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Evaluation of Antidiabetic Activity of Catharanthus Pusillus on Streptozotocin-Induced Diabetic Albino Wistar Rats

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Abstract:

The study aims to evaluate the antidiabetic activity of Catharanthus pusillus in streptozotocin-induced diabetic albino Wistar rats. Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia. Despite various synthetic drugs available for its management, natural plant-based remedies are gaining attention for their efficacy and minimal side effects. Catharanthus pusillus has been traditionally used in folk medicine for treating diabetes. This study investigates its efficacy and potential mechanisms in controlling blood glucose levels.

Keywords: Catharanthus pusillus, Antidiabetic activity, Streptozotocin, Diabetes mellitus, Albino Wistar rats.

INTRODUCTION

The complicated and diverse group of disorders known as diabetes mellitus affects how fat, protein, and carbs are metabolized. It is caused by insufficient or absent insulin secretion or by a decrease in the tissue's sensitivity to insulin. There are currently several medications available to lower hyperglycemia in people with diabetes mellitus, including biguanides and sulfonylureas. In order to overcome the side effects of these medications and solve the problems associated with diabetes, a new class of compounds must be found [1].

Insulin is a hormone produced and secreted by the pancreas, a vital organ located behind the stomach. The secretion of insulin plays a crucial role in regulating blood sugar (glucose) levels in the body. The pancreas contains clusters of cells called the Islets of Langerhans. Within these islets, beta cells are responsible for producing and secreting insulin. When blood glucose levels rise, beta cells are stimulated to release insulin.

Catharanthus Pusillus

Catharanthus pusillus is a small flowering plant belonging to the Apocynaceae family. It is commonly known as "small periwinkle" or "Madagascar periwinkle," similar to its more famous relative, Catharanthus roseus,

which is also known as the Madagascar periwinkle. These two plants share certain characteristics, but they are distinct species. Catharanthus pusillus is native to Madagascar, just like Catharanthus roseus.

Catharanthus pusillus:

1. Microscopic Analysis:

- Leaf Structure: Microscopic examination involves studying the leaf's cellular structure, including epidermal cells, stomata, trichomes, and vascular bundles.
- Identification of Specific Cells: Identifying specialized cells or structures unique to Catharanthus pusillus.

2. Macroscopic Analysis:

- Overall Plant Characteristics: This involves observing the plant's size, shape, color, and any distinctive features visible to the naked eye.
- Flower and Fruit Characteristics: Examining the flowers and fruits for specific characteristics that aid in identification.

3. Physiological Analysis:

- Metabolic Pathways: Investigating the plant's biochemical pathways, particularly those related to the production of secondary metabolites.
- Environmental Adaptations: Understanding how Catharanthus pusillus responds to various environmental conditions.

Collection of Plant:

1. Selection:

- Identification: Ensuring accurate identification of Catharanthus pusillus.
- Healthy Specimens: Choosing healthy plants for collection.

2. Timing:

- Seasonal Considerations: Collecting plants at the appropriate time of the year when their active constituents are at peak levels.

3. Parts Collected:

- Leaves, Roots, or Flowers: Depending on the specific plant, the relevant plant parts are collected.

Extraction Process:

1. Harvesting:

- Cleaning: Removing any foreign matter from the collected plant material.
- Drying: Allowing the plant material to dry, preserving it for further processing.

2. Extraction Methods:

- Solvent Extraction: Using solvents like ethanol or methanol to extract bioactive compounds.
- Steam Distillation: For essential oils.
- Cold Press Extraction: For oils from seeds.

3. Concentration and Purification:

- Concentration: Removing excess solvent to obtain a concentrated extract.
- Purification: Refining the extract through additional processes if needed.

4. Analysis:

- Chemical Analysis: Identifying and quantifying the chemical components of the extract.
- Quality Control: Ensuring the extract meets quality standards.

Please note that specific details may vary based on the plant species and the intended use of the extracted compounds. For up-to-date and specific information on Catharanthus pusillus, you may need to refer

to recent scientific literature or consult with experts in the field.

Catharanthus pusillus

Materials and Methods

Plant Material and Preparation of Extract

Catharanthus pusillus was collected, authenticated, and processed. The whole plant was dried, powdered, and extracted with ethanol using a Soxhlet apparatus. The extract was concentrated and stored at 4°C until further use.

Animals

Albino Wistar rats (180-220 g) were obtained and acclimatized under standard laboratory conditions. They were provided with standard chow and water ad libitum. All experimental procedures were conducted following ethical guidelines.

Pharmacological activity

Chemicals:
Streptozotocin was purchased from Cipla, Hyderabad. All other chemicals used for this study were analytical grade.

Induction of diabetes
The rats were fasted for 18 h prior to the experiment with water ad libitum. The rats were injected intraperitoneally with nicotinamide 100mg/kg. After 15 minutes streptpozotocin (STZ) were administered. STZ dissolved in citrate buffer at a dose of 55 mg/kg body weight. Animals were treated with 10%glucose to combat the early phase of hypoglycemia. Blood samples were collected after 72 hours of STZ treatment and the induction of diabetes mellitus was confirmed by estimation of fasting blood glucose levels (FBG). Only those rats with blood glucose levels ≥250 mg/dl were included in the study (Day 0).

Grouping of animals

After the induction of diabetes, the rats were grouped in to five different groups of each containing six animals.

Group I: (control group): Control rats receiving vehicle (0.5 ml distilled water /rat/day) orally for 60 days.

Group II: (diabetic control): Diabetic rats receiving vehicle (0.5 ml distilled water /rat/day) orally for 60 days.

Group III: (standard group): Diabetic rats receiving Glibenclamide standard drug (5mg/kg b.wt./day suspended in distilled water) orally for 60 days.

Group IV: (low dose of plant extract): Diabetic rats receiving Catharanthus pusillus extract (250 mg/kg b.wt./day suspended in distilled water) orally for 60 days

Group V: (high dose of plant extract): Diabetic rats receiving Catharanthus pusillus extract (500 mg/kg b.wt./day suspended in distilled water) orally for 12 weeks.

Treatment with drugs was started after 72 hours of STZ treatment (i.e. Day 1) and was continued for 12 weeks. All drugs were given orally as a single oral dose. Blood glucose was measured before starting the treatment (day0) and 4 weekly thereafter up to the end of the treatment and estimated fasting blood glucose by glucose-oxidase-peroxidase (GOD-POD) method. Groups that received plant extracts were compared with diabetic control group, and standard group.

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 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 fore finger 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 hyperglycemia by various mechanisms. Ether increases blood glucose levels by glycogenolysis in liver (155).

Methods of estimation

ESTIMATION OF BLOOD GLUCOSE

In this study the enzymatic; glucose oxidase-perixodase (GOD – POD) method (156) 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.

Reagents:

Reagent-1 : Glucose reagent Glucose oxidase, Peroxidase, 4-Amino antipyrine, Buffer and Stabilizers.

Reagent-2 : Glucose diluent Diluent, Phenol Preservative

Reagent-3 : Glucose standard Dextrose, Benzoic acid

Store Reagent-1 at 2-80C 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 2-80C.

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 (Semi Auto Analyzer for Biochemical parameters)

Procedure:

Pipette into tubes marked	Blank	Standard	Sample
Serum	-	-	10 µl
Glucose standard	-	10 µl	-
Working glucose reagent	1 ml	1 ml	1 ml
Mix well. Incubate at 37 0C for 10 minutes.			

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

Collection and handling: Serum should be separated within 30minutes as the rate of glycolysis is approximately 7mg/hour at room temperature. Serum is stable for 8hrs at room temperature and for up to 72 hrs at 2-8 o C.

Plant extraction

Formula: [weight of extract / weight of powdered drug] X 100

The plant material was extracted by soxhlet apparatus and the percentage yield calculated by the following formula was found to be 17.38 %.

Solubility determination

Table 1: Solubility determination of extract

S. No.	Solvent	Solubility of methanolic extract
1.	Water	Soluble
2.	Acetone	Insoluble
3.	Chloroform	Partial soluble
4.	Methanol	Soluble
5.	Petroleum ether	Partial soluble
6.	Ethylacetate	Partial soluble
7.	DMSO	Soluble

Phytochemical testing

Table 2: Phytochemical testing of extract

S. No.	Experiment	Presence or absence of phytochemical test
1.	**Alkaloids**	
1.1	Mayer's reagent test	Present
1.2	Wagner's reagent test	Present
1.3	Hager's reagent test	Present
2.	**Carbohydrates**	
2.1	Molish's test	Present
2.2	Fehling's test	Present
2.3	Benedict's test	Present
2.4	Barfoed's test	Absent
3	**Proteins and Amino Acids**	
3.1	Biuret test	Present
4.	**Flavonoids**	
4.1	Alkaline reagent test	Present
4.2	Lead Acetate test	Present
5.	**Glycoside**	
5.1	Borntrager test	Absent
5.2	Legal's test	Absent
5.3	Killer-Killiani test	Present
6.	**Tannin and Phenolic Compounds**	
6.1	Ferric Chloride test	Present
6.2	Lead Acetate test	Present
6.3	Gelatin test	Absent
7.	**Saponin**	
7.1	Foam test	Absent
8.	**Test for Triterpenoids and Steroids**	
8.1	Salkowski's test	Absent
8.2	Libbermann-Burchard's test	Present

Acute oral toxicity

The acute oral toxicity study was carried out according to OECD 423 guidelines. Four ranges of dose were ussed for toxicity studies, i.e 5mg/Kg, 50 mg/Kg, 300 mg/Kg, 2000 mg/Kg. animals were observed individually for next 4 hours after dosing for the presence of mortality during this period and 72 hours after sample administration.

Table 3: Acute oral toxicity of extract

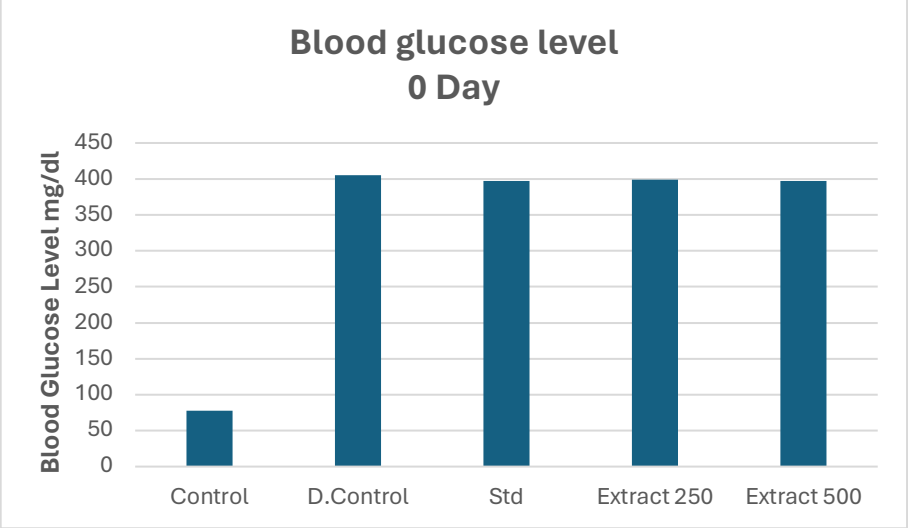
S. No.	Dose	Lethality	Mortality
1.	5 mg/Kg	0/3	Not observed
2.	5 mg/Kg	0/3	Not observed
3.	50 mg/Kg	0/3	Not observed
4.	50 mg/Kg	0/3	Not observed
5.	300 mg/Kg	0/3	Not observed
6.	300 mg/Kg	0/3	Not observed
7.	2000 mg/Kg	0/3	Not observed
8.	2000 mg/Kg	0/3	Not observed

*0/3- zero animal dead out of three animals

Estimation of Blood glucose

Table 4: Effect of plant extract on glucose during 0 day study of normal and Streptozotocin induced diabetic rats. (N=6)

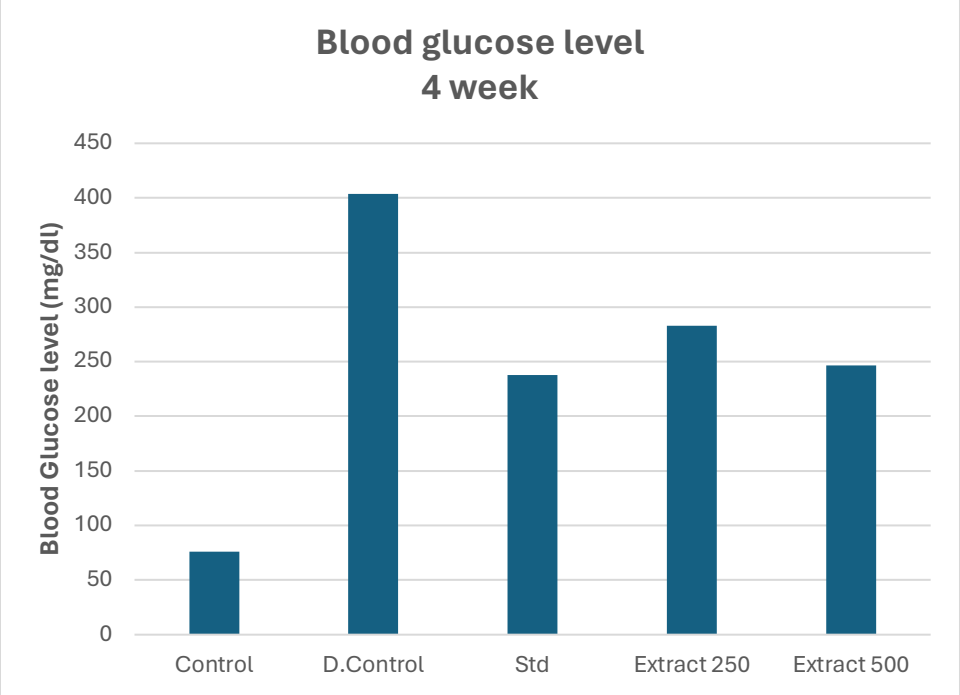
Groups	Glucose (mg/dl)						
	R1	R2	R3	R4	R5	R6	Mean±SEM
Control	75	82	83	72	69	85	77.66667±6.562
D.Control	389	402	412	413	406	411	405.5±9.093
Standard	395	387	392	403	406	399	397±7.071
Extract (250 mg/Kg)	398	406	403	385	406	394	398.6667±8.189
Extract (500 mg/Kg)	408	393	397	401	388	397	397.3333±6.831



Graph 1: Effect of plant extract on glucose during 0 day study of normal and Streptozotocin induced diabetic rats.

Table 5: Effect of plant extract on glucose during 4 week study of normal and Streptozotocin induced diabetic rats. (N=6)

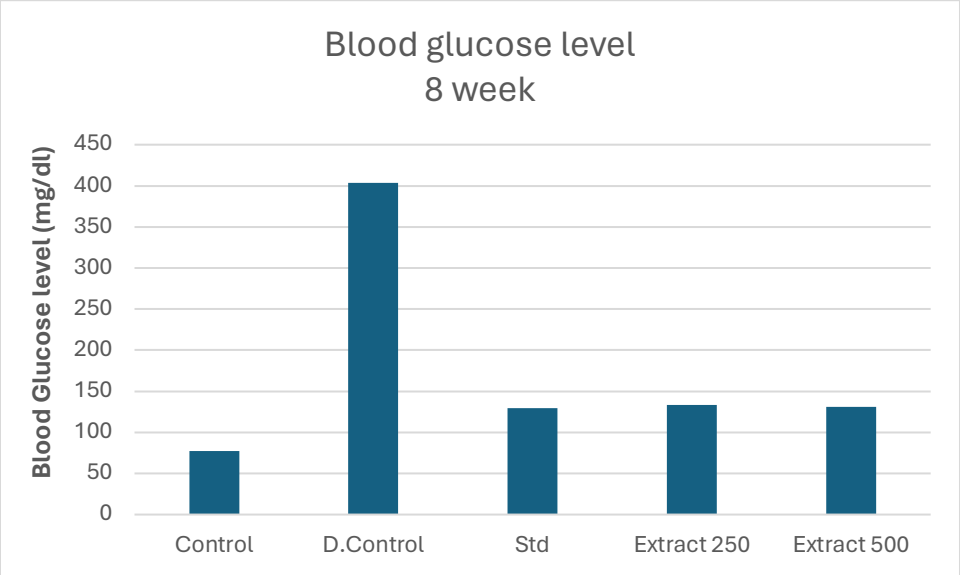
Groups	Glucose (mg/dl)						
	R1	R2	R3	R4	R5	R6	Mean±SEM
Control	72	80	69	75	80	79	75.83333±4.622
D.Control	412	387	399	402	407	416	403.8333±10.342
Standard	249	247	234	240	232	223	237.5±9.813
Extract (250 mg/Kg)	280	267	285	279	296	289	282.6667±9.892
Extract (500 mg/Kg)	256	248	243	246	233	253	246.5±8.117



Graph 2: Effect of plant extract on glucose during 4 week study of normal and Streptozotocin induced diabetic rats.

Table 6: Effect of plant extract on glucose during 8 week study of normal and Streptozotocin induced diabetic rats. (N=6)

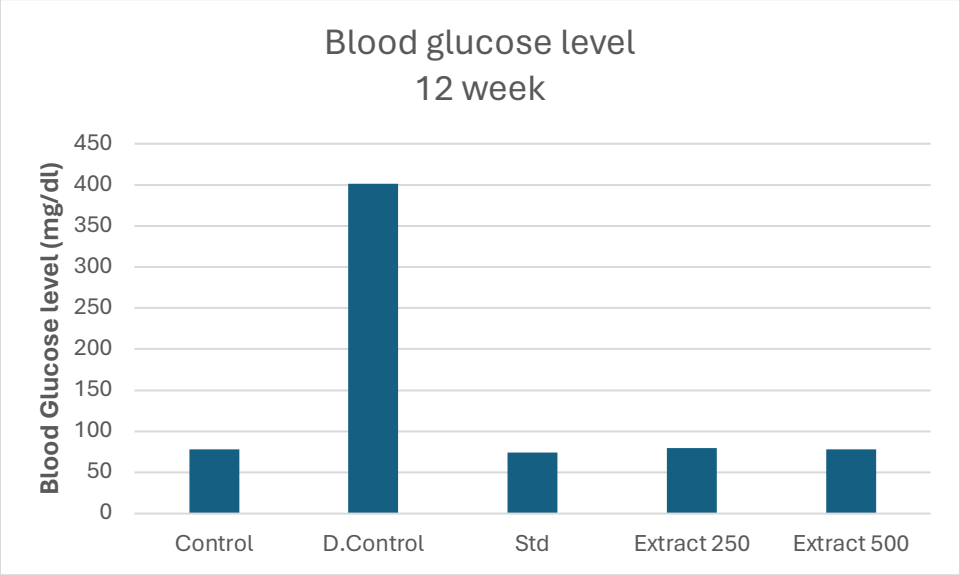
Groups	Glucose (mg/dl)						
	R1	R2	R3	R4	R5	R6	Mean±SEM
Control	83	74	75	89	64	81	77.66667±8.664
D.Control	394	421	398	396	415	395	403.1667±11.7203
Standard	141	123	112	135	137	127	129.1667±10.703
Extract (250 mg/Kg)	126	138	133	145	140	120	133.6667±9.309
Extract (500 mg/Kg)	126	136	127	132	135	128	130.6667±4.273



Graph 3: Effect of plant extract on glucose during 8 week study of normal and Streptozotocin induced diabetic rats.

Table 7: Effect of plant extract on glucose during 12 week study of normal and Streptozotocin induced diabetic rats. (N=6)

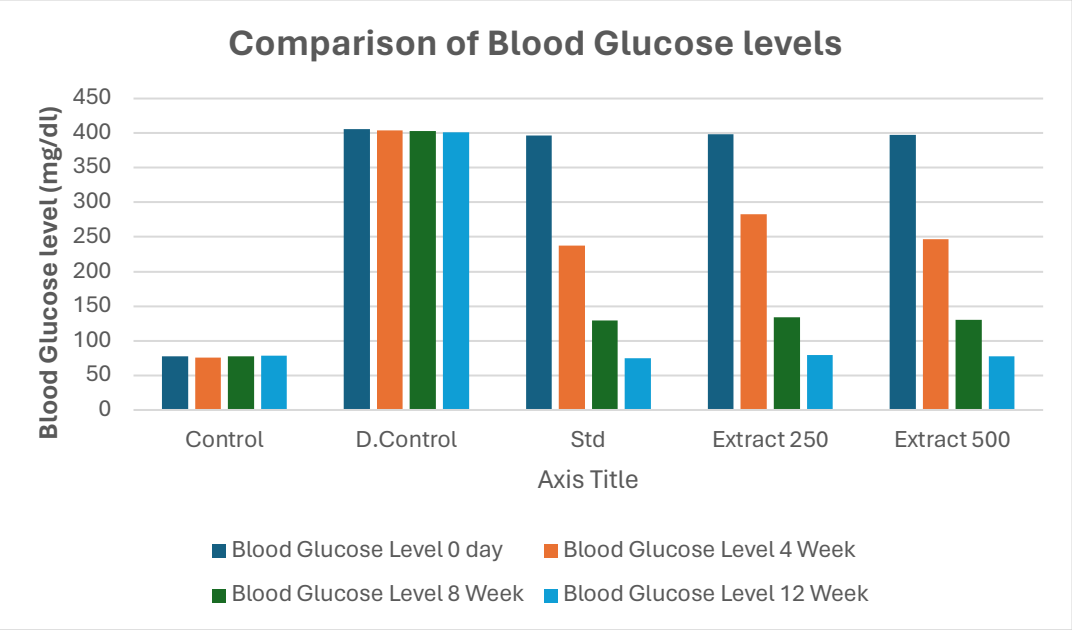
Groups	Glucose (mg/dl)						
	R1	R2	R3	R4	R5	R6	Mean±SEM
Control	78	81	79	82	76	73	78.16667±3.311
D.Control	392	397	416	404	389	409	401.1667±10.381
Standard	70	69	78	76	81	72	74.33333±4.7609
Extract (250 mg/Kg)	69	85	86	79	83	76	79.66667±6.439
Extract (500 mg/Kg)	82	68	76	75	79	87	77.83333±6.493



Graph 4: Effect of plant extract on glucose during 12 week study of normal and Streptozotocin induced diabetic rats.

Table 8: Comparison of blood glucose values during different week intervals (N=6)

Groups	Glucose (mg/dl)			
	0 Day	4 week	8 week	12 week
Control	77.666±6.562	75.83333±4.622	77.66667±8.664	78.16667±3.311
D.Control	415.6667±21.657	403.8333±10.342	403.1667±11.7203	401.1667±10.381
Standard	393.6667±25.009	237.5±9.813	129.1667±10.703	74.33333±4.7609
Extract (250 mg/Kg)	396±21.137	282.6667±9.892	133.6667±9.309	79.66667±6.439
Extract (500 mg/Kg)	393±28.579	246.5±8.117	130.6667±4.273	77.83333±6.493



Graph 5: Comparison of blood glucose values during different week intervals

In the present study, diabetes mellitus was induced in rats through a STZ injection that causes the destruction of β-cells of islets of Langerhans, As streptozotocin (STZ) is used to induce diabetes mellitus in albino Wistar rats, a poly ADP ribose inhibitor, nicotinamide was administered after 15 minutes of STZ administration to offer partial protection against the action of STZ in rats. So in the present study we used the Streptozotocin – nicotinamide model to prevent the excessive damage to the pancreas of diabetic rats. The excessive catabolism of protein to provide amino acids for gluconeogenesis during insulin deficiency results in muscle wasting and weight loss in diabetic untreated rats (Rajkumar L et al., 1991). Hyperphagia was observed in diabetic rats, increased food intake due to reduction of insulin and leptin hormone levels and which are effects on the central nervous system (CNS). In this study, treatment with the extracts significantly reduced the elevated blood glucose levels in STZ induced diabetic rats (Table 6.8). The C.pusillus leaf extract shown to have lesser antihyperglycemic activity compared to standard gliclazide in STZ induced diabetic rats.

Conclusion

Phytochemicals that are present in the diet, associated with health benefits include

alkaloids, glycosides, flavanoids, phytosterols, anthocyanins, tannins, and terpenoids. Their bioactivity has been, to some extent associated to their antioxidant properties which are involved in the onset of development of the chronic degenerative diseases (Espin J C et al., 2007).

Diabetes mellitus is a progressive degenerative disease constituting a complex syndrome initially characterized by disturbance in glucose homeostasis and is associated with many complications such as diabetic microvascular complications like retinopathy, nephropathy, neuropathy cardiomyopathy and macrovascular complications like hypertention.

Diabetic retinopathy, nephropathy, are common complications in patients with either type I or type II diabetes mellitus, has long been recognized to cause severe morbidity and mortality (Amir Farshchi et al., 2014)

Many herbs and herbal formulations were evaluated scientifically and available in the market for the treatment of diabetes and its complications (ManishaM et al., 2007). Herbal drugs are found to have less or no side effects.

Hence in the present study the selected plant extract of Catharanthus pusillus was evaluated for their beneficial role in diabetes. Diabetes was induced in experimental animals by a single injection of streptozotocin (STZ 60 mg/kg; i.p. with nicotinamide (100mg/kg) i.p).

All the selected doses of the extracts and standard gliclazide were administered to the respective groups of the animals (as described in chapter 5) for twelve weeks once daily. The blood glucose was estimated at the end of every 4 week interval of 12 week study period.

Extract of Catharanthus pusillus leaf was found to have lesser antihyperglycemic activity compared to standard gliclazide in STZ induced diabetic rats.

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