ejpmr, 2020,7(2), 516-522

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF ANTIDIABETIC ACTIVITY OF *SWERTIA CHIRAYITA* AND *PANAX GINSENG*

***Samreen Begum, Dr. A. Srinivasa Rao and M. Sri Ramachandra**

Bhaskar Pharmacy College, Moinabad, R.R District, 500075, Telangana, India.

***Corresponding Author: Samreen Begum**

Bhaskar Pharmacy College, Moinabad, R.R District, 500075, Telangana, India.

Article Received on 22/12/2019 **Article Revised on 12/01/2020** **Article Accepted on 01/02/2020**

ABSTRACT

Diabetes mellitus, one of the most common endocrine disorders has caused significant morbidity and mortality due to macro vascular and micro vascular complications. Currently available therapies for diabetes include insulin and various oral anti diabetic drugs have number of serious adverse effect; therefore the search for more effective and safer hypoglycemic agents is one of the important areas of investigation. Some medicinal plants have been reported to be useful in diabetes worldwide. The herbs like *swertia chirayata* shown to protect the liver. It contains xanthones which is reputedly effective against Malaria, Tuberculosis. It also cures constipation and used for treating dyspepsia with all other properties the *swertia chirayita* shows good anti diabetic activity. The other herb which was used to carry out the experiment *panax ginseng* is well effective in case of anti-sterility in men, it prevents cancer and fight chemical dependency (anti proliferative). The study was conducted to examine the possible antidiabetic activity of *swertia chirayata and panax ginseng* leaf extraction on male wistar rats. Gold thio glucose method was used to induce diabetes in rats. Initially blood glucose levels were increased abruptly after induction. After giving the oral administration of ethanolic extract of *swertia chirayat* (100mg/ Kg, 200mg/kg) and *panax gingseng* (250mg/kg, 100mg/kg). Finding of this research showed that ethanolic extract of a plant *swertia* possess phytochemicals like steroids, alkaloids, tannins, flavonoids and *panax ginseng* possess alkaloids, carbohydrates, flavonoids and tannins significant ($P < 0.05$) anti diabetic activity. The results were compared with standard drug metformin (400mg/kg).

KEYWORDS: *Swertia chirayita*, *panax ginseng*, Antidiabetics.

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.

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 no 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 hyperglycaemia 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), foetal malformation and congenital heart disease. It requires careful medical supervision during the pregnancy (Lawurence *et 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 hemoglobin test (HbA1c)

Materials and source

Sodium citrate-Virat labs, Hyd, India
Diethyl ether-Finar chemicals limited, Ahmadabad.
Methanol -E-Merk, Mumbai, India.
Normal saline-Claris life sciences, Ahmadabad, India.
Formaldehyde- Finar chemicals limited, Ahmadabad, India
Chloroform- Molychem, Mumbai, India.
Gold thio glucose -Sigma, St Louis, U.S.A.
Metformin-MSN Formulations, HYD, India.

EQUIPMENTS USED

Centrifuge –Remiequipments Pvt, Ltd, Hyd, India.
Shimadzu electronic balance- Toshvin Analytical Pvt. Ltd, India
Shimadzu UV-spectrophotometer- Toshvin Analytical Pvt. Ltd, Mumbai.
Inverted microscope- Boeckl + co, Hamburg.

GOLD THIOGLUCOSE

Gold thio glucose is diabetogenic compound, which is induced hyperphagia and severe obesity induced Type -2 diabetes.

Chemical Properties

• It is derivative of sugar glucose.
• Gold thio glucose is precipitated with methanol and recrystallized with water and methanol.

Mechanism of Action

Gold thio glucose developed obesity induces diabetes in genetically normal mouse strains. Gold thio glucose treated DBA/2 (Dilute Brown Non- Agouti), C57BLKs, and BDF1 Rats gained weight rapidly and significantly increase non fasting plasma glucose level within 8-12 weeks. These Rats showed impaired insulin secretion, mainly in early phase after glucose load and reduced insulin content in pancreatic islets.

METHODS

Collection and authentification of Plant Material

The whole plant of swertia chirayita and Panax ginseng are collected and authenticated by Dr k madhava chetty, department of botany, Sri Venkateswara University, Tirupathy.

Extraction of Plant to a coarse powder with the help of suitable grinder.

Cold Extraction (Methanol Extraction)

In this work the cold extraction process was done with the help of methanol. About 200gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750 ml of methanol. The

container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool.

Evaporation of Solvent

The filtrates (methanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vacuum desiccator for 7 days.

PHYTOCHEMICAL SCREENING

GROUPSP	TEST PERFORMED	OBSEVATIONS
Alkaloids	Mayer's test	Yellow colored precipitate
Carbohydrates	Molisch's test	Violet ring at the junction
Saponin	Froth test	1 cm layer of foam
Steroids	Salkowaski's test	Golden yellow color
Phenols	Ferric chloride test	Bluish black color
Tanins	Gelatin test	White precipitate
Flavonoid	Leaad acetate test	Yellow color precipitate

Animals

Healthy Swiss Rats (Wistar strain) weight about 40-60 g were kept in individual polyethylene cages and maintained standard condition (12 h dark and 12 h light circle; 25 ± 5°C; 40-60% humidity), and the animals were fed ad libitum with normal laboratory chow standard pellet diet, purchased from the Sanzyme pvt. Limited, Hyderabad, India. The animals were allowed to acclimatize for 5 days before commencing the experiments. All the studies were conducted in accordance with the Animal Ethical Committee.

Acute toxicity studies

The Acute oral toxicity test of the extracts was determined prior to the experimentation on animals according to the OECD (Organization for Economic Co-operation and Development) guidelines no 423. Female Albino Swiss Rats (40-60 g) were taken for the study and dosed once with 2000 mg/kg (*swertia chirayita*) 5000mg/kg (*panax ginseng*). The treated animals were monitored for 14 days to observe general clinical signs and symptoms as well as mortality. No mortality was observed till the end of the study revealing the 2000 mg/kg (*swertia*) and 5000mg/kg (*panax)* dose to be safe. Thus, 1/10 and 1/20 doses of 2000 mg/kg i.e. 200 mg/kg and 100 mg/kg (*swertia*) and 500mg/kg and 250mg/kg (*panax)* were chosen for subsequent experimentation.

Experimental procedure of diabetes induction

After 1 week of acclimatization, Rats of each strain were divided into control or GTG groups. Because the expected rates of GTG-induced obesity ranged from 40 to 80% depending on the mouse strain, a large number of Rats (12–20 Rats) were assigned to GTG groups with the aim of obtaining six to eight obese Rats per group. The Rats received an intra peritoneal administration of saline or GTG (Cat. No. 1045508; USP, Rockville, MD) at the following optimal doses: 0.8 g/kg for Rats groups, respectively. After 4 or 6 weeks, Rats that developed obesity (GTG-obese Rats) were selected for further studies on showing a greater weight gain compared with the average weight gain measured in the control Rats.

The development of obesity-induced diabetes was investigated in control and GTG-obese Rats of each strain (n = 6–8 per group) for 12–14 weeks. Body weights were measured every 2 or 4 weeks and the development of diabetes was monitored by measuring non fasting plasma glucose levels between 9:00 and 10:30 am.

Experimental Study Design of *swertia chirayita* for Diabetic screening

Diabetic rats were divided in to five groups with each group four animals.

- Group-I: Rats served as normal control group.
- Group-II: served as diabetic/disease control.
- Group-III: Diabetic rats treated with *swertia chirayita* at a dose 100 mg/kg.
- Group-IV: Diabetic rats treated with *swertia chirayita* at a dose of 200 mg/kg
- Group V: Diabetic rats treated with Metformin (standard drug) at 450mg/kg.

The treatment was given for 14days and blood samples were collected at different intervals.

Experimental Study Design of *Panax ginseng* for Diabetic screening

Diabetic rats were divided in to five groups with each group four animals.

- Group-I: Rats served as normal control group.
- Group-II: served as diabetic/disease control.
- Group-III: Diabetic rats treated with *Panax ginseng* at a dose 250 mg/kg.
- Group-IV: Diabetic rats treated with *Panax ginseng* at a dose of 500 mg/kg
- Group V: Diabetic rats treated with Metformin (standard drug) at 450mg/kg.

The treatment was given for 14days and blood samples were collected at different intervals.

Experimental Study Design of *swertia chirayat* and *Panax ginseng* for Diabetic screening

- Diabetic rats were divided in to five groups with each group four animals.
- Group-I: Rats served as normal control group.
- Group-II: served as diabetic/disease control.
- Group-III: Diabetic rats treated with *ME-SCPG* at a dose 250 mg/kg.
- Group-IV: Diabetic rats treated with *ME-SCPG* at a dose of 500 mg/kg
- Group V: Diabetic rats treated with Metformin (standard drug) at 450mg/kg

Collection of blood samples

Blood samples were collected from all the groups of animals at 0, 7, 15th day intervals.

EVALUTION PARAMETER

Glucose Method[23]: GOD/POD method

Principle

D-glucose + H_2O + O_2 glucose oxidase ⟶ (GOD) gluconic acid + H_2O_2

H_2O_2 + 4-AAP + Phenol peroxidase ⟶ (POD) Quinoneimine dye + H_2O

RESULTS

Swertia chirayita

%Yield value of Methanolic Extract from *swertia chirayita* was found to be **12.27%.**

Preliminary Phytochemical Screening

Investigation revealed the presence of steroid, Alkaloid, Tannins & Flavonoid in Methanolic Extract of *swertia chirayita.*

Table: Phytochemical screening.

Phytochemical	Results
Steroid	+
Alkaloid	+
Tannin	+
Carbohydrate	-
Phenol	-
Flavonoid	+
Saponin	-

(+) Present **(-)** Absent

Procedure

- Wavelength/filter : 505 nm (Hg 546 nm) / Green
- Temperature : 37^0 C / R.T.
- Light path : 1 cm
- Pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T)

Mix well and incubate at 37^{0}C for 10 min or at room temperature (25^{0}C) for 30 mins. Measure absorbances of the Standard (Abs.S) and Test Sample (Abs.T) compare these against the Blank within 60 mins.

Additional sequence	B (ml)	S (ml)	T (ml)
Glucose reagent L1	1.0	1.0	1.0
Distilled water	0.01	-	-
Glucose standard (s)	-	0.01	-
Sample	-	-	0.01

STATISTICAL ANALYSIS

All the values will be expressed as mean ±standard deviation (S.D). Statistical comparisons between different groups will be done by using one way analysis of variance. P value <0.05 will be considered as statistically significant.

Table: Effect of *swertia chirayita* extract on serum glucose levels (mg/dl) in diabetic rat.

Groups/Interval	0th Day	7th Day	15th Day
Normal	83.3±4.23	79.1±5.36	77.7±5.62
Diabetic control	283.8±5.01	286.4±12.4	300.3±8.64
MESC(100mg/kg)	293.1±9.83	152.9±6.91**	110.1±17.1**
MESC(200mg/kg)	280.5±42.4	85.5±7.20***	64.7±20.7***
Metformin(450mg/kg)	211.0±34.7	79.7±10.2***	68.3±2.4**

MESC-METHANOLIC EXTRACT OF *SWERTIA CHIRAYITA*

*All the values of mean ± SD; n=6; ** indicates p<0.01, *** indicates [a]p<0.001 vs diabetic control.*

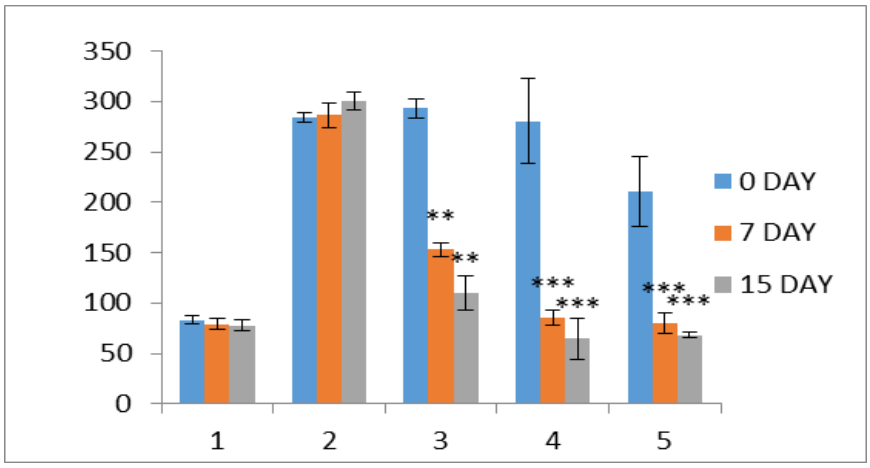

Fig. 3: Effect of *swertia chirayita* methanolic extract on serum glucose levels (mg/dl) in diabetic rats.
*All the values of mean ± SD; n=6; ** indicates p<0.01, *** indicates [a]p<0.001 vs. diabetic control.*

Panax ginseng

%Yield of Ethanolic Extract from Aerial Parts of *panax ginseng* was found to be **12.43%.**

Table: 7 Preliminary Phytochemical Screening
Glucose

Phytochemical	Results
Steroid	-
Alkaloid	+++
Tannin	+
Carbohydrate	++
Phenol	-
Flavonoid	+
Saponin	-

(+) Present. **(-)** Absent

Table: Effect of *Panax ginseng* (EPG) on serum glucose levels (mg/dl) in diabetic rats.

Groups/Interval	0th Day	7th Day	15th Day
Normal	65.48±0.35	67.89±0.18	69.96±0.78
Diabetic control	185.65±0.64	188.64±0.65	189.67±0.95
EEPG (250mg/kg)	164.48±0.48	159.74±0.74	145.86±0.64
EEPG (500mg/kg)	142.65±0.46	138.65±0.71	135.75±0.48
Metformin (450mg/kg)	120.34±0.75	115.75±0.48	111.34±0.69

All the values of mean ± SD; n=3.

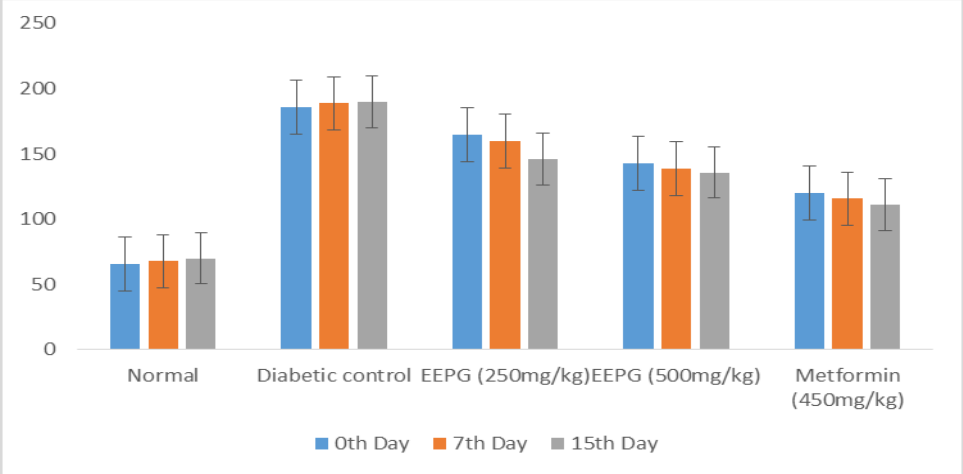

FIG: Effect of *Panax ginseng (EEPG)* on serum glucose levels (mg/dl) in diabetic rats.

MIXED EXTRACTS OF *SWERTIA CHIRAYITA* AND *PANAX GINSENG*

Table: Effect of *mixed* extract on serum glucose levels (mg/dl) in diabetic rats.

Groups/Interval	0th Day	7th Day	15th Day
Normal	81.3±4.1	72.0±5.6	74.7±7.9
Diabetic control	269.0±12.6	277.5±12.7	306.0±10.2
ME-SCPG (250mg/kg)	260.1±14.1	152.2±15.2**	110.0±11.2**
ME-SCPG (500mg/kg)	270.7±14.2	97.8±11.1***	72.6±4.1***
Metformin(450mg/kg)	260.1±16.8	90.2±9.5***	71.2±7.1***

*All the values of mean±SD; n=6; ** indicates p<0.01, *** indicates [a]p<0.001 vs diabetic control.*

ME-SCPG=MIXED EXTRACTS OF *SWERTIA CHIRAYITA* AND *PANAX GINSENG*

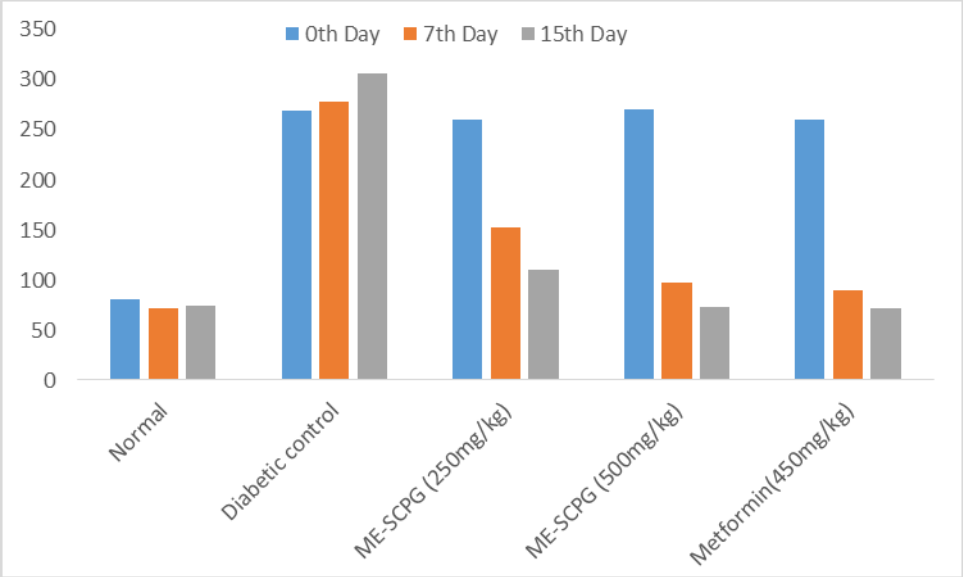

FIG:. Effect of *mixed* extract on serum glucose levels (mg/dl) in diabetic rats.

DISCUSSION

This study aimed to evaluate the synergestic antidiabetic activity of *sertia chirayata* and *panax ginseng.* Leaves were used to prepare the extract of *swertia chirayata* and *panax ginseng*. The physical appearance of the extract was dark greenish in color, non-sticky in nature and odourless.

Phytochemical screening of methanolic extract revealed the presence of phytochemicals in *swertia chirayata* such as steroids, alkaloids, tannins and flavonoids, and in *panax ginseng* alkaloids, carbohydrates, flavonoids and tannins.

Gold thioglucose method (GTG) was used to induce diabetes in rats. The rats received an intraperitonial administration of GTG at the optimal dose of 0.8gm/kg. After 2 weeks rats develop diabetes. Gold thioglucose causes hypertrophy of pancreatic islets associated with degranulation of β-cells. It causes hyperinsulinemia, hyperglycemia, glucosuria and depressed insulin sensitivity.

Therefore the development of diabetes was monitored by measuring non fasting plasma glucose levels between 9-10:30 am. Which was defined as diabetes, because non fasting plasma glucose levels values were higher than 300mg/dl.

The extract of the plants *swertia chirayata* and *panax ginseng* was administered orally at the dose of 100, 200 mg/kg (*swertia chiarayata)*, 250, 500 mg/kg (*panax ginseng*) and mixed extract the dose of 500mg/kg for 14 days.

In disease treated animals diabetes was reduced by α-amylase inhibition, decrease in cholesterol, triglycerides and blood glucose levels in *swertia chirayata*. And in *panax ginseng* it worked by protecting pancreatic islets and inhibition of β-cells. It also modulates the production or secretion of insulin, glucose metabolism and uptake and obesity reduction.

Gold thioglucose induced diabetes treated at the dose of 200mg/kg when compared to 100mg/kg of *swertia chirayata* shows more anti diabetic activity and in case of *panax ginseng* the higher dose 500mg/kg shows more antidiabetic activity than the lower dose 250 mg/kg.

Mixed extract of *swertia chirayata* and *panax ginseng* showed synergistic effect at the higher dose 500mg/kg when compared to lower dose 250mg/kg with the reference drug metformin 450mg/kg.

CONCLUSION

- *Swertia chirayita* have many medicinal properties useful to cure anorexia, ulcers etc. *swertia chirayita*

have different medicinal properties due to its active phytochemical constituents and able to treat diabetes & diabetics complications.

- In the literature review the acute toxicity studies (OECD guidelines no. 423) shows methanolic extract of *swertia chirayita* is safe to use up to the dose of 2000mg/kg.
- The methanolic extract of *swertia chirayita* was found to be in dose dependent way against GTG induced diabetes in rats. The reduction of the elevated blood glucose levels in diabetic rats on treatment with the extract at two different concentrations confirmed that methanolic extract of *swertia chirayita* possess antidiabetic activity & has shown significant effect when compared to GTG administration.
- The acute toxicity studies revealed that the ethanolic extracts were safe for oral administration is safe upto 5000mg/kg. and the results for anti diabetic activity of *Panax ginseng* against GTG induction is found to be dose dependent and gave positive results.
- It needs comprehensive investigations for developing a safe and effective herbal drug. Further research is required to isolate the biomolecules responsible for the antidiabetic and antidiabetic complications.

ACKNOWLEDGEMENT

The research was supported by Bhaskar Pharmacy College. I am thankful to almighty Allah for showering his never ending bounties. I am also thankful to my parents for their love and support. I am extremely thankful to my guide and principal D r. A. Srinivasa Rao for his assistance this research.

REFERENCES

1. Evaluation of antioxidant, antibacterial and antidiabetic potential of two traditional medicinal plants of india*: swertia cordata and swertia chirayita* by Priyanka Roy, Fatima abdulsalam, Dk panday, Aniruddha Bhattacharjee, Naveen Reddy eruvaram, Tabarak malik, Pharmacognosy research, 2015; 7(suppl 1): s57.
2. A review of *swertia chirayita* (Gentianaceae) as a traditional medicinal plan by vijay kumar, Johannes van staden, Frontiers in pharmacology, 2016; 6: 308.
3. *Ginseng* and diabetes: the evidences from in vitro animal and human studies by Hai-dan yuan, Jung Tae kim, Sung hoon kim, sung hung chung, Journal of ginseng, 2012; 36(1): 27.
4. Antidiabetic effect of *ginseng* in human and rodents by Zhan xiang wang, Hongji Zhang J metabolic synd1, 2012; (106): 2167-0943. 1000106.
5. Antidiabetic activity of ethanolic extract of *swertia chirayita* Buch ham by Arya renu, Kumar sunil, Kumar dinesh, malika ajay, kumar tarun, Int Res J Pharm, 2011; 2(1): 230-232.
6. Antidiabetic effect of whole plant extract an fraction of *swertia chirayita* Buch ham by R arya, SK Shara, S Singh, Planta medica, 2011; 77(05): P-138.
7. Obesity induced diabetes in mouse strains treated with Gold thioglucose: a novel animal model for studying β- cell dysfunction. By Hiroshi karawasa, Kiyosumi takaishi, Yoshihiro Kumagae, 06 September 2012- Wiley online library.
8. Potential analgesicand anti inflammatory activities of *panax ginseng* head butanolic fraction in animals by Je- Hyuk Lee- Coon –sil Jeong, December 2008 pages 3749-3752 Science direct.
9. Invite an in vivo antioxidant effect of ethanolic extract of *swertia chirayita* by Yue chen, Bo huang, Jingsheng He, Li Han, Yichaoo zhae, Youwei Wang, Journal of ethanopharmacology, 2011; 136(2): 309-35.