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GRT Golden Research Thoughts

EVALUATION OF ANTIDIABETIC ACTIVITY OF RHIZOMES OF GLYCYRRHIZA GLABRA

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose concentration (hyperglycemia) caused by insulin deficiency often combined with insulin resistance (Rang and Dale, 2008). The Diabetes Mellitus has been rise across the world affecting over 150 million people. Over 20% diabetics are Indians. At present, it is higher in developed than in developing countries. The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025. The major part of this numerical increase will occur in developing countries. By the year 2025, greater than 75% of people with diabetes will reside in developing countries. The countries with the largest number of people with diabetes are, and will be in the year 2025, India, China, and U.S. (King H et al.1998).

The synthetic anti diabetic drugs can produce a series of side effects including gastrointestinal disturbances, hypoglycemia, weight gain, hypertension and hypersensitivity reactions etc. Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones. Herbal drugs claimed to relieve not only the symptoms of diabetes mellitus, but also they can regenerate and repair the dysfunctional pancreas. In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale Traditional Medicines derived from medicinal plants are used by about 60% of the world's population. Some examples of medicinal plants with proven antidiabetic activity are Allium sativum, Eugenia jambolana,

Momordicacharantia, Ocimum sanctum, Phyllanthusamarus, Pterocarpusmarsupium etc. (Seth et al, 2004). The present study was undertaken to evaluate the effect acute and chronic administration of methanolic extract of rhizome of Glycyrrhizaglabra on various biochemical parameters in normal and diabetic rats.

KEYWORDS :evaluation, metabolic, hypoglycemia.

INTRODUCTION :

DIABETES MELLITUS

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose concentration (hyperglycemia) caused by insulin deficiency often combined with insulin resistance (Rang and Dale, 2008). Diabetes mellitus refers to the group of diseases that leads to high blood glucose level due to defect in either insulin secretion or insulin action in the body (Rother, 2007). Hyperglycemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis. When the renal threshold for glucose reabsorption is exceeded, glucose spills over into the urine (Glycosuria) and causes an osmotic diuresis (polyuria), which in turn results in dehydration, thirst and increased drinking of water (polydipsia).

The characteristic symptoms of diabetes mellitus are polyuria, polydipsia, polyphagia (increased hunger), blurred vision, these symptoms may be absent if the blood sugar is only mildly elevated. Diabetes mellitus is major public health problem in the developed as well as developing countries. It is ranked seventh among the leading causes of death and third when all its fatal complications are taken into accounts (Burke, 2003).

EPIDEMIOLOGY

In 2000, according to the World Health Organization, at least 171 million people worldwide suffer from diabetes i.e. 2.8% of the population (Wild. S et al, 2004). Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will almost double. Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030 (Wild. S et al, 2004). The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet. This has suggested an environmental (i.e., dietary) effect, but there is little understanding of the mechanism(s) at present, though there is much speculation, some of it most compellingly presented.In 2004 Williams and Pick up said majority (90%) of patients with type-II diabetes is diagnosed before the age of 30.

TYPES OF DIABETES MELLITUS (Senthil vasan et al, 2008)

1.Type I diabetes mellitus
2.Type II diabetes mellitus
3.Gestational diabetes mellitus
4.Other specific types :

A.PANCREATIC DISEASES

- Fibrocalcific pancreatitis
- Pancreatectomy

•Cystic fibrosis

B.ENDOCRINOPATHIES

•Acromegaly
•Cushing's syndrome
•Phaeochromocytoma
•Hyperthyroidism

C.INFECTIONS

•Mumps
•Cytomegalo virus
•Congenital Rubella

d.Drug induced

•Glucocorticoids
•Thyroid harmones
•Thiazides

IMPORTANT TYPES OF DIABETES MELLITUS

A. TYPE I DIABETES MELLITUS

Type I diabetes mellitus is characterized by loss of the insulin producing beta cells of the islets of Langerhans in the pancreas leading to insulin deficiency. Type I diabetes can be further classified as immune mediated or idiopathic. Type I diabetes is majorly of the immune mediated variety, where beta cell loss is a T-cell mediated auto immune attack (Rother, 2007). Type I diabetes is also called as juvenile diabetes (childhood) or insulin dependent diabetes mellitus (IDDM). There is no preventive measure that can be taken against this type I diabetes. Diet and exercise cannot reverse or prevent type I diabetes. Sensitivity and responsiveness to insulin are usually normal especially in early stages.

B. TYPE II DIABETES MELLITUS

Type II diabetes mellitus is characterized differently and it is due to insulin resistance or reduced insulin sensitivity and it may be absolutely due to reduced insulin secretion in some of the cases. Insulin receptor sensitivity decreases on insulin receptors. Type II diabetes is also called as adult onset diabetes mellitus, maturity onset diabetes mellitus or non insulin dependent diabetes mellitus (NIDDM) Type II diabetes mellitus is characterized by insulin resistance, impaired glucose induced insulin secretion and inappropriately regulated glucagon secretion which in combination eventually results in hyperglycemia and in the longer term micro vascular and macro vascular diabetic complications. There are numerous theories as to the exact cause and mechanism in type II diabetes. Central obesity (fat concentrated around the waist in relation to abdominal organs, but not subcutaneous fat) is known to predispose individuals to insulin resistance. Abdominal fat is especially active hormonally, secreting a group of hormones called adipokines that may possibly impair glucose tolerance. Obesity was found to be the reason in approximately 55% of patients diagnosed with type II diabetes.

C. GESTATIONAL DIABETES MELLITUS

Gestational diabetes develops during pregnancy and it may persists or disappear after delivery. Gestational diabetes may damage the health of foetus or mother, and about 20%-50% of women with

gestational diabetes develop type II diabetes later in life. Gestational diabetes mellitus (GDM) occurs in about 2%-5% of all pregnancies, including high birth weight (Macrosomia), fetal malformation and congenital heart disease. It requires careful medical supervision during the pregnancy (Lawurenceet al., 2008).

CAUSES OF DIABETES MELLITUS: (Arlal Rosen bloom et al., 2003)

- Shortage or defective imperfect insulin produced by the body.
- Stress, fear and tension
- Hereditary either parent suffering from diabetes
- Frequent administration of steroids
- Insomnia
- Alcoholic habituation
- Addiction to sweets
- Swollen pancreas
- Smoking
- Malnutrition

COMPLICATIONS OF DIABETES MELLITUS

- Kidney (Diabetic nephropathy)
- Nerves (Diabetic neuropathy)
- Retina (Diabetic retinopathy)
- Testes (Infertility in males)
- Coronary thrombosis
- Cerebral thrombosis
- Hemorrhage

DIAGNOSIS OF DIABETES MELLITUS

The following tests performed when the patient complaint symptoms suggesting diabetes.

- Urine test for glucose and ketones
- Measurement of random blood glucose, plasma electrolytes
- Measurement of fasting blood glucose levels
- Glucose tolerance test(GTT)
- Glycosylated heamoglobin test (HbA1c)

DRUGS USED IN DIABETES MELLITUS (KD. TRIPATHI, 2008)
IN TYPE I DIABETES MELLITUS OR IDDM

- Short and rapid acting insulin

a)Insulin, onset 0.5 to 1 hour, duration of action 6-8 hours.
b)Prompt insulin zinc suspension, onset 1 hour, duration of action 12-16 hours.

- Intermediate acting

a)Insulin zinc suspension, onset 1-2 hour, duration 20-24 hours.

- Long acting

Extended insulin zinc suspension, onset 4-6 hour, duration 24-36 hours.

IN TYPE II DIABETES MELLITUS OR NIDDM

I

a)Sulfonylureas

First generation Second generation Tolbutamide Glibenclamide Chlorpropamide Glipizide, Gliclazide, Glimepamide

b)Biguanides

Eg: Metformin

c)Meglitinide analogues

Eg: Nateglinide, Repaglinide

d)α – glucosidase inhibitors

Eg: acarbose, miglitol

e)Thiazolidinediones

Eg: Rosiglitazone, Pioglitazon

II.OBJECTIVES

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. A worldwide survey reported that diabetes mellitus is affecting nearly 10% of the population every year. The treatment of diabetes mellitus in clinical practice has been confined to use oral hypoglycemic agents and insulin, the former being reported to be endowed with characteristic profiles of serious side effects. This leads to increasing demand for herbal drugs with anti-diabetic factor with little side effects. A large number of plants have been recognized to be effective in the treatment of diabetes mellitus.

Herbal drugs claimed to relieve not only the symptoms of diabetes mellitus, but also they can regenerate and repair the dysfunctional pancreas. *Glycyrrhizaglabraplant* is widely used in folk medicine for various purposes.The present study is aimed at evaluating the antidiabetic activity of a *Glycyrrhizaglabramethanolicextract*. *Glycyrrhizaglabrais* reported to have many biological activities including antibacterial, *immunostimulant, antidiarrhoeal*, antioxidant, anti-inflammatory, anti HIV, antipyretic, antimalarial. Its hypoglycemic and antihyperglycemic activities are not well reported.

Hence present study is carryout with an objective to evaluate the effect of acute and chronic administration of methanolic extract of *Glycyrrhizaglabrain* STZ induced type I and II diabetic rats by measuring various biochemical parameters.

THE PRESENT STUDY IS CARRIED WITH THE FOLLOWING OBJECTIVES:

•To study the effect of single dose of Glycyrrhizaglabramethanolic extract on blood glucose level in normal rats.

•To study the effect of single dose of Glycyrrhizaglabramethanolic extracton blood glucose level in streptozotocin induced type I and type II diabetic rats.

•To study the effect of chronic administration of Glycyrrhizaglabramethanolic extract on diabetic animals for a period of 7 days. The following parameters are evaluated before and after the administration of the extract.

- Oxidative stress
- Lipid profile
- Blood glucose

- Nootropic activity
- Immunostimulant activity
- Liver glycogen
- Cerebroprotective activity.

III.REVIEW OF LITERATURE

Past work done on antidiabetic activity of Glycyrrhizaglabra

In 2000, Xiang-Fan Zhang et al., investigated the ethanolic extract of the aerial parts of *Glycyrrhizaglabra* for antihyperglycaemic and antioxidant effects in normal and streptozotocin-induced type I diabetic rats. Oxidative stress is evident in streptozotocin-diabetic rats and indicate that the ethanolic extract of Glycyrrhizaglabranot only possesses an antihyperglycaemic property, but may also reduce oxidative stress in diabetic rats.

In 2000, Zhang et al.,reported theethanolic extract of *Glycyrrhizaglabrapossesses* antidiabetic property. Its antidiabetic effect may be attributed at least in part to increased glucose metabolism. Its hypotriglyceridemic effect is also reported.

In 2006, A.S.Syahrin et al., reported that the AP extract increased the survival rate and endocrine cell density, improved estrous cycle and reduced the ”insulin resistance‘ phenomenon in STZ- induced diabetic rats.

In 2006, B.A.S. Reyes et al., reportedthat*Glycyrrhizaglabra possesses* anti-diabetic potential in alloxan induceddiabetic rats. Moreover, this is the first physiological evidence that the anti-diabetic potentials of *Momordicacharantia* and *Glycyrrhizaglabr*a restore the impaired estrous cyclicity in alloxan-induced diabetic rat model.

In 2006, NalamoluKoteswaraRao,tested the chloroform extract of *Glycyrrhizaglabra* roots for its antihyperglycemic activity in alloxan induced diabetic rats using chronic and acute studies. The extract significanly inhibited the induction of albuminuria, proteinemia and uremia. From the results it indicated a significant antidiabetic activity with the chloroform extract of A. paniculata roots and supports the traditional usage of the plant by Ayurvedicphy-sicians for the control of diabetes. Also the extract is useful in preventing the incidence of long-term complication, diabetic nephropathy.

In 2007, Mariam Ahmad et al., concluded that hypoglycemic tests in normal and diabetic rats showed a reduction in fasting blood glucose and insulin levels in rats compared to the pre-treatment levels after oral administration of 50 and 95% ethanol extracts of A. paniculata at 1 g/kg for 14 days. The hypoglycemic test and the results can be utilized for screening antidiabetic activity and for further mechanistic studies to indicate the exact mechanism of action of the extracts.

In 2007, Md. AlamgirHossain et al.,studied that the aqueous and ethanolic extractives of *Glycyrrhizaglabra* are capable to exhibit significant blood sugar lowering effects in both glucose-loaded and alloxan induced diabetic rat. The lowering of blood glucose levels by the aqueous and ethanolic extracts is also comparable. Both extractives are capable to reduce the sugar level almost identical.

IV.METHODOLOGY

GLYCYRRHIZAGLABRA

Glycyrrhizaglabra, (AP), also known commonly as "Liquorice," is a member of the plant family Leguminoceae and is prominent in atleast 26 Ayurvedic formulas.

NOMENCLATURE

BOTANICAL NAME:*GLYCYRRHIZAGLABRA.*

TAXONOMICAL CLASSIFICATION:

Kingdom : Plantae;
Division : Angiosperma
Class : Equisetopsida
Sub class : Magnoliidae
Order : Fab
Family : Leguminoceae
Genus : Glycyrrhiza
Species : glabra

ENGLISH NAME: LIQUORICE

FIGURE 4.1: GLYCYRRHIZAGLABRA

BOTANICAL DESCRIPTION:

ORIGIN AND DISTRIBUTION:

The crop is found common in stony lines in forests and in wastelands. It is widely distributed in Bangladesh, Pakistan, all South East Asian and SAARC countries andthroughout plains of India from Himachal Pradesh to Assam, Mizoram, Gujrat, Bihar,Madhya Pradesh, Karnataka, Tamil Nadu and Kerala.

DESCRIPTION:

Annual herb, up to 1 m high, erect, stem acutely quadrangular. Leaves simple, opposite, lanceolate, acute, glabrous, antire-slightly undulate, 2 to 12 cm long and 1 to 3 cm wide, upper ones often bracteiform; petiole short. Inflorescence patent, in panicle, 10 to 30 long; terminal and axillary, bract small, pedicel short.Calyx 5-partite, small, linear. Corolla tube narraw about 6 mm long; limb not shorter than the tube, bilabiate; upper lip oblong, white with a yellowish top; lower lip broadly cuneate, trifid white with violet marking. Stamens 2, inserted in the throad and far exserted. Capsule erect, linear-oblong, 1 to 2 cm long and 2 to 5 mm wide, compressed, longitudinally furrowed on the broad faces, thinly glandular-hairy. Seed small, subquadrate.

HABITAT:

An erect annual herb, chiefly found throughout the Indian plains from Himachal Pradesh to Assam and Mizoram and all over South India. The plant is gregarious and grows abundantly in moist, shady waste grounds and sometimes in dry forests.

PARTS USED:AERIAL PART LEAVES AND WHOLE PLANT

MORPHOLOGICAL CHARACTERISTICS OF DRUG:

Drug consists of dried leaves and tender shoots; yields not less than 1% of andrographolide on dry wt.basis. Normally powdered drug is used.

Chemical Composition

The major constituents are diterpene lactones (free and in glycosidic forms), including Glycyrrhizin, glycyrrhizanic acid, 11, 12-didehydro-14-deoxyglycyrrolide, neoglycyrrolide.

PHARMACOLOGICAL ACTIVITIES

ANTIBACTERIAL ACTIVITY

An ethanol extract of the leaves inhibited the growth in vitro of *Escherichia coli* and *Staphylococcus aureus* (George et al, 1949). A 50% methanol extract of the leaves inhibited growth in vitro of Proteus vulgaris (Nakanishi et al, 1965). However, no in vitro antibacterial activity was observed when dried powde from the aerial parts was tested against *E.Coli, Staphylococcus aureus, Salmonella typhior Shigella species* (Leelarasamee A et al, 1990).

ANTI-HUMAN IMMUNODEFICIENCY VIRUS (HIV) ACTIVITY

Aqueous extracts of the leaves inhibited HIV-1 infection and replication in the lymphoid cell line MOLT-4(Yao et al, 1992). A hot aqueous extract of the aerial parts reduced the percentage of HIV antigen-positive H9 cells (Chang RS et al, 1988). Dehydroglycyrrolide inhibited HIV-1 and HIV-1 (UCD123) infection of H9 cells at 1.6mg/ml and 50mg/ml, respectively, and also inhibited HIV-1 infection of human lymphocytes at 50mg/ml(Chang RS et al, 1991). A methanol extract of the leaves suppressed syncytia formation in co-cultures of uninfected and HIV-1-infected MOLT cells (median effective dose [ED50] 70mg/ml) Otakeet a,1995.

IMMUNOSTIMULATORY ACTIVITY

Intragastric administration of an ethanol extract of the aerial parts (25mg/kg body weight) or purified glycyrrolides (1 mg/kg body weight) to mice stimulated antibody production and the delayed-type hypersensitivity response to sheep red blood cells (Puriet al, 1993). The extract also stimulated a non-specific immune response in mice, measured by macrophage migration index, phagocytosis of [14C] leucine-labelled E. coli, and proliferation of splenic lymphocytes(Vedavathyet al, 1991).The extract was more effective than either glycyrrolides or neoglycyrrolide alone, suggesting that other constituents may be involved in the immunostimulant response (Madavet al, 1995).

ANTIDIARRHOEAL ACTIVITY

ANTI-INFLAMMATORY ACTIVITY

Intragastric administrationof deoxyglycyrrolide, glycyrrolide, or 11,12-didehydroglycyrrolide to mice inhibited the increase in cutaneous or peritoneal capillary permeability induced by xylene or acetic acid, and reduced acute exudation in Selye granulocysts treated with croton oil. 11, 12 Didehydrodeoxyglycyrrolide had the most potent anti-inflammatory activity in vivo (Nazimudeen et al,

1978)

ANTIMALARIAL ACTIVITY

A 50% ethanol extract of the aerial parts inhibited the growth of Plasmodium berghei both in vitro (100 mg/ml) and in mice after intragastric administration (1 g/kg body weight). Intragastric administration of a 1-butanol, chloroform or ethanol–water extract of the aerial parts to *Mastomys natalensis* inhibited the growth of P. berghei at doses of 1–2 g/kg body weight.

ANTIVENOM ACTIVITY

Intraperitoneal injection of an ethanol extract of the aerial parts (25 g/kg body weight) to mice poisoned with cobra venom markedly delayed the occurrence of respiratory failure and death. The same extract induced contractions in guinea-pig ileum at concentrations of 2 mg/ml. The contractions were enhanced by physostigmine and blocked by atropine, but were unchanged by antihistamines. These data suggest that extracts of the aerial parts do not modify the activity of the nicotinic receptors but produce significant muscarinic activity, which account forits antivenom effects(Nazimudeen et al, 1978).

ANTIHEPATOTOXIC ACTIVITY

The aerial parts and their constituent glycyrrolides have anti-hepatotoxic activity in vitro and in vivo(Visen et al, 1993). Intraperitoneal administration of a methanol extract of the aerial parts (861.3 mg/kg body weight) to mice reduced hepatotoxicity induced by carbon tetrachloride (CCl4), and reversed CCl4-induced histopathological changes in the liver (Chander et al,1995, Bhaumik et al, 1993). Intraperitoneal administration of a methanol extract of the aerial parts (500 mg/kg body weight) to rats also suppressed the CCl4-induced increase in the activity of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase and bilirubin(Chander et al,1995). Intragastric administration of an aqueous extract of the aerial parts (500mg/kg body weight) to ethanol-treated rats decreased the activity of serum transaminases and suppressed histopathological changes in the liver(Nazimudeen et al, 1978).

ANTIOXIDATIVE EFFECT:

Extract of A. paniculata possesantioxidative property (Chanwitheesuk et al., 2002; Singh et al., 2001; Zhang and Tan, 2000) and it was reported that neoglycyrrolide is an antioxidant of superoxide free radicals (Kamdem et al., 2002).

HYPOGLYCEMIC EFFECT:

Ethanolic extract of A. paniculata showed hypoglycemic effect in diabetic rats induced with streptozotocin (STZ) (Zhang and Tan, 2000). Water extract of A. paniculata at dosage of 10 mg/kgBW can decrease blood glucose level after induction of hyperglycemia with glucose orally at 2 mg/kg BW but cannot reduce blood sugar activated by adrenaline. However, prolong administration up to 6 months showed no hypoglycemic activity (Husen et al., 2004). Water extract of A. paniculata at 50 mg/kgBW showed 52.90% reduction of blood glucose in diabetic rats treated with STZ while 61.81% reduction of blood glucose was found in diabetic rats treated with freeze-dried form at dosage 6.25 mg/kgBW. Determination of active constituents in G.glabra was studied and found that glycyrrhizin was one of hypoglycemic agents. After orally given of glycyrrhizin into STZ-induced diabetic rats, it showed hypoglycemic effect in dose-response manner. In addition, glycyrrhizin at 1.5 mg/kgBW can reduce

elevated blood sugar level after infusion of glucose intravenously. The mechanism of hypoglycemic effect might be due to glucose uptake capability of the muscle (Yu et al., 2003).

ANIMALS USED IN STUDY:

Wistar albino rats of either sex were procured from Mahaveer Enterprises, Hyderabad, India. The animals were maintained on a 12 hour light and 12hour dark cycle. They were fed, ad libitum regular grain chow (Rayans Biotechnologies Pvt. Ltd., Hyderabad). Diet containing 56% grain derived carbohydrate, 21% protein, 6.7% moisture, 3.58% total oil, 2.58% dietary fiber, 5.5% cellulose, 0.8% calcium, 0.6% phosphrous, 0.3 % sodium chloride. The animal housing and handling were in accordance with CPCSCA guidelines. The prior permission for the study was obtained from our Institutional Animal Ethics Committee (IAEC).

SUBSTANCES USED IN STUDY:

- Methanolicextract of Glycyrrhizaglabra
- Streptozotocin (Sigma chemicalsltd., USA)
- Nicotinamide (Sigma chemicalsltd., USA)
- Citrate buffer PH 4.5 (I.P)

Unless otherwise specified all the chemicals and reagents used are of analytical grade.

INDUCTION OF TYPE I AND TYPE II DIABETES MELLITUS

PREPARATION OF STZ:

STZ is stable at PH4.5, and stability decreases rapidly at a higher or lower PH so STZ was dissolved in citrate buffer with the adjusted to PH 4.5 and then the solution was injected within a short time (with in five minutes).

EXPERIMENTAL WORK

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia which is due to insulin deficiency or insulin resistance. Diabetes mellitus has been on the rise across the world effecting over 150 million people. 20% of diabetics in the world are Indians. The global prevalence of diabetes is estimated to increase, from 4% in 1995 to 5.4% by the year 2025. WHO has predicted that the major burden will occur in developing countries (Seth et al, 2004).

Diabetes is an important human ailment afflicting many from various walks of life in different countries. Though there are various approaches to reduce the ill effects of diabetes and its secondary complications, herbal formulations are preferred due to lesser side effects and low cost (ManishaModak et al, 2007). Herbal drugs claimed to relieve not only the symptoms of diabetes mellitus, but also they can regenerate and repair the dysfunctional pancreas. Some examples of medicinal plants with proven antidiabetic activity are *Allium sativum, Eugenia jambolana, Momordicacharantia, Ocimum sanctum, Phyllanthusamarus, Pterocarpusmarsupium.*

Glycyrrhizaglabra plant is widely used in folk medicine for various purposes. Hence present study is carryout with an objective to evaluate the effect of methanolic extract of Glycyrrhizaglabra in STZ induced type I&II diabetic rats.

PROCEDURE:

Albino rats of either sex were fasted over night before injecting with STZ. Streptozotocin was dissolved in citrate buffer pH4.5 at a dose of 40mg/kg body weight immediately before use and injected

in to the tail vein of rats which are lightly anaesthetized with ether. Blood glucose levels were estimated after 48 hrs for the confirmation of diabetes induction.

To induce type I diabetes 40 mg/kg dose of STZ is given through tail vein of the rat. To induce type II diabetes 65 mg/kg dose of STZ is given, before giving this dose of STZ, 150 mg/kg dose of Nicotinamide was given which is helpful to produce partial destruction of pancreas.

Glucose levels were estimated weekly once and before going to test the pharmacological activity to ensure require blood glucose levels in the rats.

COLLECTION OF BLOOD SAMPLES FROM RATS:

MATERIALS:

1) Micro-centrifuge tubes (1.5 ml capacity)
2) Micro-capillary tubes (1 mm diameter)
3) Absorbent cotton

Blood was collected from the retro orbital plexus of rats. It is the best method, if small amounts (0.1 to 0.5 ml) of blood samples are required. A fine capillary is inserted gently in the inner angle of the eye, and then the capillary was slided under the eye ball at 45 degree angle and over the bony socket to rupture the fragile venous capillaries of the ophthalmic venous plexus. The passage is about 10 mm. The tip of the capillary is slightly retracted and the blood collected in the orbital cavity flows out from the capillary which is collected in a micro-centrifuge tube. After collecting the desired volume, capillary is removed with simultaneous release of pressure by forefinger and thumb. Any residual blood droplet around the eye ball is wiped off by absorbent cotton swab. In this study unanaesthetized animals were used because, anesthesia causes hyperglycaemia by various mechanisms. Ether increases blood glucose levels by glycogenolysis in liver (Dohm et al., 1983). Halothane increases blood glucose by inhibiting release of insulin from pancreas, inhibit the effect of insulin on tissues and decreased rate of glycogen synthesis in liver (Scott et al., 1982). The same procedure was carried out for collection of blood samples from diabetic rats after induction of diabetic state by STZ.

ESTIMATION OF BLOOD GLUCOSE LEVEL IN RATS:

In this study the enzymatic; glucose oxidase-perixodase (GOD – POD) method (Trinder, 1964) was used.

GLUCOSE OXIDASE-PEROXIDASE (GOD/POD) METHOD

Glucose kit based on Trinder's method in which glucose oxidase (GOD) and peroxidase (POD) enzymes were used along with the chromogen 4-aminoantipyrine and phenol. This method is one step, simple and rapid.

PRINCIPLE:

Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. In a subsequent peroxidase catalyzed reaction the oxygen liberated is accepted by the chromogen system to give a red colored quinoneimine compound. The red colourquinoneimine dye so developed is measured at 505 nm using semi auto analyzer (Screen master 3000).

Glucose oxidase

Glucose + H_2O ⟶ Gluconic acid + H_2O_2Peroxidase H2O2+4-Aminoantipyrine + Phydroxy benzoate ⟶ Quinoneimine dye

REAGENTS:

REAGENT-1:Glucose reagent Glucose oxidase, (9 vials) Peroxidase, 4 - aminoantipyrine,Buffer, Stabilizers.

REAGENT-2: Glucose diluent Diluent, Phenol preservative (1*450 ml)

REAGENT-3:Glucose standard Dextrose, Benzoic acid (100mg/dl) (1*3 ml)

Store Reagent-1 at 2-8^{0}C and Reagent-2 at room temperature.

Working Reagent Preparation: The contents of 1 vial of Reagent-1 are transferred quantitatively to a clean black colored plastic bottle provided in the kit. The bottle was reconstituted with 50 ml of glucose diluent (Reagent-2).

Storage of working reagent: The working reagent is stable for 12 months from the date of reconstitution when stored at 2-8^{0}C.

Specimen Collection: The collected blood was made to stand without adding any anticoagulant. The clot that is formed is disturbed using a glass rod and was then centrifuged at 3000 rpm for 10 min. The serum is separated and used for the analysis.

EQUIPMENT: SCREEN MASTER 3000
(AUTO ANALYZER FOR BIOCHEMICAL PARAMETERS)
PROGRAMME:

The basic assay parameters are

Mode	: End point
Wavelength	: 505 nm (490-550 nm)
Temperature	: 37 ^{0}C
Optical path length	: 1 cm
Blanking	: Reagent blank
Incubation	: 10 min at 37^{0}C
Sample volume	: 10 µl
Working reagent volume	: 1 ml
Concentration of standard	: 100 mg/dl
Linearity	: up to 500 mg/dl
Stability of color	: 1 hour
Units	: mg/dl

PROCEDURE:

Pipette into tubes marked	*blank*	*standerd*	*test*
Serum	-	-	10µl
Glucose Standard	-	10µl	-
Working glucose reagent	1 ml	1 ml	1 ml

Mix well. Incubate at 37 ^{0}C for 10 minutes.

They were mixed well and glucose values are noted using semi auto analyzer.

COLLECTION AND HANDLING:

Serum should be separated within 30 minutes as the rate of glycolysis is approximately 7

mg/hour at room temperature. Serum is stable for 8 h at room temperature and for up to 72 h at 2-8 °C.

LIPID PROFILE

For lipid profile estimation following tests are performed

1) Total lipids: Principle lipids react with sulphuric acid, phosphoric acid and vanillin to form pink color complex. Normal values 400-1000 mg/dl

2) Phospholipids: It is a fully enzymatic method which uses three different enzymes- phospholipase D, Choline oxidase and per oxidase to developed color which is measured at 500nn. Normal range 160-270 mg/dl

3) Triglycerides:

4) Cholesterol:

5) HDL:

6) LDL: Cholesterol – HDL + VLDL

7) VLDL: TG/

ESTIMATION OF SERUM TRIGLYCERIDES

Introduction: Elevated levels of triglycerides in plasma have been considered as risk factors related to atherosclerotic diseases. The hyperlipidemias can be inherited trait or they can be secondary to a variety of disorders of diseases including nephrosis, diabetes mellitus, biliary obstruction and metabolic disorders associated with endocrine disorders.

Method: Acetyl-acetone. (Kinetic method)
Normal range: 10-190 mg/dl

PRINCIPLE:

The serum lipids are extracted by isopropanol, which also precipitates serum proteins. The interfering phospholipids, containing glycerol as integral part, are removed by adsorption on alumina. Filtrate is used for saponification and glycerol is separated from triglycerides. Action of metaperiodate converts glycerol into glyceraldehydes; in this the rate of reaction was measured. The intensity of the rate of reaction is measured at 410 nm (Violet filter).

CHEMICAL PRINCIPLE OF TEST:

Lipoprotein, Lipas

$$\text{Triglycerides} + H_2O \longrightarrow \text{Glycerol} + \text{fatty acids}$$

Glycerol kinase

$$\text{Glycerol} + ATP \longrightarrow \text{Glycerol} - 3 - \text{phosphate} + ADP$$

Glycerol phosphate oxidase

$$\text{Glycerol–3-phosphate} + O_2 \longrightarrow \text{Dihydroxy acetone phosphate} + H_2O_2$$

PEROXIDASE

$$H_2O_2 + \text{4-amino-phenazone} + \text{p-chlorophenol} \longrightarrow \text{colored complex}$$

Working reagent preparation: The contents of 1 vial of Reagent-1 are transferred quantitatively to a clean black colored plastic bottle provided in the kit. The bottle was reconstituted with 50 ml of

triglyceride diluent (Reagent-2).

Storage Of Working Reagent: The working reagent is stable for 12 months from the date of reconstitution when stored at 2-8^{0}C.

Specimen Collection: The collected blood was made to stand without adding any anticoagulant. The clot that is formed is disturbed using a glass rod and was then centrifuged at 3000 rpm for 10 min. The serum is separated and used for the analysis.

EQUIPMENT:

SCREEN MASTER 3000 (AUTO ANALYZER FOR BIOCHEMICAL PARAMETERS)

PROGRAMME:

The basic assay parameters are

Mode	: Kinetic method
Wavelength	: 505 nm (490-550 nm)
Temperature	: 37 ^{0}C
Optical path length	: 1 cm
Blanking	: Reagent blank
Incubation	: 0 min at 37 ^{0}C
Sample volume	: 10 µl
Working reagent volume	: 1 ml
Concentration of standard	: mg/dl
Linearity	: up to 500 mg/dl
Units	: mg/dl

PROCEDURE:

Pipette into tubes marked	*blank*	*standerd*	*test*
Serum	-	-	10µl
triglyceride Standard	-	-	-
Working triglyceride reagent	1 ml	-	1 ml

Mix well. Take reading immediately.

They were mixed well and triglyceride values are noted using semi auto analyzer.

Technical Contents: Using Kit Of Serum Triglycerides.

Estimation Of Serum Cholesterol (total)

Introduction: Cholesterol is a fat-like substance that is found in all body cells. The liver makes all of the cholesterol the body needs to form cell membranes and to make certain hormones. The determination of serum cholesterol is one of the important tools in the diagnosis and classification of lipidemia. High blood cholesterol is one of the major risk factors for heart disease.

PRINCIPLE

The serum lipids are extracted by isopropanol, which also precipitates serum proteins. The interfering phospholipids, containing glycerol as integral part, are removed by adsorption on alumina. Cholesterol forms Liebermann-Burchard reaction, in this the rate of reaction was measured. The intensity of the rate of reaction is measured at 410 nm (Violet filter).

METHOD

Liebermann-Burchard reaction (Kinetic method)

Normal Range Serum cholesterol varies from 150-240 mg/100 ml in healthy young adults. The level rises with age and may go up to 300 mg/100 ml in the elderly.

Working Reagent Preparation The contents of 1 vial of Reagent-1 are transferred quantitatively to a clean black colored plastic bottle provided in the kit. The bottle was reconstituted with 50 ml of Total cholesterol diluent (Reagent-2).

Storage Of Working Reagent: The working reagent is stable for 12 months from the date of reconstitution when stored at 2-8^{0}C.

Specimen Collection: The collected blood was made to stand without adding any anticoagulant. The clot that is formed is disturbed using a glass rod and was then centrifuged at 3000 rpm for 10 min. The serum is separated and used for the analysis.

EQUIPMENT: Screen Master 3000 (Auto Analyzer for Biochemical parameters)

The basic assay parameters are

Mode : Kinetic method
Wavelength : 505 nm (490-550 nm)
Temperature : 37 ^{0}C

Procedure:

Pipette into tubes marked	*blank*	*standerd*	*test*
Serum	-	-	10µl
Total cholesterol Standard	-	-	-
Working cholesterol reagent	1 ml	-	1 ml

Mix well. Take reading immediately.

They were mixed well and Total cholesterol values are noted using semi auto analyzer.

ESTIMATION OF SERUM HDL CHOLESTEROL

INTRODUCTION

In the presence of phosphotungstic acid and magnesium chloride, LDL, VLDL & chylomicrons are precipitated. Centrifugation leaves only the HDL in the supernatant. Cholesterol in the HDL fraction can be tested by the usual methods.

METHOD

Watson : (Kinetic method)
Normal range
Men : 30-60 mg/dl.
Women : 40-70 mg/dl.

WORKING REAGENT PREPARATION:

The contents of 1 vial of Reagent-1 are transferred quantitatively to a clean black colored plastic

bottle provided in the kit. The bottle was reconstituted with 50 ml of HDLcholesterol diluent (Reagent-2).

Storage Of Working Reagent: *The working reagent is stable for 12 months from the date of reconstitution when stored at 2-8^{0}C.*

Specimen Collection: *The collected blood was made to stand without adding any anticoagulant. The clot that is formed is disturbed using a glass rod and was then centrifuged at 3000 rpm for 10 min. The serum is separated and used for the analysis.*

EQUIPMENT: Screen Master 3000 (Auto Analyzer for Biochemical parameters)Programme:

The basic assay parameters are

Mode	*: Kinetic method*
Wavelength	*: 505 nm (490-550 nm)*
Temperature	*: 37 0C*
Optical path length	*: 1 cm*
Blanking	*: Reagent blank*
Incubation	*: 0 min at 37 0C*
Sample volume	*: 10 µl*
Working reagent volume	*: 1 ml*
Concentration of standard	*: mg/dl*
Linearity	*: up to 500 mg/ dl*
Units	*: mg/ dl*

PROCEDURE:

Pipette into tubes marked	*blank*	*standerd*	*test*
Serum	*-*	*-*	*10µl*
HDL cholesterol Standard	*-*	*-*	*-*
WorkinHDL cholesterol reagent	*1 ml*	*-*	*1 ml*

Mix well. Take reading immediately.

They were mixed well and HDLcholesterolvalues are noted using semi auto analyzer.

TECHNICAL CONTENTS: USING KIT OF HDL CHOLESTEROL

DETERMINATION OF MDA LEVELS IN SERUM

Reagents

1) Potassium chloride (1.15%) solution: *1.15 g of Potassium chloride was weighed, transferred to a volumetric flask and the volume made upto 100 ml with distilled water.*

2) Thiobarbituric acid (0.8%) solution *0.8 g of Thiobarbituric acid was weighed transferred to a volumetric flask and the volume made up to 100 ml with distilled water.*

3) Sodium dodecyl sulphate (8.1%) solution: *8.1 g of Sodium dodecyl sulphate was weighed transferred to a volumetric flask and the volume made to 100 ml with distilled water.*

4) Acetic acid (20%) solution: *20 ml of Glacial acetic acid was transferred to a volumetric flask and the volume was made to 100 ml with distilled water. The pH was adjusted to 3.5 with drop wise addition of N/10 Sodium hydroxide.*

PROCEDURE:

MDA levels in the serum were measured by the method developed by Ohkawa et al 1979. To a sample of 0.2 ml of serum, 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid solution, and 1.5 ml of 0.8% aqueous solution of TBA were added. The mixture was made up to 5 ml with distilled water and then heated in an oil bath at 950C of 60 min using a glass ball as a condenser. After cooling with tap water, 5 ml of the mixture of n-butanol and pyridine (15:1, v/v) was added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance was measured at 532 nm. The serum MDA levels were measured from the standard curve and expressed as nmol/g tissue.

ESTIMATION OF ENDOGENOUS ANTIOXIDANT ENZYMES

PROTEIN ESTIMATION

REAGENTS

1) Tris buffer (0.25 M) solution:3.0285 g of Tris buffer was weighed, transferred to a volumetric flask and the volume made upto 100 ml with distilled water

2) Solution A: 1 g of sodium carbonate and 200 mg of sodium hydroxide were weighed, transferred to a volumetric flask and the volume made up to 50 ml with distilled water.

3) Solution B: 100 mg of Copper sulphate and 200 mg of Sodium potassium tartarate were weighed, transferred to a volumetric flask and the volume made up to 10 ml with distilled water.

4) Alkaline copper solution:50 ml of Solution A and 1 ml of Solution B were transferred to a volumetric flask.

5) Folin – ciocalteau phenol reagent: Folin – ciocalteau phenol solution and distilled water were mixed in 1:1 ratio in a volumetric flask.

6) Bovine serum Albumin (0.1%) Solution: 10 mg of Bovine serum Albumin was weighed, transferred to a volumetric flask and made up to 10 ml with distilled water

PROCEDURE:

Protein was estimated by the method developed by Lowry et al 1955. To 0.1 ml Serum, 0.9 ml of water and 4.5 ml of alkaline copper solution were added in the test tube, vortexed and kept at room temperature for 10 min. To the above solution 0.5 ml of Folin-ciocalteau phenol reagent was added and vortexed and kept for 30 min and the absorbance was measured at 640 nm. Protein level was determined by comparing known concentration of standard bovine serum albumin. The protein level was estimated.

DETERMINATION OF SOD LEVELS IN SERUM

1) Tris buffer (0.25 M) solution:3.0285 g of Tris buffer was weighed, transferred to a volumetric flask and the volume made up to 100 ml with distilled water .and expressed as mg protein. g-1 tissue

2) Solution A: 1 g of sodium carbonate and 200 mg of sodium hydroxide were weighed, transferred to a volumetric flask and the volume made up to 50 ml with distilled water.

3) Solution B:100 mg of Copper sulphate and 200 mg of Sodium potassium tartarate were weighed, transferred to a volumetric flask and the volume made up to 10 ml with distilled water.

4) Alkaline copper solution:50 ml of Solution A and 1 ml of Solution B were transferred to a volumetric flask.

5) Folin – ciocalteau phenol reagent:Folin – ciocalteau phenol solution and distilled water were mixed in 1:1 ratio in a volumetric flask.

6) Bovine serum Albumin (0.1%) Solution:10 mg of Bovine serum Albumin was weighed, transferred to a volumetric flask and made upto 10 ml with distilled water.

PROCEDURE:

Superoxide dismutase (SOD) activity was determined by the method developed by Kakkar et al. 1984. To 0.1 ml serum was added to 1.2 ml of 0.052 M sodium pyrophosphate buffer (pH 8.3) followed by addition of 0.1 ml of 186 □M phenazoniummethosulphate, 0.3 ml of 300 □M nitrobluetetrazolium, 0.2 ml of 780 □M NADH. Reaction mixture was incubated for 90 sec at 30°C, and the reaction was stopped by the addition of 1.0 ml of glacial acetic acid. Reaction mixture was stirred vigorously and shaken with 4.0 ml of n-butanol and centrifuged at 4000 rpm for 10 min. The absorbance of organic layer was measured at 560 nm. A control was prepared using 0.1 ml of distilled water devoid of 0.1 ml of homogenate. One unit of the enzyme activity is defined, as enzyme concentration required inhibiting the absorbance of chromogen production by 50% in control sample under the assay conditions. The SOD level was expressed as Units mg protein^{-1}.

ESTIMATION OF HEPATIC GLYCOGEN

Most of the glucose in the body is established to generate ATP. However glucose may take part in several anabolic reactions. One is the synthesis of glycogen, if glucose is not needed immediately for ATP production; it is combined with many other molecules of glucose to form a long chain molecule called glycogen. This process is termed as glycogenesis (glycol-sweet, genesis-to generate) about 500mgs of glycogen can be stored, 25% in the liver, and 75% in skeletal muscle fibers. In the process of glycogenesis, glucose that enters the cells is first phosphorylated to glucose-6-phosphate, this is then converted to glucose-l-phosphate, then to uridinediphosphate glucose and finally to glycogen, glycogenisis is stimulated by insulin. It was hypothicated that glycogen formation, which is impaired in diabetic mellitus is due to, ionic derangement correction of this electrolyte balance may have better results. The animals, which were contributed in the study of, anti-diabetic activity antihyperlipidemic activity and influence on sodium levels in urine and serum, were finally sacrificed to confirm the effectiveness of the formulation.

MATERIALS

1) 30%potassium hydroxide (Sd.fine.chem)
2) 95% alcohol
3) 60% alcohol
4) 2N sulphuric acid (Qualigens)
5) phenol red indicaror (BDH)
6) 1N sodium hydroxide (Sdfine.chem)
7) centrifuge tubes, volumetric flasks
8) albino rats (Ghosh enterprises)

PROCEDURE

l) 6ml 30% KOH were taken into a 50ml centrifuge tubes, and were kept in place with rubber stoppers
2) Rat was removed from the cage gently, to avoid excitation and it was stunned by a blow on the head and decapitated quickly.
3) And immediately liver was separated, about lgm of the sample was taken for study, then the liver sample was minced immediately and transferred to the centrifuge tube containing 6ml of 30% KOH.

4) Remove the stopper, and place the tube upright in boiling water both for 15 to 20 mins, agitating to ensure thorough disintegration.
5) Add 7ml of 95% alcohol to the centrifuge tube, mix by tapping, immerse in water bath until boiling just begins (care must be taken to avoid loss by sudden foaming). Allow the tubes to cool at room temperature for about 2 hours.
6) Centrifuge, decant and discard the supernatant fluid, drain and wash the precipitate twice with 5-ml portions of 60%by centrifuging decanting and draining as before.
7) Expel the last traces of alcohol by immersing the tubes in boiling water just long enough to dry the glycogen. Then add 10ml distilled water and stir until a uniform suspension is obtained.
8) Pippete 5ml of the suspension into a clean test tube add,5ml,2N sulfuric acid solution, insert a small funnel in the mouth of the test tube to minimize loss by evaporation, and heat in a boiling water for 3 to 4 hrs to hydrolyze the glycogen.
9) Cool, add a drop of phenol red indicator and neutralize cautiously with lN, sodium hydroxide, with constant stirring. Transfer the neutralized solution quantitatively to a 50ml volumetric flask, dilute to volume with water and mix.
l0) Analyse the solution for glucose content, and express in mcg glucose equ/gm of tissue.

IMMUNOSTIMULANT ACTIVITY

Adaptogenic drugs that help an organism to cope better during stress and retard aging process are well recognized in Ayurvedic medicine. Herbal drugs are known to have immunomodulatory properties. Many plants are claimed to have immunostimulant activity. Immunopharmacological properties of Picrorhizakurroa, Ocimum sanctum were studied. Immunomodulatory action is mostly concerned with cellular involvement of haemopoietic, lymphoid tissue. Immune stimulation of host immune system in terms of tumor growth inhibition, preventing the metastasis and increasing survival time.

MATERIALS USED

- Leishman,s stain
- Alcohol
- Haemocytometer
- Cotton

MEASUREMENTS OF BLOOD LYMPHOCYTE, SPLENIC LYMPHOCYTE AND PERITONEAL MACROPHAGE COUNTS.

Blood lymphocyte count was carried out using Leishman stain in microscope. To determine splenic lymphocyte count, spleen was dissected, macerated and washed with 10 ml balanced salt solution (BSS pH 7.2) and pellets were resuspended in 2ml of BSS and counting was done with haemocytometer. Peritoneal macrophages were collecte at different days of treatment by washing peritoneal cavity with chilled BSS. The peritoneal fluid was incubated at 37 $^{\circ}$C for 60 min in glass petridish. 2% cold EDTA was added to the petridish and flushed gently and kept at 4°c for 30 min. Suspension was taken off from petridish and centrifuged and suspended in 1 ml of BSS. Counting was done with haemocytometer in the presence of neutral red.

EXPERIMENTAL PROTOCOL

PREPARATION OF DRUG SOLUTION:

The *Glycyrrhizaglabrawas* suspended in the 1% sodium carboxy methyl cellulose (CMC) mucilage with continuous trituration.

Route of Administration: Oral

PROCEDURE:

ANIMALS USED:

Albino Wistar rats of either sex weighing 180g-250g

Number of animals in each group: six (n=6)

The rats were divided into 6 groups each group contains 6 rats, type I diabetes is induced by STZ administration as described earlier and type II diabetes is induced by STZ+Nicotinamide administration as described earlier.

Group I : Normal control rats treated with 1% Sod .CMC 1ml.
Group II : Normal rats treated with Glycyrrhizaglabra100 mg/kg.
Group III : Type I Diabetic control rats treated with 1% sod. CMC 1ml.
Group IV : Type II Diabetic control rats treated with 1% sod. CMC 1ml.
Group V : Type I Diabetic rats treated with Glycyrrhizaglabra 100 mg/kg.
Group VI : Type II Diabetic rats treated with Glycyrrhizaglabra 100 mg/kg.

In the present study the Albino Wistar rats of either sex supplied by Mahaveer Enterprises, Hyderabad, weighing 180g to 250g were used .The rats were kept on fasting for 18 hours before the experiment. The blood samples were collected from the retro orbital plexus of rats. After collection of blood sample, the serum was separated by centrifuge at 3000 rpm for 10 min. The serum glucose estimation was done with the Screen Master 3000 (Auto Analyzer for Biochemical parameters).

The fasting serum glucose level was noted which is considered as 0 hour reading. The methanolic rhizome extract of Glycyrrhizaglabra at a dose of 100mg/kg was given orally to the normal, type I and type II diabetic rats. The blood samples were taken as well as serum glucose levels were estimated at 0,1st, 2nd, 4th, 6th, 8th, 12th and 24th hour as mentioned above. The percent blood glucose reduction was calculated for each group.

V.RESULTS & DISCUSSIONS

Statistical Analysis

All values were expressed as (Mean ± S.E.M.). Differences between means were tested using One-way ANOVA.Individual groups were compared using,Dunnett's multiple comparision test. Differences with $P< 0.05$ were considered as statistically significant. Statistical analysis was performed using Graph pad prism (version 5).

TABLE 5.1: CONSOLIDATED TABLE SHOWING THE PERCENTAGE REDUCTION OF BLOOD GLUCOSE (MG/DL) IN NORMAL, TYPE I AND TYPE II DIABETIC RATS

Dose: 100mg/kg methanolic extract of Glycyrrhizaglabra

Time (hr's)	Normal control	Normal treated	Type-I control	Type-I treated	Type-II control	Type-II treated
1	2.36	7.59	3.09	5.95	2.80	13.66
2	4.85	10.83	6.71	11.20	6.85	20.59
4	5.20	19.14	7.91	15.77	7.31	35.91
6	6.71	23.82	6.11	12.57	5.49	29.10
8	7.03	18.97	5.20	8.65	3.45	26.50
12	6.89	12.69	4.11	7.62	6.58	23.22
24	8.39	6.90	4.63	7.91	5.39	19.76

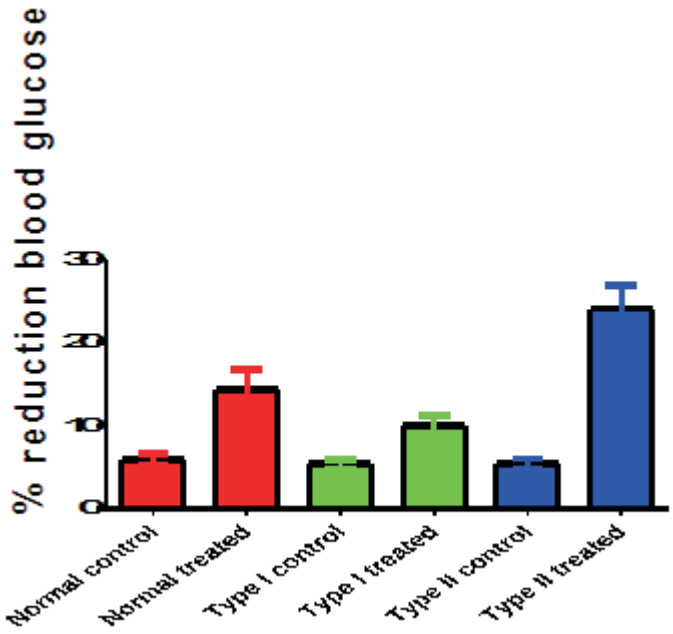

Figure 5.1: Reduction in blood glucose levels

Results and Discussion:

1)As the result obtained from the table, a good amount of significant hypoglycaemia is observed at 6th hour (23.82%) in normal rats treated with the methanolic extract of *Glycyrrhizaglabra.*

2)In type I diabetic rats treated with the extract, a small reduction in the blood glucose was observed where as in type II diabetic rats treated with the extract significant antihyperglycaemic activity is observed and it is sustained upto 8th hour.

3) In type II diabetic rats, maximum reduction was observed at 4th hour (35.91%) From the above observations, it is found that the extract at a dose of 100mg/kg is having hypoglycaemic as well as antihyperglycaemic activity in type II diabetic rats. Because it is having hypoglycaemic and

antihyperglycaemic activity in type II diabetic rats, the extract might have exhibited sulfonyl urea like activity.

From the results, it was found that the methanolic rhizome extract of Glycyrrhiza glabrasignificantly reduced the blood glucose levels in normal and diabetic rats at a dose 100mg/kg. Borhanuddin et al., 2008, reported that aqueous extract of *Glycyrrhizaglabrasignificantly* prevented induction of hyperglycaemia induced by oral administration of glucose in rabbits.

R. Subramanian., et al, 2008, reported that Glycyrrhizaglabramay have an insulin sensitizing effect, by attenuating the impairment of insulin stimulated glucose disposal in insulin resistant rats. This probably indicates that the extract may increase the activity of endogenous insulin to improve insulin resistance condition. *Glycyrrhizin* can increase the glucose utilization to lower plasma glucose in diabetic rats lacking insulin. Previous study Xiang-Fan zhang et al., 2000 reported that ethanolic extract reduced blood glucose in diabetic rats but not in normal rats.Husenet al., 2004and Zhang et al., 2000,have reported the antihyperglycemic property of *Glycyrrhizaglabra* in streptozotocin-induced hyperglycemic rats but enough evidence was not available to confirm the hypoglycemic activity of the various extracts on different hyperglycemic conditions. In this paper, we report the hypoglycemic activity of hot water and ethanol extracts of the aerial parts of Glycyrrhizaglabra growing in glucose-loaded hyperglycemic and alloxan-induced diabetic rats. NalamoluKoteswaraRao, 2006, investigated theAntihyperglycaemic activity of chloroform extract is reported and also investigated the antihyperglycemic action of andrographolide in streptozotocin-induced diabetic rats (STZ-diabetic rats) (Yu Bc et al., 2003).

From above observations *Glycyrrhizaglabra* shows the hypoglycemic, antihyperglycaemic activity in type II diabetic rats.

It was observed that the degree of percent reduction of blood glucose was more in typeII diabetic rats when compared to type I diabetic rats. Because the compound is having antihyperglycaemic activity in type II diabetic rats at a dose of 100mg/kg, it is proposed to evaluate the dose dependent antihyperglycaemic activity of the extract in type II diabetic rats by using various doses. Hence dose dependent effect of methanolic extract of Glycyrrhizaglabrawas evaluated in type II diabetic rats.

PROTOCOL FOR DOSE DEPENDENT ACTIVITY.

The rats were divided into 5 groups each group contains 6 rats, methanolic extract of Glycyrrhizaglabrais given by oral route.

Group I: Type II Diabetic control rats treated with 1% sod. CMC.
Group II: Type II Diabetic rats treated with Glycyrrhizaglabra100 mg/kg.
Group III: Type II Diabetic rats treated with Glycyrrhizaglabra 200 mg/kg.
Group IV: Type II Diabetic rats treated with Glycyrrhizaglabra 400 mg/kg.
Group V: Type II Diabetic rats treated with reference compound Gliclazide 1mg/kg.

TABLE 5.2: CONSOLIDATED TABLE SHOWING PERCENTAGE REDUCTION OF BLOOD GLUCOSE (MG/DL) OF VARIOUS DOSES OF *GLYCYRRHIZAGLABRA*

Time (hr)	control	100mg/kg	200mg/kg	400mg/kg	Gliclazide 1mg/kg
1	2.80	13.66	15.99	17.37	36.96
2	6.85	20.59	23.45	22.41	29.00
4	7.31	35.91	30.44	31.81	21.38
6	5.49	29.10	32.46	43.37	34.98
8	3.45	26.50	38.35	47.34	29.26
12	6.58	23.22	29.08	38.83	24.12
24	5.39	19.76	21.91	27.40	19.59

RESULTS AND DISCUSSION:

From the observations, when compared to various doses of methanolic extract of *Glycyrrhizaglabrawith* diabetic control, there is a dose dependent significant reduction of blood glucose is observed($p<0.05$, Dunnett's multiple comparision test). A significant dose dependent percent blood glucose reduction was observed 6th onwards but no significant dose dependent blood glucose was observed upto 4th hour in type II diabetic rats. In 100 mg/kg extract treated rats show maximum peak reduction at 4th hour and its antihyperglycaemia is sustained up to 8th hour. 100mg/kg dose produces significant percent reduction of blood glucose and is comparable with 1mg/kg gliclazide (reference compound).

Dose dependent antihyperglycaemic activity is observed with the 100mg/kg, 200mg/kg, 400mg/kg methanolic extract of Glycyrrhizaglabra. Our results show dose dependent antihyperglycaemic activity which is supported by Zhang et al., 2000, Oral administration of the extract at different doses (0.1, 0.2, and 0.4 g/body weight) significantly reduced the fasting serum glucose level in STZ-diabetic rats compared to the vehicle (distilled water), but not in normal rats. This effect was dose-dependent.

Nalamolukoteswararao, 2006, reported that the chloroform extract of Glycyrrhizaglabraroots produced a dose-dependent hypoglycemia in alloxan-induced diabetic rats in acute study. It produced signifi-cant reduction in blood glucose with doses of 50, 100 and 150 mg/kg body weight compared to control group.

Oral administration of glycyrrolide decreases the plasma glucose concentrations of STZ induced diabetic rats in a dependent manner (Yu B.C. et al, 2003).

Effect of Chronic administration (7 Days) of methanolic extract of *Glycyrrhizaglabraon* various biochemical parameters in normal and Type II Diabetics rats

Preparation Of Drug Solution:

The *Glycyrrhizaglabrawas* suspended in the 1% sodium carboxy methyl cellulose (CMC) mucilage with continuous trituration.

Route of Administration: Oral
Animals used: Albino Wistar rats of either sex weighing 180g-250g
Dose: 100mg/kg methanolic extract ofGlycyrrhizaglabra
Treatment: 7 days.
Number of animals in each group: six (n=6)

The rats were divided into 4 groups each group contains 6 rats. Both methanolic extract of

Glycyrrhizaglabraand vehicle (1% sodium CMC) are administered by oral route to narmal and diabetic rats for a period of 7 days.

Group I : Normal control rats treated with 1% Sod .CMC 1ml.
Group II : Normal rats treated with Glycyrrhizaglabra100 mg/kg.
Group III : Type II Diabetic control rats treated with 1% sod. CMC 1ml.
Group : Type II Diabetic rats treated with Glycyrrhizaglabra100 mg/kg.

STATISTICAL ANALYSIS

All values are expressed as MEAN ± S.E.M. Differences between means were tested using paired T-tests. P value less than 0.05 was considered significant.

1. Effect of methanolic extract of *Glycyrrhizaglabraon* blood glucose levels in normal and type II diabetic rats.

Hyperglycemia occurs because of uncontrolled hepatic glucose output and decreased uptake of glucose by skeletal muscle with reduced glycogen synthesis. During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation. A large number of plants have been recognized to be effective in the treatment of diabetes mellitus. These *include, Allium sativum, Eugenia jambolana, MomordicacharantiaOcimum sanctum, Phyllanthusamarus, Pterocarpusmarsupium* etc. Hence in the present study, the effect of *Glycyrrhizaglabraon* blood glucose was evaluated and the results are given below.

TABLE 5.3: EFFECT OF METHANOLIC EXTRACT OF GLYCYRRHIZAGLABRAON BLOOD GLUCOSE LEVELS IN NORMAL AND TYPE II DIABETIC RATS.

S.No.	Normal control (group-I)		NORMAL EXTRACT TREATED (group-II)		DIABETIC CONTROL (group-III)		DIABETIC EXTRACT TREATED (group-IV)	
	Before	After	Before	After	Before	After	Before	After
1	84	90	103	85	296	287	331	184
2	96	91	113	91	325	311	305	157
3	135	121	91	68	336	345	287	143
4	105	112	93	76	326	334	306	163
5	91	99	87	69	311	321	328	187
6	101	117	121	96	318	329	311	196
Mean± SEM	102 ±7.3	105 ± 5.5	102.33± 5.6	80.83 ± 4.75	318.66± 5.7	321.16± 8.3	311.33±6.6	171.6±8.3
% reduction	-2.96		21.01		-0.78		44.86	

RESULTS AND DISCUSSION:

A significant reduction in the blood glucose level was observed in normal rats and type 2 diabetic rats treated with the methanolic extract of *Glycyrrhizaglabra.*

The degree of percent reduction was more in type II diabetic rats and the good amount of percent reduction in the blood glucose was significant in type II diabetic treated rats.

Glycyrrhizaglabra is producing hypoglycemic as well as antihyperglycemic activity in type II diabetic rats. It might have exhibiting insulin sensitizing effect, by attenuating the impairment of insulin stimulated glucose disposal in insulin resistant rats. This probably indicates that the extract may increase the activity of endogenous insulin to improve insulin resistance condition. It may be following sulfonyl urea group of actions

EFFECT OF METHANOLIC EXTRACT OF *GLYCYRRHIZAGLABRAON* HEPATIC GLYCOGEN IN NORMAL AND TYPE II DIABETIC RATS.

It is promoting glycogen synthesis in type 2 diabetic rats treated with G.g.extract. Most of the glucose in the body is established to generate ATP. However glucose may take part in several anabolic reactions. One is the synthesis of glycogen, if glucose is not needed immediately for ATP production; it is combined with many other molecules of glucose to form a long chain molecule called glycogen. This process is termed as glycogenesis (glycol-sweet, genesis-to generate) about 500mgs of glycogen can be stored, 25% in the liver, and 75% in skeletal muscle fibers. In the process of glycogenesis, glucose that enters the cells is first phosphorylated to glucose-6-phosphate, this is then converted to glucose-l-phosphate, then to uridinediphosphate glucose and finally to glycogen, glycogenisis is stimulated by insulin. It was hypothecated that glycogen formation, which is impaired in diabetic mellitus is due to, ionic derangement correction of this electrolyte balance may have better results.

TABLE 5.4: EFFECT OF METHANOLIC EXTRACT OF *GLYCYRRHIZAGLABRAON* HEPATIC GLYCOGEN IN NORMAL AND TYPE II DIABETIC RATS.

S.No.	Normal control (group-I)	NORMAL EXTRACT TREATED (group-II)	DIABETIC CONTROL (group-III)	DIABETIC EXTRACT TREATED (group-IV)
1	210	215	148	169
2	178	183	156	183
3	194	189	152	174
4	166	175	142	178
5	169	173	158	196
6	183	189	132	185
Mean±SEM	183.33±6.73	187.3±6.18	148±3.96	180.83±3.85
% increase of hepatic glycogen	-	2	-	32.83*

S: Significant at p<0.5(in comparision to diabetic control)

RESULTS AND DISCUSSION:

In diabetic control the hepatic glycogen levels were found to be decreased. In diabetic treated animals, which received the extract, were showed a improvement in glycogen levels, when compared to diabetic control. There is no signifint difference between normal control and normal treated rats.

DISCUSSION:

Most of the glucose in the body is established to generate ATP. However glucose may take part

in several anabolic reactions. One is the synthesis of glycogen, if glucose is not needed immediately for ATP production; it is combined with many other molecules of glucose to form a long chain molecule called glycogen. This process is termed as glycogenesis (glycol-sweet, genesis-to generate) about 500mgs of glycogen can be stored, 25% in the liver, and 75% in skeletal muscle fibers. In the process of glycogenesis, glucose that enters the cells is first phosphorylated to glucose-6-phosphate, this is then converted to glucose-l-phosphate, then to uridinediphosphate glucose and finally to glycogen, glycogenisis is stimulated by insulin. It was hypothecated that glycogen formation, which is impaired in diabetic mellitus is due to, ionic derangement correction of this electrolyte balance may have better results.

Umamaheswari et al., reported that oral administration of an ayurvedic formulation fortified with Glycyrrhizaglabra, 50mg/kg and 100mg/kg for 60 days results in significant increasing levels of hepatic glycogen in diabetic animals. R. Subramanian also reported that increased hepatic glycogen levels in STZ induced diabetic rats.

The possible mechanism may a possible extrapancreatic mode of action, by stimulating peripheral glucose utilization or enhancing glycolytic and glycogenic processes with concomitant decrease in glycogenolysis, gluconeogenesis and increase in glycogenesis.

3. EFFECT OF METHANOLIC EXTRACT OF *GLYCYRRHIZAGLABRAON* LIPID PROFILE IN NORMAL AND TYPE II DIABETIC RATS.

Diabetes is also associated with hyperlipedemia and hypertriglyceridemia (De Sereday, et al., 2004). The rise in blood sugar is accompanied with the increase in TC, TGS, VLDL, and fall of HDL. Diabetes induced hyperlipedemia is attributed to excess mobilization of fat from the adipose due to underutilization of glucose (Krishna kumar, et al., 2000).

Hence in the present study, the effect of *Glycyrrhizaglabraon* lipid profile was evaluated and the results are given below.

5.5: Effect of methanolic extract of *Glycyrrhizaglabraon* % variation of Lipid Profile in normal and Type II Diabetic rats..

PARAMETER	NORMAL CONTROL (group-I)	NORMAL EXTRACT TREATED (group-II)	DIABETIC CONTROL (group-III)	DIABETIC EXTRACT TREATED (group-IV)
TC(mg/dl)	1.86	12.12	-1.8	11.9
TGS(mg/dl)	1.51	18.56	3.58	16.88
HDL(mg/dl) (% increase)	(+)5.89	(+)14.25	3.99	(+)33.21
LDL(mg/dl)	6.42	19.81	6.29	60.56
VLDL(mg/dl)	1.85	21.88	-1.8	11.9

RESULTS AND DISCUSSION:

A significant decrease in the TC, TGS, VLDL and LDL, a significant increase was observed in HDL levels in normal rats and type II diabetic rats treated with the methanolic extract of Glycyrrhizaglabra.

The degree of percent reduction in the TC, TGS and VLDL was observed in normal treated rats whereas percent reduction in LDL is significant in type 2 treated rats. Significant percent increase in HDL levels was more in type II diabetic treated rats. Thus methanolic extract of *Glycyrrhizaglabrais*

producing antihyperlipidemic activity in diabetic rats and hypolipidemic activity in normal rats.

Rammohan Subramanian et al., demonstrates that glycyrrhizin at a dose of 10 mg/kg and the ethanol extract of Glycyrrhizaglabra, at dose levels of 500 and 1000 mg/kg, p.o., exhibited hypolipidemic in adult STZ type II diabetes mellitus. Hypotriglyceridemic effect is also reported (Zhang et.al., 2000).

4. Effect of Methanolic extract of *Glycyrrhizaglabraon* Oxidative stress (Serum MDA and SOD) in normal and Type II Diabetics rats:

Increase in lipid peroxide was found in diabetes mellitus with complication Elevated levels of lipid peroxide (MDA) in diabetes mellitus may be due to the alteration of function of erythrocytes membrane. This inhibits the activity of superoxide dismutase (SOD) enzyme leading to accumulation of superoxide radicals which cause the maximum lipid peroxidation and tissue damage in diabetes (Freitas, et al., 1997). The increase of free radicals in diabetic condition is suggested to be due to the increased lipid peroxidation and the damage of antioxidant defense system mda is one of the lipid peroxidation products frequently used to determine the oxidant /antioxidant balance in diabetic patients (Mc Cord JM (1993), (Baynes JW (1991).

Hence in the present study, the effect of Glycyrrhizaglabraon antioxidant activity was evaluated and the results are given below.

TABLE 5.6: EFFECT OF METHANOLIC EXTRACT OF *GLYCYRRHIZAGLABRAON* SERUM MDA (N.MOLES/IU) IN NORMAL AND TYPEII DIABETIC RATS.

S.No.	Normal control (group-I)		NORMAL EXTRACT TREATED (group-II)		DIABETIC CONTROL (group-III)		DIABETIC EXTRACT TREATED (group-IV)	
	Before	After	Before	After	Before	After	Before	After
1	4.2	4.0	4.0	3.7	5.6	5.2	6.0	4.5
2	4.6	4.3	3.9	4.4	6.3	6.0	4.9	3.8
3	3.9	4.1	3.8	3.4	6.0	6.4	5.5	3.6
4	4.4	4.1	4.3	3.7	5.8	6.2	5.9	4.2
5	4.0	3.8	4.6	3.1	5.2	5.4	5.6	3.9
6	4.6	4.2	3.8	2.9	5.2	5.6	5.7	3.6
Mean± SEM	4.28± 0.12	4.08 ± 0.07	4.06 ± 0.13	3.53 ± 0.21	5.68± 0.18	5.8± 0.19	5.6± 0.16	3.93 ± 0.15
%reduction	4.67		13.05		-2.11		29.82	

Table 5.6: Effect of methanolic extract of *Glycyrrhizaglabraon* serum SOD (IU/gm of serum protein) in normal and TypeII Diabetic rats.

S.No.	Normal control (group-I)		NORMAL EXTRACT TREATED (group-II)		DIABETIC CONTROL (group-III)		DIABETIC EXTRACT TREATED (group-IV)	
	Before	After	Before	After	Before	After	Before	After
1	148	155	156	169	127	135	139	149
2	152	146	148	159	129	132	131	161
3	156	154	153	161	134	131	141	165
4	134	143	146	164	122	130	137	154
5	147	149	146	158	128	121	132	157
6	145	155	138	145	136	140	126	148
Mean± SEM	147 ± 3.1	150.33± 2.1	147.83± 2.6	159.3± 3.29	129.33± 2.1	131.5± 2.6	132.66 ± 2.2	155.66± 2.7
% INCREASE	(+)2.26		(+)7.75		(+)1.67		(+)17.33	

RESULTS AND DISCUSSION:

A significant decrease in MDA level and significant increase in SOD levels was observed in normal rats and diabetic rats treated with *Glycyrrhizaglabra*. The degree of percent reduction in the MDA and a significant increase in SOD levels was observed more in type II diabetic treated rats. *Glycyrrhizaglabrashowing* antioxidant activity which is supported by several studies.Xiang-Fan Zhang et al., 2000,investigated the ethanolic extract of the aerial parts of Glycyrrhizaglabrafor antihyperglycaemic and antioxidant effects in normal and streptozotocin-induced type I diabetic rats. Oxidative stress is evident in streptozotocin-diabetic rats and indicate that the ethanolic extract of *Glycyrrhizaglabranot* only possesses an antihyperglycaemic property, but may also reduce oxidative stress in diabetic rats.

Extract of *Glycyrrhizaglabrapossess* antioxidative property (Chanwitheesuk et al., 2002; Singh et al., 2001; Zhang and Tan, 2000) and it was reported that neoglycyrrolide is an antioxidant of superoxide free radicals (Kamdem et al., 2002).

The beneficial combination of antidiabetic, hypolipidemic, and antioxidant properties would be of great therapeutic application in the management of T2DM and the associative abnormalities in lipid profiles.

VI. CONCLUSIONS

- The study was carried out with a single dose of *Glycyrrhizaglabramethanolic* extract on blood glucose level in normal rats and diabetic rats and observed that Glycyrrhizaglabra is shown significant hypoglycaemic and antihyperglycaemic activity in type II diabetic rats but no significant antihyperglycaemic activity in type I diabetic rats.
- *Glycyrrhizaglabraat* a dose of 100mg/kg is shown significant antihyperglycaemic activity which is comparable with the 1mg/kg gliclazide.
- *Glycyrrhizaglabrais* also producing dose dependent antidiabetic activity with the doses of 100mg/kg, 200mg/kg and 400mg/kg.
- The probable mechanism may have an insulin sensitizing effect, by attenuating the impairment of insulin stimulated glucose disposal in insulin resistant rats and also it may follow sulfonyl urea group mechanism.

- Upon the chronic administration of *Glycyrrhizaglabramethanolic* extract for a period of 7 days to normal and type II diabetic rats shown.

- ✓ Significant hypoglycaemic and antidiabetic activity.
- ✓ Lowering of lipid profile
- ✓ Antioxidant activity
- ✓ Increasing the hepatic glycogen in diabetic rats but not in normal rats.

- Thus the folk use of this plant in treating diabetes is justified and also it helps in preventing diabetic complications and serves as a good adjuvant in the present armamentarium of antidiabetic drugs.
- Further investigation is in progress to find out its mechanism of action and to establish its potential in the treatment of macro vascular complications of diabetes.

VII. BIBLIOGRAPHY

[1]Rother, KI. "Diabetes Treatment –Bridging the Divide". N Engl J Med 2007,356 15:1499-1501.
[2]Rang and Dale, 7th edition-2008, pg no:356-358.
[3]Wild S Roglic G, Green A, Sicree R, King H (May 2004). "Global prevalence of diabetes: estimates for the year 2000 and projections for 2030". Diabetes care 27 5:1047-53.
[4]Lawrence JM, contrearas R, Chen W, Sacks DA (May 2008). "trends in the prevalence of preexisting diabetes and gestational Diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999-2005". *Diabetes care* 31 (5):899-904.
[5]KD Tripathi. Essentials of medical pharmacology 5th edition . page no 325.
[6]George M, Pandalai KM. Investigations on plant antibiotics. Part IV. Further search for antibiotic substances in Indian medicinal plants. *Indian Journal of Medical Research*, 1949, 37:169–181.
[7]Nakanishi K. Phytochemical survey of Malaysian plants: preliminary chemical and *pharmacological screening. Chemical and Pharmaceutical Bulletin*, 1965, 13: 882–890.
[8]Leelarasamee A. Undetectable antibacterial activity of *Andrographis paniculata (Burm) Wall. ex Nees. Journal of the Medical Association of Thailand,* 1990, 73:299–304.
[9]Yao XJ. Mechanism of inhibition of HIV-1 infection in vitro by a purified extract of *Prunella vulgaris. Virology*, 1992, 187:56–62.
[10]Chang RS, Yeung HW. Inhibition of growth of human immunodeficiency virus in vitro by crude extracts of Chinese medicinal herbs. *Antiviral Research,* 1988, 9: 163–175.
[11]Chang RS. Dehydroandrographolide succinic acid monoester as an inhibitor against the human immunodeficiency virus (43225).P*roceedings of the Society of Experimental Biology and Medicine*, 1991, 197:59–66.
[12]Otake T. Screening of Indonesian plant extracts for anti-human immunodeficiency virus type 1 (HIV-1) activity. *PhytotherapyResearch*, 1995, 9:6–10.
[13]Puri A. Immunostimulant agents from Andrographis paniculata. Journal of Natural Products, 1993, 56:995–999.
[14]Vedavathy S, Rao KN. Antipyretic activity of six indigenous medicinal plants of Tirumala Hills, Andhra Pradesh, India. *Journal ofEthnopharmacology*, 1991, 33: 193–196.
[15] Madav S. Analgesic and antiulcerogenic effects of andrographolide. *Indian Journal of Pharmaceutical Science*, 1995, 57:121–125.
[16]Deng W. Comparison of pharmacological effect of four andrographolides. Chinese Pharmaceutical

Bulletin, 1982, 17:195–198.
[17] Gupta *S. Antisecretory* (antidiarrhoeal) activity of Indian medicinal plants against Escherichia coli enterotoxin-induced secretion in rabbit and guinea-pig ileal loop models. *International Journal of Pharmacognosy*, 1993, 31:198–204.
[18]Gupta S. Antidiarrhoeal activityof diterpenes of Andrographis paniculata (kalmegh)against *Escherichia* coli enterotoxin in invivo models. *International Journal of Crude Drug Research,* 1990, 28:273–283.
[19] Chiou W-F, Lin J-J, Chen C-F. Andrographolide suppresses the expression of inducible nitric oxide synthase in macrophages and restores the vasoconstriction in rat aorta treated with lipopolysaccharide. *British Journal of Pharmacology*, 1998, 125:327–334.
[20] Misra P. Antimalarial activity of traditional plants against erythrocytic stages of Plasmodium berghei. International Journal ofPharmacognosy, 1991, 29:19–23.
[21]Misra P. Antimalarial activity of Andrographis paniculata (kalmegh) against P*lasmodium berghei NK 65 in Mastomysnatalensis. International Journal of Pharmacognosy*, 1992, 30:263–274.
[22]Nazimudeen SK. Effect of *Andrographis paniculata on snake venom-induced death and its mechanism. Indian Journal of Pharmaceutical Sciences*, 1978, 40: 132– 134.
[23]Chander R. *Antihepatotoxic activity of diterpene of Andrographis paniculata (kalmegh) against Plasmodium berghei*-induced hepatic damage in Mastomys natalensis. International Journal of Pharmacognosy, 1995, 33:135–138.
[24]Bhaumik A, Sharma MC. Therapeutic effect of two herbal preparations in induced hepatopathy in sheep. *Journal of Research in Indian Medicine*, 1993, 12:33–42.
[25] Kapil A. Antihepatotoxic effects of major diterpenoid constituents of *Andrographis paniculata. Biochemical Pharmacology,* 1993, 46:182–185.
[26]Visen PKS. *Andrographolide protects rat hepatocytes against paracetamol-induced damage.Journal of Ethnopharmacology,* 1993, 40:131–136.
[27]Manisha Modak, Priyanjali Dixit, Jayant Londhe, Saroj Ghaskadbi, and Thomas Paul A. Devasagayam. Indian Herbs and Herbal Drugs Used for the Treatment of Diabetes. *J Clin Biochem Nutr.* 2007 May; 40(3): 163–173.
[28]Seth S.D., Sharma B. Medicinal plants of India. Indian J. Med. Res. 2004;120:9–11.
[29]Chanwitheesuk, A., Rakriyatham, N., Teerawutgukrag, A. ang Wonchanapiboon, T., 2002 Screening of antioxidants from Thai vegatables and herbs. Chaing Mai J Sci, 29(1): 1-5
[30]Singh, R. P., Banerjee, A. and Rao, A. R., 2001, Modulatory influence of Andrographis paniculata on mouse hepatic and extrahephatic carcinogen metabolizing enzymes and antioxidant status. Phytother Res 15: 382-390
[31]Zhang, X. F. and Tan, B. K., 2000, Antihyperglycaemic and anti-oxidant properties of Andrographis paniculata in normal and diabetic rats. Lin Exp Pharmacol Physiol, 27(5-6): 358-363
[32]Kamdem, R. E., Sang, S., and Ho, C. T., 2002, Mechanism of superoxide scavenging activity of neoandrographolide – a natural product from Andrographis paniculata Nees. J Agric Food Chem, 50(16): 4662-4665
[33]Zhang, X. F. and Tan, B. K., 2000, Anti-diabetic property of ethanolic extract of Andrographis paniculata in streptozotocin-diabetic rats. Acta Pharmacol Sin, 21 (12): 1157-1164
[34]Husen, R.,Pihie, A. H. and Nallappan, M., 2004, Screening for antihyperglycaemic activity in several local of Malaysia. J Ehtnopharmacol, 95(2-3): 205-208
[35]Yu, B. C., Hung, C. R., Chen, W. C. et al., 2003, Antihyperglycaemic effect of andrographolide in streptozotocin-induced diabetic rats. Planta Med, 69(12): 1075-1079

[36]Xiang-Fan Zhang and Benny Kwong-Huat Tan., 2000,Antihyperglycaemic and anti-oxidant properties of Andrographis paniculata in normal and diabetic rats. *Clinical and Experimental Pharmacology and Physiology,* 27: 358-363

[37]A.S.Syahrin, S.S.Amrah, K.L.Chan, B.Y.Lim, N.Hasnan, S.S.J.Mohsin., 2008, Effect of spray-dried ethanolic extract of *Andrographis paniculata* on streptozotocin-induced diabetic female rats. Int J diab ctries, 26(4): 163-168

[38]King ,H. Albert , RE., Herman, W. H., Global burden of diabetes, 1995-2025- prevalence, numerical estimates and projections; Diabetes care, (1998), 21:1414-31.

[39] Borhanuddin, M., Shamsuzzoha, M. and Hussian,A.H., 1994 Hypoglycaemic effects of Andrographis paniculata Nees on non-diabetic rabbits. Bangladesh Med. Res. Counc.,20, 24-26.

[40]Rammohan Subramanian, Mohd. Zaini Asmawi1, Amirin Sadikun., 2008,effect of ethanolic extract of andrographis Paniculata (burm. F.) Nees on a combination ofFat-fed diet and low dose streptozotocin induced Chronic insulin resistance in rats. Diabetologia Croatica 37-1, 2008.

[41]Saxena A, Vikram NK (2004): Role of selected Indian plants in management of T2DM: A review. J Alter Complement Med10: 369–378.