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In-Vitro Models for the Investigation of Antidiabetic activity for the treatment of Diabetes mellitus type-1

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Abstract : There is prevalence of Diabetes mellitus type-1 in the country as well as worldwide. The available treatment for Diabetes Mellitus type-1 is Insulin treatment given i.m which causes various symptoms like phelebitis, pain at the site of injection, Severe hypoglycemia which leads to increased risk of heart disorders, kidney complication, liver complication. Various drug used for the therapy like glargine increased risk of breast cancer. However, No single monotherapy like *Digitalis* is available. Current therapy includes Insulin along with it oral hypoglycemic agent and immunosuppressant agent has to be taken. The objective of the study is to determine drug which lowers down serum glucose, and reduces all the micro and macro complication which causes mortality in the Diabetic patient. For this purpose various invitro models were performed like "Antioxidant model", "Hypoglycemic Model". By performing the experiment results were obtained like 0.69 ± 0.01, 0.87 ± 0.05 respectively. Results of hypoglycemic model was found to be 75±0.005 and 83± 0.001 respectively. The suspension of *digitalis purpurea* found to have better hypoglycemic and antioxidant property compared to that of standard.
Keywords : Diabetes mellitus type-1, *Digitalis Purpurea*, In-vitro models.

Introduction and Experimental:

Diabetes Mellitus is a major endocrine disorder characterized by elevated blood glucose level which results due to absence of pancreatic insulin secretion Or loss of insulin action.[1] It is considered to be due to the destruction of beta cell of pancreas.[1] Diabetes is nowadays considered as global problem affecting nearly 10%of the total population all over the world.[1] According to the WHO report the number of diabetic cases were 171 million in 2000 and which has increased up to 365 million.[1]

Type-1 DM is also considered as insulin dependent diabetes mellitus which is mostly seen in childhood. It is considered to be due to the auto destruction of the beta cell of pancreas [2]. Due to which there is loss of insulin secretion and its action [2]. The only efficient therapy available for the disease is insulin injection of

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about 5U/6U is given to the patient daily by the route of i.m which has several disadvantage of pain, phlebitis, pain at the site of injection.[2] It also has drawback which is due to the hypoglycemia post meals and difficulty to manage basal glucose and stable glucose level which leads to fatigue & generation of other symptoms. [2]There are different types of Diabetes mellitus like: [3]

- Autoimmune Diabetes mellitus
- Idiopathic Diabetes Mellitus.

Type-1 Dm:

Juvenile diabetes is characterized by beta cell destruction caused by an autoimmune process usually leading to loss of insulin release or leading to insulin resistance.[3]Type-1 DM is characterized by the presence of Anti-glutamic acid decarboxylase, islet cell or insulin antibodies which are considered as marker for the destruction of beta cell. [3]Insulin therapy is essential to maintain normal glucose level for patient suffering from Type-1 DM.[3]

Various Factor For Type-1 DM: genetic factor, enviournmantal factor, viral factors and other factor like intestinal microbiota, vaccines, hygiene, toxin& chemical compound, beta cell stress. Post translational modification etc.[4]

Genetic Factor:

Different 6 genes at six different loci had been lead responsible for the disease. The HLA genes encode highly polymorphic proteins, which are essential in self-immune recognition.[5] The HLA gene (class-1 mol) are expressed & present as intracellular antigen to CD8+T-cell. [5]Class II molecule compound are expressed mainly on APC, DC, macrophages, B-lymphocyte &thymus epithelium.[5]

Available treatment:

Insulin and it's novel formulation

Insulin therapy:

Various doses of insulin in the unit of 5U/6U per day is given to the patient.it is normally given as therapy to diabetic patient along with it adjunctive drug therapy is also given as insulin resistance also plays role in diabetes mellitus type-1.[8]

RAPID	Humalog or Lispro	< 15 min	60-90 min	3-5 hrs	• Inject 10-15 min before mealtime • Typically used in conjunction with longer-acting insulin.
	Novolog or Aspart	< 15 min	60-120 min	3-5 hrs	
	Apidra or Glulisine	< 15 min	60-90 min	1-2.5 hrs	
SHORT	Regular (R) Humulin, Actrapid or Novolin	30-60 min	2-5 hrs	6-8 hrs	• Inject at least 20-30 minutes before mealtime
	Velosulin	30-60 min	2-3 hrs	2-3 hrs	
INTERMEDIATE	NPH (N)	1-2 hrs	4-12 hrs	18-24 hrs	• Commonly used twice daily • Often combined with rapid- or short-acting insulin
	Lente (L)	1-2.5 hrs	3-10 hrs	18-24 hrs	
LONG	Ultralente (U)	30 min- 3 hrs	10-20 hrs	20-36 hrs	• Covers insulin needs for 24 hrs • If needed, often combined with rapid- or short-acting insulin
	Lantus or Glargine	1-1.5 hrs	No Peak	20-24 hrs	
	Levemir or Detemir	1-2 hrs	6-8 hrs	Up to 24 hrs	
PRE-MIXED	Humulin 70/30	30 min	2-4 hrs	14-24 hrs	• Combination of intermediate- and short-acting insulin • Commonly used twice daily before mealtime
	Novolin 70/30	30 min	2-12 hrs	Up to 24 hrs	
	Novolog 70/30	10-20 min	1-4 hrs	Up to 24 hrs	
	Humulin 50/50	30 min	2-5 hrs	18-24 hrs	
	Humalog 75/25	15 min	30 min-2.5 hrs	16-20 hrs	

[Fig 1: Table of different forms of insulin with its duration of action and its recommended use]

Insulin pen : it is the most better and convenient way of administrating insulin. There is built-in dial that allow us to determine the exact amount which needs to be delivered. It is mostly used when pre-mixed type of insulin is to be injected. [8]

Insulin pump : it is a small device like size of pager which can be attached to the belt or pocket near to the abdomen of the patient. They consist of small reservoir of insulin attached to a tube and followed by attachment to the cathereter.By which slowly administration of insulin takes place. It mimics the action of insulin and releases insulin according to the level of glucose.[8]

Insulin Patch : it is the formulation which is under development. As absorption of insulin is very difficult from the skin. The patch is designed to slowly release insulin.[8]

Insulin inhalers : They are similar to that of the asthma pump. It delivers dry powder of insulin directly into the bloodstream. It delivers powdered insulin through lungs. Drawback of the therapy is only 10% of the drug reaches the lungs or systemic circulation. It has been approved for use in U.S.A [8]

Adjunctive therapy: [9]

- **Biguanides**
- **Sulphonyl urea**
- **Thaizolindiones**
- **SGL2-Inhibitors**
- **Amylin analogues**
- **Incretin based analogues**
- **DPP4 inhibitors**
- **S.c Insulin infusion**

*Digitalis purpurea :*Digitalis purpurea (foxglove, common foxglove, purple foxglove or lady's glove) is a species of flowering plant in the plantain family Plantaginaceae, native to and widespread throughout most of temperate Europe.[10]

(Fig 2: Digitalis purpurea plant (common foxglove)

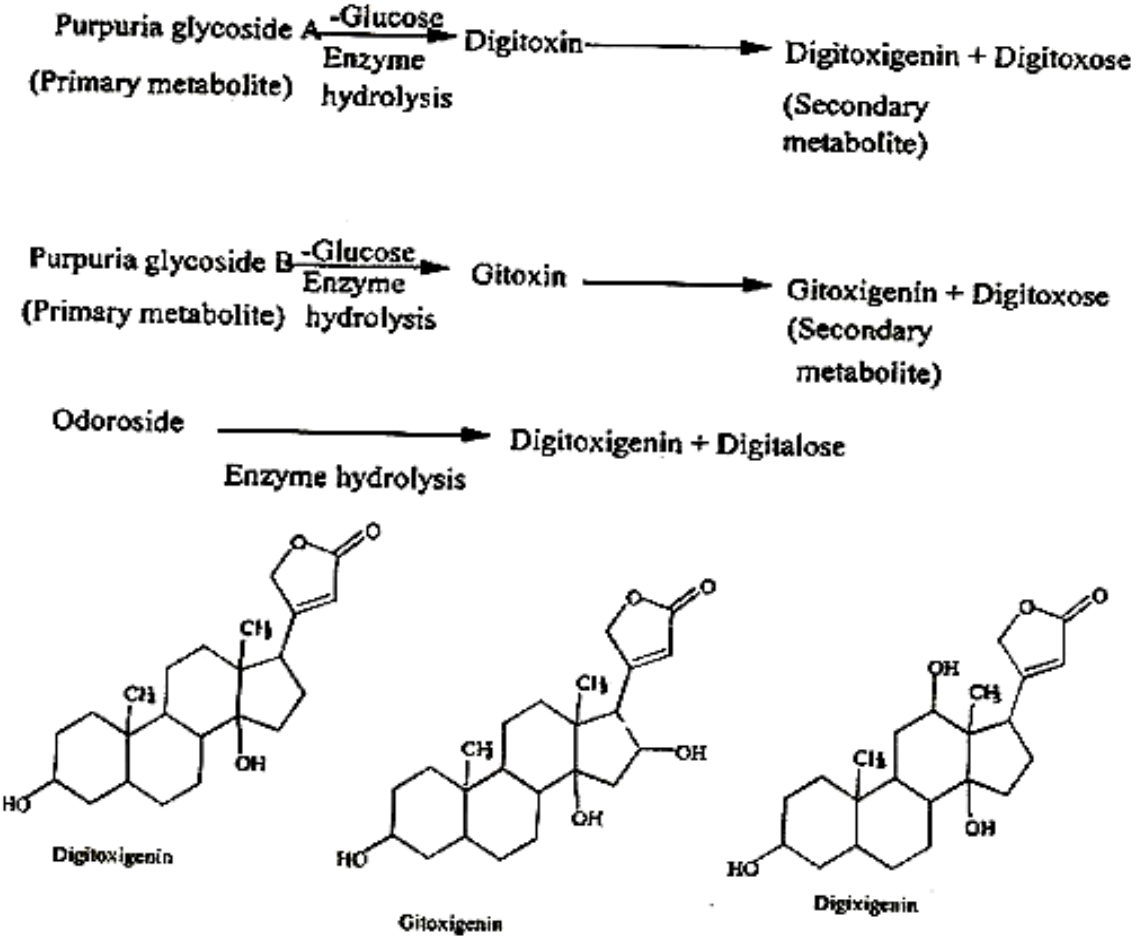
Morphological characters

The flowering stem develops in the second year, typically 1–2 m (3.3–6.6 ft.) tall, sometimes longer. The flowers are arranged in a showy, terminal, elongated cluster, and each flower is tubular and pendent. The flowers are typically purple, but some plants, especially those under cultivation, may be pink, rose, yellow, or white. The inside surface of the flower tube is heavily spotted. The flowering period is early summer, sometimes with additional flower stems developing later in the season. The plant is frequented by bees, which climb right inside the flower tube to gain the nectar within. [10]

The fruit is a capsule which splits open at maturity to release the numerous tiny 0.1-0.2 mm seeds. [10]

Digitalis purpurea contains 35 glycosides:[11]

1. The primary glycosides are purpurea glycosides A and B.
2. It also contains Odoroside H, glucogitaloxin.
3. Verodoxin and glucoverodoxin.
4. The digitoxigenin, Digitoxin, Gitoxigenin, gitaloxin are also important medicinal compounds. They are also called secondary glycosides.
5. They contain anthraquinones derivatives like digitolutin, methoxy-2 methyl anthraquinones, etc.



Digoxin which act as major chemical constituents found to have better heart protective properties. Foxglove contain chlorogenic acid and other chemical constituent which have found to show hypoglycemic activity. Mechanism of action is not clear .Chlorogenic acid shows to increase glucose uptake through activation of AMPK. It is found to act by decreasing hepatic glucose production and fatty acid synthesis.It increased glucose metabolism.[11]

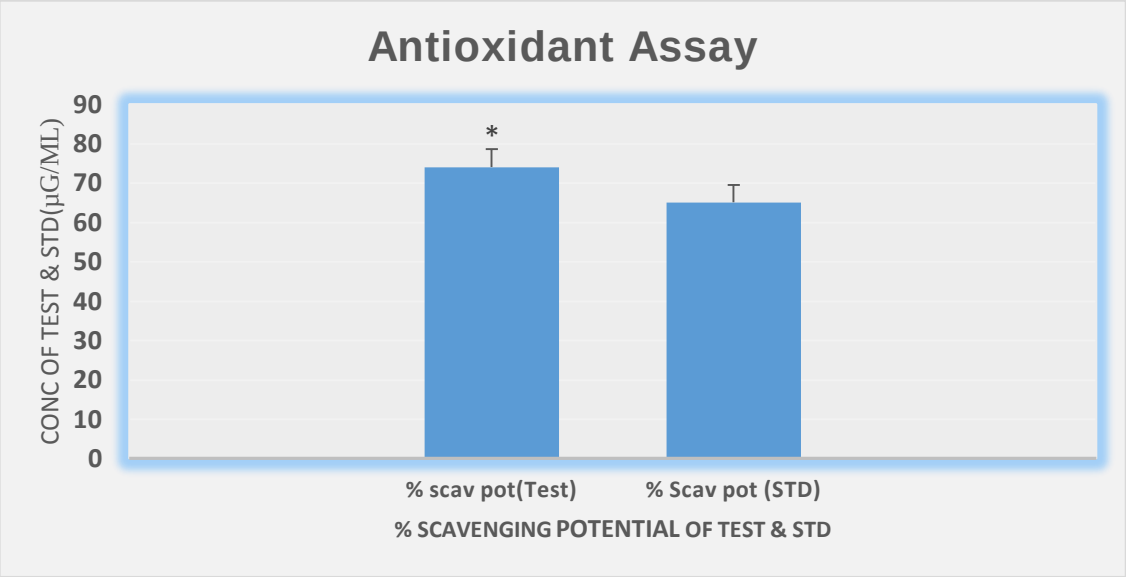
Experiment:

Acquirement : *Digitalis purpurea* was acquired from Medicinal garden of R.k University.[11]

Authentification as *Digitalis purpurea* powder : Glycoside is dissolved in a mixture of 1 % ferric sulphate solution in (5%) glacial acetic acid. Add one or two drop of concentrated sulphuric acid. A blue colour develops due to the presence of deoxy sugar.[11]

Xanthydrol test : The crude is heated with 0.1 to 5% solution of Xanthydrol in glacial acetic acid containing 1% hydrochloric acid. A red colour is produced due to the presence of 2-deoxysugar.[11]

Antioxidant Assay : Antioxidant assay was performed using Nitric oxide assay



***=Significantly different from control animals. (P<0.05)**

Fig 1: Statistical analysis chart of antioxidant assay of test (*Digitalis Purpurea suspension*) and standard(Metformin)

Principle : The procedure is based on the principle that,sodium nitroprusside in aqueous solution at pysiological PH spontaneously generate nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated by griess reagent.Scavengers of nitric oxide competes with oxygen,leading to reduced production of nitrite ionsLarge amount of NO responsible for damage to the tissue. [12]

Procedure :[12]After preparation of stock and standard solution. Two different sets of test tube was labeled as std and test.In one of the test set add 0.05 ml of the test drug was added and were diluted with DMSO up to 10 ml.To each tube 2.0 ml of sodium nitroprusside was added. The solution were incubated for 150 minutes. Similar procedure was followed for the std sample and were incubated Blank consist of sodium nitroprusside and DMSO as the solutionAfter incubation,5ml of griess reagent was added in each tube.And were measured at 546 nm.

Calculation: It is detected by finding % scaveging property by adding absorbance of control and blank in the equation:**% scaveging =[Absorbance of control –absorbance of test sample/absorbance of control**

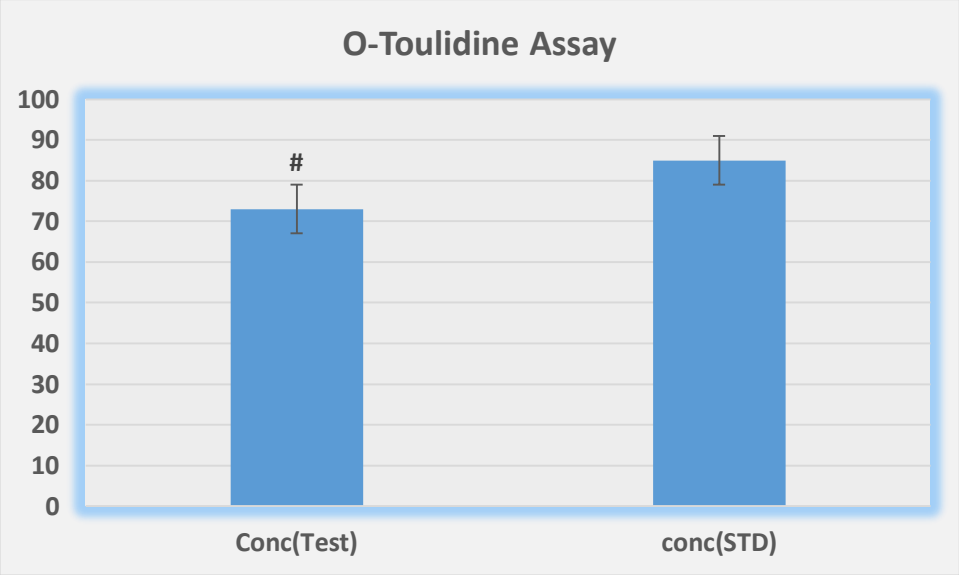
To determine hypoglycemic activity of Digitalis purpurea using O-Toluidine model:

Principle : It involves condensation reaction of aldehyde of glucose with the amine group of o-toulidine reagent.It leads to the formation of blue –green derivative(imine).Acc to the glucose concentration in solution more imine is formed and detected at 630 nm.[13]

Procedure (o-toluidine method) [13]First two sets of test tube were taken and labeled properly. Then in both set 0.05 ml of glucose solution (20,40,60,80,100µg/ml) was added in separate test-tube. After that addition of 0.05 ml of test extract in tube of one set and incubated fin dark for 4 hours. Comparative Metformin solution were also prepared as comparative with test solution.Finally,4ml of o-toluidine was mixed in all test-tube and heated for 15 min.Absorbance was measured at 630 nm.

Calculation: Conc of glucose: (AK/AU) ×C

AU: absorbance in presence of sample
AK: absorbance of standard glucose
C means: concentration of STD glucose



 # = Significantly different from Conc (std) P<(0.001)

Fig 2: Statistical analysis chart of Hypoglycemic assay of test (*Digitalis Purpurea suspension*) and standard(Metformin)

Results:

Results of digitalis purpureea as test extract in antioxidant assay and hypoglycemic assay ius shown in table 1 given below.Stastical studies was done using Instat software one way anova following Tuckey's test.It showed better response as compared to that of the standard drug.Digitalis suspension showed lower glucose concentration as compared to standard drug.Stastically showing higher scaveging property indicates its potential to cure Type-1 diabetes mellitus and all the complication associated with it.

Table 1: In-Vitro models to determine antioxidant and hypoglycemic activity of *Digitalis purpurea*

Sr.No	Parameters Analyzed	Mean ± SEM(std)	Mean± SEM(Test)
1.	% Scavenging Activity	65.07 ± 0.791	74.07 ± 0.65
2.	Concentration of glucose(µg/ml)	70.21± 0.006	65 ± 0.005

Discussion:

From the above models performed it can be proved that test drug has property to prevent Reactive oxygen species solely responsible for all the micro and macro complication which may be helpful to prevent mortality in patients. It also showed antidiabetic activity which showed lower concentration of glucose compared to that of the standard. It can be said *digitalis purpurea* oral formulation prepared further can be better alternative than insulin and other hypoglycemic agent, immunosuppressant.

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