118**	0.062**	0.060**	0.066**

Values with the different superscripts in the same column are statistically significant at ($p<0.01$) Here ** denotes 1% level of significance. Figures indicate the Mean ± SE (standard Error).

N.B: T_0 = Negative Control (Normal animals), T_1 = Positive Control (Steroid induced hyperglycemia), T_2 = Hyperglycemia with ethanolic extract of insulin plant (150 mg/kg body weight i.e. 2.5 ml/kg).

In this study table 4.3 represents the lipid profile of normal and treated rat groups which were significantly different. Present study shows that lipid profile was normal on negative control group T_0 (Normal animal) with rate of Cholesterol 54.42 mg/dl, HDL 53.23 mg/dl, LDL 10.37mg/dl, Triglyceride 167.2mg/dl while the positive control group T_1 showed an increased level of Cholesterol 120.5mg/dl, LDL 13.38 mg/dl, Triglyceride 147.2 mg/dl and decreased HDL 50.23 mg/dl. In addition, lipid profile was at peak on T_2 group with 21st days of treatment and the rate was Cholesterol 140.5 mg/dl, LDL 18.42 mg/dl and Triglyceride 292.3 mg/dl with decreased HDL 13.60 mg/dl which were statistically significant.

Table 4.4: Effect of *Gynura procumbens* plant extracts on HbA1c level (%) and Insulin level (ng/ml) in steroid induced hyperglycemic rats (n=5) at the end of experiment

Parameter	Group			P Value
	T_0 (Mean ± SE)	T_1 (Mean ± SE)	T_2 (Mean ± SE)	
HbA1c (%)	$1.56^{c} \pm 0.02$	$2.27^{b} \pm 0.02$	$2.87^{a} \pm 0.02$	0.002**
Insulin (ng/ml)	$0.344^{b} \pm 0.01$	$0.408^{a} \pm 0.01$	$0.356^{ab} \pm 0.01$	0.001**

Values with the different superscripts in the same column are statistically significant at ($p<0.01$) Here ** denotes 1% level of significance. Figures indicate the Mean ± SE (standard Error).

N.B: T_0 = Negative Control (Normal animals), T_1 = Positive Control (Steroid induced hyperglycemia), T_2 = Hyperglycemia with ethanolic extract of insulin plant (150 mg/kg body weight i.e. 2.5 ml/kg).

Table 4.4 indicates HbA1c and plasma insulin level. The HbA1c levels of different groups show a significant difference. Treatment group denotes the highest level T_2 (2.87± 0.02) and steroid induced hyperglycemia group T_1 represents (2.27± 0.02) which is higher than normal animal group T_0 (1.56± 0.02) and were statistically significant. While in case of insulin level T_1 represents the highest value (0.408± 0.01) and T_0, T_2 shows (0.344± 0.01), (0.356 ± 0.01) respectively.

4.1 Histopathological Examination

4.1.1 Normal Rat Pancreas

The pancreas of normal rat contains islets of Langerhans which contain alpha, beta and delta cells that produce glucagon, insulin and somatostatin respectively. These are commonly known as the insulin producing tissue. The alpha cells of the islets of Langerhans produce an opposing hormone, glucagon which helps to release glucose from the liver and fatty acids from fat tissue. The following slides contain the secreting glands in the periphery with Langerhans cells in the middle.

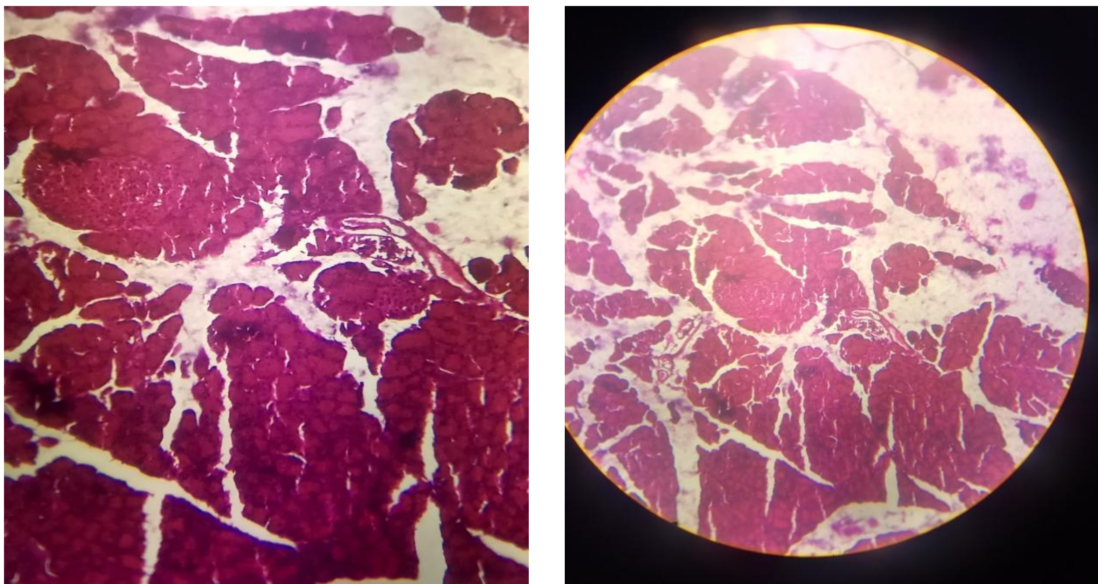


Figure 4.1: Normal Rat Pancreas

4.1.2 Pancreas of Hyperglycemic Rat

Steroid induced hyperglycemic rat pancreases showed a fragmented Langerhans cells. One of the Langerhans cells was intact and it contains alpha, beta and delta cells inside. On the contrary another one was empty without any cells inside.

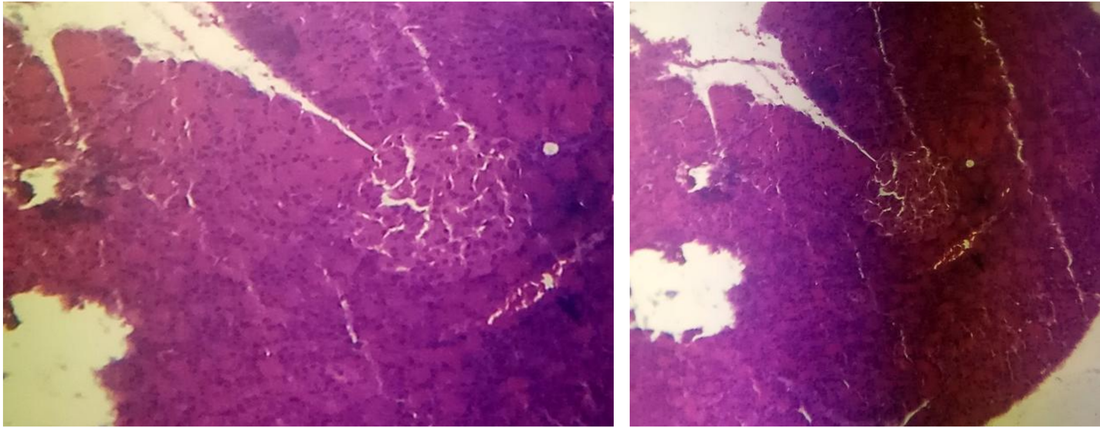


Figure 4.2: Pancreas of Hyperglycemic rat

4.1.3 Pancreas of Treated Rat

Rats treated with *Gynura procumbens* ethanolic extract showed a negative response on the pancreatic islets cells. Only the secretory cells were present surrounding the area of Langerhans cells. No distinct islets of Langerhans were found. It might develop hyperplastic growth over the Langerhans cells.

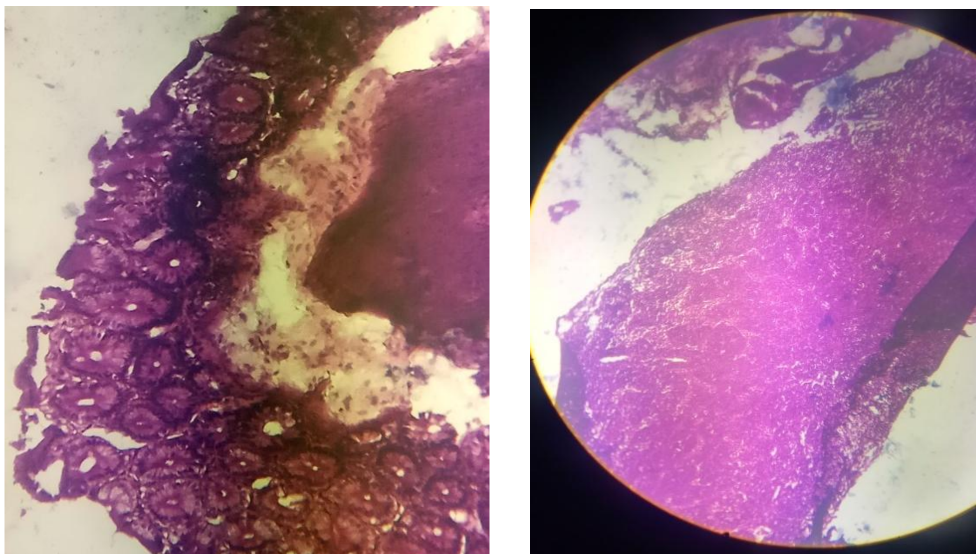


Figure 4.3: Rat Pancreas of Treatment Group

CHAPTER V

DISCUSSION

The experiment was conducted to determine the consequences of steroid induced hyperglycemia on blood glucose level, HbA1c, body weight, lipid profile and insulin levels against traditional hypoglycemic plant extract in rat. Microscopic changes were also compared with the normal and treated groups. To perform the experiment 15 longevan male rats were randomly divided into 3 equal groups named T_0, T_1, T_2 and5 rats in each group. Dexamethasone (Dextason, each ampule contains 5 mg in 1 ml) was injected intramuscularly @ 5 mg/kg body weight on T_1 and T_2 groups for induction of hyperglycemia. Group T_0 rats were kept as normal (negative control) without giving steroid therapy and any other treatment. Group T_1 rats were kept as steroid induced hyperglycemia (positive control) without giving any extract treatment. Group T_2 rats were treated with traditional hypoglycemic plant (*Gynura procumbens*) ethanolic extract @ 150 mg/kg body weight for 21 consecutive days after confirming hyperglycemia. All groups were closely observed during 21 days of treatment period.

5.1 Blood Glucose and HbA1c Level

Present study revealed that the blood glucose levels were normal in the negative control T_0 (4.15±0.07) group on 21st day. Whereas the remaining two groups met an increased blood glucose level i.e; positive control group (Steroid induced hyperglycemia) T_1 (7.14±0.07) and the treatment group T_2 (8.35±0.07) on 21st day. However, little or no significant response was observed on treatment group (T_2) with the ethanolic extract of insulin plant. Here the blood glucose levels were significantly increased in the positive control (T_1) treated with dexamethasone and the treatment group (T_2) with dexamethasone and plant extract compared to the negative control (T_0) group. This result showed that the blood glucose levels were significantly ($P<0.01$) increased with the treatment of *Gynura procumbens* ethanolic extract.

According to Saad *et al.,* 1993, diabetes was induced in 100% of animals with a dose of dexamethasone only 4-8%. Here present study complies with the findings of Saad *et al.,* 1993.

In this study the treatment fails to decrease the blood glucose level due to severe induction. These observations are similar to the findings of Algariri *et al.,* 2013 who reported that at an acute dose (1 g/kg), the extracts of *G. procumbens* did not produce any significant effect on fasting blood glucose**,** even after 7 h.

Lee *et al.,* 2011 also described that diabetic rats treated with ethanolic extract of *G. procumbens* leaves at 50, 100 and 150 mg/kg b.w. showed 49.0, 56.8 and 55.7% reduction in fasting blood glucose in a dose independent manner. It might be due to the presence of antagonistic substances at higher doses of the ethanolic extract. But the present study fails to meet the anti-hyperglycemic effect and is opposed to the findings of Lee *et al.,* 2011.

Fatima *et al.,* 2010 said that the extracts may be considered to have good antihyperglycemic active principles without causing any hypoglycemic effect in normal rats. Zurina *et al.,* 2010 reported that *G. procumbens* water extract (1,000 mg/kg) and metformin had significantly decreased fasting blood glucose levels while in this study ethanolic extract shows an inverse result.

Saad *et al.,* 1993 described that glucocorticoids therapy increases visceral fat which contributes to the insulin resistance and hyperglycemia. Glucocorticoids have a directactions on muscle, liver, and other tissues. Thus the plant extracts become irresponsive to this steroid induced hyperglycemia.

In this study results indicate that the HbA1c level increased significantly ($P<0.01$) in positive control T_1 group (2.27±0.02) and treatment group T_2 (2.87±0.02) compared to the negative control group T_0 (1.56±0.02).

According to (Pari and Maheswari, 1999) chronic hyperglycemia may leads to glycosylated hemoglobin (HbA1c), which resulted from the reaction between excess glucose in the blood with hemoglobin. The present study reveals the same findings and is hyperglycemic animals showed an increased HbA1c level compared to the normal.

Rajasekaran *et al.,* 2005 described that Oral administration of *G. procumbens* aqueous and ethanolic extracts have shown significant reduction on the HbA1c level of the STZ-diabetic rats which was due to improved glucose metabolism as well as increased hemoglobin synthesis. Another finding from Palsamy and Subramanian, 2008 is *Gynura procumbens* extract reduces HbA1c level and it confirmed its ability in prevention of

oxidative damage resulted from protein glycosylation reactions during diabetic condition, thus reducing the risk of diabetic complication pathogenesis. But in this study the condition was opposite and the extract fails to reduce the HbA1c level in the treatment group.

5.2 Body Weight

The present study indicated that the body weight of all groups were similar at the initial days whereas positive control group (Steroid induced hyperglycemia) T_1 (156.0±0.13) was significantly decreased from the negative control T_0 (204.6±0.13) at the last 21st day and similarly followed by the treatment of traditional insulin plant exreact for 21 days it showed an increased body weight at T_2 group (163.0±0.13) comparing to the positive control group.

Body weight is a sensitive indicator that reflects the state of health of experimental animals. In the present study, it was found that the final body weight of negative control group and the treatment group were significantly higher ($P<0.01$) than the positive control groups (hyperglycemia) that showed similarity with the findings of Algariri *et al.* (2013). He found that the extracts of *G. procumbens* showed significant recovery in body weight gain after 14 days administration, body weight gain is an indicator of efficient glucose homeostasis.

Atangwho *et al.,* (2012) described that in hyperglycemic condition the body cells rarely access glucose, and body weight losses due to fats and tissue proteins are breakdown for energy supply (muscle wasting). Heywood, 1983 mentioned that decrease in body weight correlates with defects in body metabolism that is due to toxicity. Saad *et al.,* 1993 described weight gain as distressing side effect of long term glucocorticoid therapy.

The study revealed that final body weight was decreased during hyperglycemic condition which complies with the findings of Atangwho *et al.,* (2012). And the increase in body weight on extract treated group might be due to long term glucocorticoid therapy which is similar to the findings of Saad *et al.,* 1993.

5.3 Lipid Profile

Present study represents serum triglyceride, cholesterol, LDL, HDL level innegative control, positive control and treatment groups. Statistical analysis suggests that

cholesterol level was normal (54.42±0.20) on negative control group but it increases in case of positive control (120.5±0.20) and treatment group (140.5±0.20). Likewise LDL and Triglyceride levels were significantly ($P<0.01$) increased in both positive control (13.38±0.14), (147.2±0.15) and treatment groups (18.42±0.14), (292.3±0.15) comparing to the negative control group (10.37±0.14), (167.2±0.15) respectively. HDL was higher in negative control group (53.23±0.14) and decreased statistically ($P<0.01$) in positive control T_1 (50.23±0.14) and Treatment group T_2 (13.60±0.14).

The result of current research show that mean serum Triglyceride, LDL and cholesterol level is significantly higher than negative control group in both hyperglycemic and treated groups. According to Poorsoltan *et al.,* 2013 the triglyceride levels are higher than normal groups in both diabetes and severe diabetes groups. Bardini *et al.,* 2012 reported lipid profile abnormalities in both diabetes and severe diabetes group. Moreover, results of other studies (Shemesh and Zafrir, 2019) are similar to our study which reveals that hypertriglyceridemia is a complication of diabetes leading to acute pancreatitis and hyperviscosity syndrome in severe condition.

Nazri *et al.,* 2019 suggested that the phenolic acid constituent in the *G. procumbens* extract might have an effect towards the cholesterol metabolism. *G. procumbens* extract reduced plasma concentrations of TG, TC, and LDL and increased plasma HDL.

A previous study by Murugaiyah*et al.,* 2018 reported that phenolic acids were able to reduce TC and LDL and increase HDL. Another study by Zhang X and Tan B 2000 also reported that serum TC and TG in hyperlipidaemic rat were reduced with *G. procumbens* supplementation.

But the present study are dissimilar with (Nazri *et al.,* 2019), (Murugaiyah *et al.,* 2018) and (Zhang and Tan, 2000) they reported a reduced cholesterol, LDL and triglyceride with an increased HDL by the treatment of *G. procumbens.*

5.4 Insulin Level

In case of plasma insulin positive control group T_1 represents the highest value (0.408±0.01). No significant changes in the plasma insulin levels were found between the positive control group$T_1$and treatment group T_2 (0.356±.01), either before or after treatment which is similar with the findings by Zurina *et al.,* 2010.

Lee *et al.,* 2011 described that, oral administration of *G. procumbens* extracts did not stimulate insulin secretion.

Zurina *et al.,* 2010 mentioned that *G. procumbens* water extract acts at the peripheral level and it did not stimulate insulin secretion and inhibit endogenous insulin production. *G. procumbens* water extract-treated rats demonstrated minimal immunochemical staining for insulin, showing no activation of the β cells of pancreas.

Current study suggest that *Gynura procumbens* extracts do not possess insulinotropic activity and is similar to the findings of Zurina *et al.,* 2010 and Lee *et al.,* 2011.

5.5 Microscopic examinations

Hadi *et al.,* (2016) reported that the size and number of pancreatic islets were decreased in hyperglycemic rats in comparison to normal rats which were in consistent with histopathological data obtained from the current study.

El-Esawy *et al.,* (2016) described that the pancreatic tissue of hyperglycemic rat demonstrated degeneration and vacuolizations in the islet of Langerhan's cells, decreasing in islets size, decreasing in β-cell number and also in the architecture of the islets. So, the serum insulin level was decreased and the glucose concentration was increased. These findings are similar with the current study.

Histopathological observations in the present study reveals that plant extracts failed to improve the islets cells and acini compared to the steroid hyperglycemic groups.

CHAPTER VI
CONCLUSION

In conclusion, steroid treatment increased blood glucose, HbA1c, plasma insulin, lipid profile values except HDL whereas *Gynura procumbens* extract has no significant effect on blood glucose, HbA1c, plasma insulin, lipid profile and body weight. Histopathological evaluation indicates hyperplastic growth over the Langerhans cells in pancreas of steroid treatment rat which were not altered by *Gynura procumbens* treatment. Finally this study shows that chronic steroid therapy induced hyperglycemia in rat is irresponsive to *Gynura procumbens* treatment. This steroid hyperglycemia is very dangerous and difficult to control by traditional hypoglycemic plant extract.

For further research it can be recommended that *Gynura procumbens* plant extract can be compared with the synthetic insulin therapy in steroid induced rats.

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