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Biological Evaluation of Plant Extract of *Tinospora cardifolia* in Diabetes Mellitus in the Animal Model

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ABSTRACT:- Aim of the present investigation was to develop an animal model mimicking human type 2 diabetes. High fat fed wistar rats after 28 days became obese and the blood glucose level reached 110 mg/dl. In this animal model obesity causes the border case exerting stress on beta cells to produce compensatory insulin. The stress, damaged the beta cells of islets of Langerhans- resulting hyperglycemia, thus leading to type 2 diabetes.

The present work was to evaluate the therapeutic efficacy of *T. cordifolia* stem extract on type 1 and 2 diabetes induced animal model wistar rat various biochemical parameters investigated include blood glucose, after treatment with *T. cordifolia* stem extract with 150 and 250 mg/kg/day to diabetic group elevated levels were restored to control group level. Whereas glucose level decreased in diabetic group to that of control group of for treatment with *T. cordifolia* stem extract. The studies on the islets of Langerhans suggest that the plant extract treatment to diabetic group (150 and 250 mg/kg/day) to type1 and 2 diabetic rats resulted in the recovery of damaged islets and restoring the beta cell number, hence enhanced the insulin secretion, thus establishing the homeostasis of blood glucose

The plant extract of *T. cordifolia* stem extract has improved the damaged Langerhans and enhanced insulin secretion ofbeta cells in type 1 and 2 diabetes. Therefore the plant extract of *T. cordifolia* has a therapeutic efficacy in alleviating type 1 and 2 diabetes but type 2 diabetes is totally invisible.

Index Terms - Diabetes, Insulin, Extract, Animal model, Obese, etc.

I. INTRODUCTION:-

Diabetes mellitus is a disorder of metabolism of carbohydrate, protein and fat associated with a relative or absolute insulin secretion and with various degrees of insulin resistance. It is recognized as one of the leading causes of morbidity and mortality in the world.

According to the World Health Organization, the prevalence of the disease will grow from 171 million in 2000, to 366 million in 2030, which amount to an increase of 144% over the next 30 years. Deaths related to diabetes are estimated at about 9%of global mortality. Over all direct health care cost of diabetes range from 2.5 to 15% of annual health care budgets, depending on local diabetes prevalence and treatments available. Diabetes mellitus is subdivided into four different types, which appear to differ in causes and in pathogenesis.

1- Type 1: Insulin Dependent Diabetes Mellitus (IDDM).
2- Type2: Non-Insulin Dependent Diabetes Mellitus(NIDDM).
3- Malnutrition related Diabetes Mellitus (MRDM).
4- Gestational Diabetes. [1-3]

Medicinal plants and diabetes

The ethno-botanical information reports about 800 plants that may possess anti- diabetic potential. However, diabetes mellitus is also treated in Indian traditional medicine using anti-diabetic medicinal plants. Indian plants which are most effective and the most commonly studied in relation to diabetes and their

complications are: *Allium cepa, Allium sativum, Aloe vera, Cajanus cajan, Coccinia indica, Caesalpinia bonducella, Ficus bengalenesis, Gymnema sylvestre, Momordica charantia, Ocimum sanctum, Pterocarpus marsupium, Swertia chirayita, Syzigium cumini, Tinospora cordifolia* and *Trigonella foenum graecum. M. charantia, Eugenia jambolana, Mucuna pruriens, Murraya koeingii and Brassica juncea.* All plants have shown varying degree of hypoglycemic and anti- hyperglycemic activity.[4]

Tinosoro cordifolia

T. cordifolia is widely used in Ayurvedic medicine in India as tonic, vitalizer and as a remedy for diabetes mellitus and metabolic disorders. It is a large, glabrous, deciduous climbing shrub belonging to the Family Mehispermacea. It is found in forests throughout India. It is used as vitalizer and as remedy for diabetes mellitus and metabolic disorders. *T. cordifolia* (Gauduchi) is a widely used shrub in folk and Ayurvedic systems of medicine. It is distributed throughout the tropical Indian subcontinent China, Burma and Ceylon.[5]

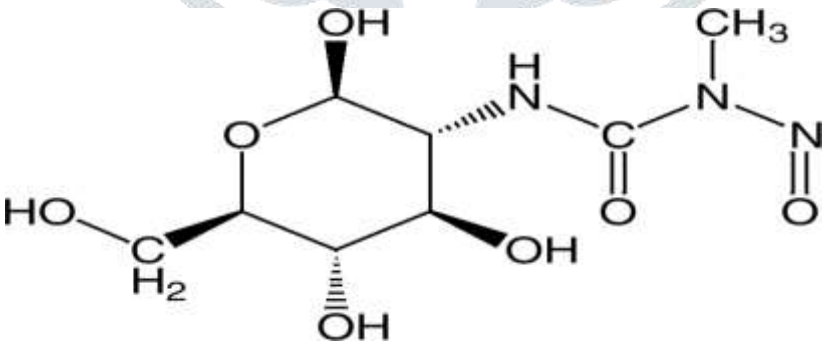
Aerial roots and stem of the plant are sources of the drug preparation. A number of different active principles including alkaloids, bitter compounds (tinosporin, tinosporic acid and tinosporal), essential oil and a mixture of fatty acids, have been identified as contributing to the observed medical effects. In addition, *T. cordifolia* is also found to have an anti stress effect. *T.cordifolia* has been known in India as beneficial in treating a wide variety of diseases *T. cordifolia* is a plant of recognized medicinal values and is widely used. as antibacterial, analgesic , antipyretics and also for the treatment of jaundice, skin disease, diabetes, diabetes, anemia etc. studied the effect of *T. cordifolia* on blood glucose and total lipids of normal and alloxan diabetic rabbits. Stanley, reported hypoglycemic action of *T. cordifolia* roots on alloxan induced diabetic rat.[6]

MATERIALS AND METHODS

The present work was carried out on mature virgin male and female Wistar *Albino* rats. They were used for all the experiments. The Wistar albino strain is a laboratory rat and this was developed from the Norwegian Rat *(Rattus nowegicus,* Family: Muridae, Order: Rodentia) by an American physiologist, Henry Donaldson, who started a breeding colony in 1906 at the Wistar Institute in Philadelphia. These are quite moderately prolific strain, rather resistant to infection, wide head, long ears and tail length always less than the body length. These rats were procured from the animal house of the Department of Zoology, University of oriental, Indore. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocols in the present study. The animals were maintained in a room with a 12 h light /dark cycle temperature of 22+2°C, and humidity of 44-63%. The animals were fed with a balanced commercial diet and water.[7]

Strepozotocin (STZ) SG13G-1G

IUPAC name -N-[Methylnitrosocarbamoyl] —D-glucoseamine. Structural formula



Molecular weight — 265.2.

Molecular formula— $C_6H_{15}N_3O_7$

STZ is an antibiotic which is a product of *Strepomyces achromogenes* variant Streptozoticus - a microorganism. STZ has been particularly useful in treating functional, malignant pancreatic islet cell tumors. It affects cells in all stages of the mammalian cell cycle. It is a nitrosourea derivative having high affinity for B-cell of the islets of Langerhans and causes diabetes in experimental animals. Strepozotocine enters the B-cells via glucose transporter (GLUT2) and causes alkylation of DNA. It damages the B-cell membrane and induction of. DNA strand breaks, leading to activation of poly (ADP-ribose) synthetase and NDA depletion. Strepozotocine liberates toxic amounts of nitric oxide that inhibits aconites activity and participates in DNA damage, as a result of its action; B-cells undergo the destruction by necrosis.STZ is a

better diabetes inductor (and it causes B-cell necrosis and diabetes supervenes within 1-2 days.[8,9]
The administration of STZ to rats resulted in polyphagia, polydipsia, hyperglycemia, hypoinsulinemai, increased cholesterol, triglycerides, blood urea levels and decrease weight. Strepozotocine induced diabetes in rodents results in development of nephropathy similar to early stage clinical diabetic nephropathy. STZ monohydrate was procured from Himedia Laboratories, Mumbai India.[10]

Glucometer

The glucometer was used to determine of blood glucose levels. This is an EZ Smart Diabetes monitoring system and registered trademark of Roche giagnostics GMBH. Every week blood was drawn from tail of conscious rats and glucose was estimated by glucometer. Similarly blood glucose was estimated every week until autopsy and recorded weekly in every group.[11]

Procurement and preparation of the plant material

The plant material was obtained from the botanical garden of our University. It was identified as *T.cordifolia* by a botanist and its sample is preserved and documented in the herbarium of our department. Pieces of the stem of the plant were washed well and dried in shade in room temperature and were powdered using an electric mixer.
The present experiment was carried out to study the effect of ethanol stem extract of *T. cordifolia.* The powder weighing 100 g was soaked with 1000 ml of 90% ethanol or at room temperature and left for overnight with occasional shaking. The extract was filtered with Whatman filter paper Nol. The filtrate was evaporated using a soxhlet evaporator until drying and was dried to obtain 8.5 g dried extract.[12]

EXPERIMENTAL DESIGN

Experiments were conducted, in the first set of experiment type 2 diabetes was induced by STZ and treated by T. cordifolia extract.

Protocols of type 2 diabetes (Experiment)

The animals of irrespective of sex with body weight ranging between 70 to 90 g were distributed into four groups at 6 weeks of age, each group consisting of eight animals as follows. Control group (I), Diabetic control group (II), Diabetic group treated with 150 mg/kg/day (III) and Diabetic group treated with 250 mg/kg/day *T.cordifolia* stem extract (IV). Animals of groups II, III and IV received a diet with high fat from the initial day of the experiment to the 50° day, which induces obesity, insulin resistance (OB/IR) and leads to a typical pre-diabetic state, while group(I) received normal rat diet. Animals of groups II, III and IV were rendered diabetic by single intra peritoneal cZ (i.p) injection of STZ-10 mg/kg prepared in citrate buffer (pH 4.5) on the 40° day and received oral glucose dose of 2 g/kg body weight until 70 day. Group I animals were injected with buffer alone. After 52 h of STZ injection, blood was drawn from tail of conscious rats and glucose was estimated by glucometer. Animals with blood glucose ranging above 140 mg/dl were considered as potential to develop diabetes. From the 51 “day, animals of all the groups received commercial normal diet. The animals of group Ill received 150mg/kg b.w/day and group IV received 250 mg/kg/day *T. cordifolia* stem extract orally from the 51st day to the 100° day.
Body weight was recorded at the beginning and termination of the experiment period; animals were fasted for overnight and autopsied under light ether anesthesia.
Blood was collected by superior and inferior vena cava punctures in 5% EDTA vials for measurement of biochemical parameters. Plasma was separated for the estimation of insulin.[13-15]

Measurement of biochemical parameter

This verity of diabetic state was judged by the failure of normal growth and by the blood glucose measurements. After autopsy plasma glucose was estimated by Trinder, methods using GOD/POD kit. The glucose kit in which glucose oxidase (GOD) and peroxidase (POD) enzymes are used along chromogen4-aminoantipyrine and phenol. The enzyme GOD gives D- glucomic acid and hydrogenperoxide. Hydrogen peroxide in the presence of the enzyme POD oxidizes phenol, which combines with 4- amino antipyrine to produce red coloured quinoneinne dye. Plasma insulin levels were determined in duplicate using insulin RIA Kit with rat insulin as a standard.
Blood urea was estimated by urea’s-glutamate dehydrogenise (GLDH) method. Positive urinary glucose test son routine urinalysis should alert the physician to the need for further screening or diagnostic procedures, where as a negative test does not rule out the presence of diabetes [16,17]

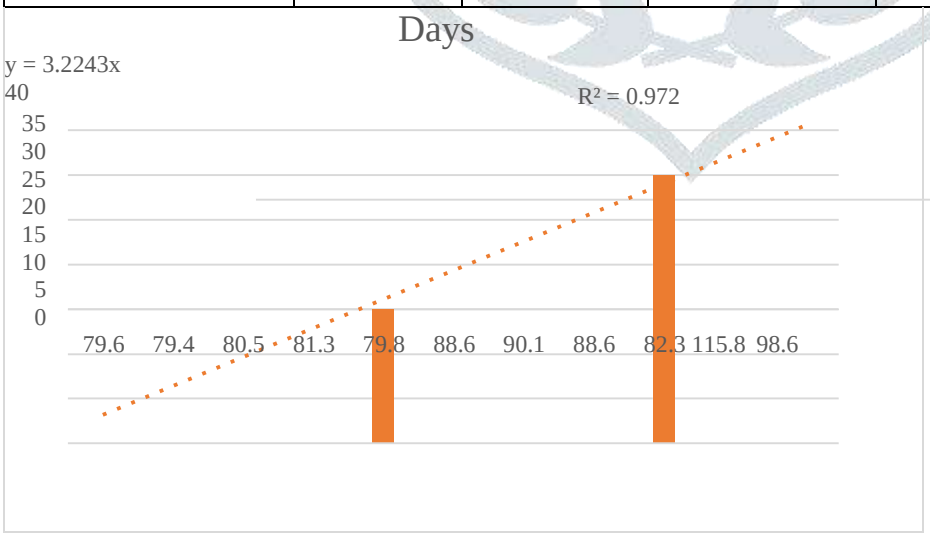
OBSERVATIONS RESULT AND DISCUSSION

Table (1) and Fig. (1) show the body weight in type 2 diabetes experimental group of animals. Control group of animals (I) gradually gained the body weight reaching 119.6 during 70 days of the experiment. This body weight gain was significantly higher than the initial body weight of 59+2.27g. There was a gradual increase in body weight in groups II, III and IV from the 0“ day to the 45th days since they were fed with high fat diet. Further there was a slight decrease in body weight by the 70 day. However, the final body weight was significantly higher in all the groups compared to the initial body weight ($P<0.05$). Final body weight of group IV 119 gm was significantly higher than that of diabetic group 98.

Table (2) and Fig. (1 to 7) show the details of fasting blood glucose level for 70 days. Control rats (group I) did not show any significant variation in the blood glucose throughout the experimental period. It ranged from79.2+3.71mg/dlto80.9mg/dl. Blood glucose level gradually increased from pre diabetic stage79.4mg/dl in group II, III and IV to the diabetic stage on the 45'h day (79.7 mg/d1, 126.4 mg/dl and110.6mg/d1respectively) after oral glucose load was given from the15“day to 60 days. Blood glucose level indicating diabetic state in groupie remained throughout the experiment ranging from 88.6 mg/dl to 147.3 mg/dl. The blood glucose level significantly reduced to non-diabetic state 101.6 mg/dl and 125.9 mg/d1 in group III and group IV respectively after treatment with *T. cordifolia* stem extract (150 and 250 mg/kg/day respectively).

Table no.1 Effects of *T. cordifolia* stem extract on body weight (g) of control and experimental groups of type 2 diabetic

Duration and groups	**0 Day**	**15Days**	**30Days**	**45Days**	**70Days**
Control I	64.3	70.3	78.3	90.0	119.6
Diabetic II	59.6	88.1	115.5	110.1	98.1
Diabetic +T.c 150 mg/kg III	59.2	90.0	100.2	119.8	117.3
Diabetic +T.c 250 mg\kg IV	63.3	78.1	89.3	123.4	119.3



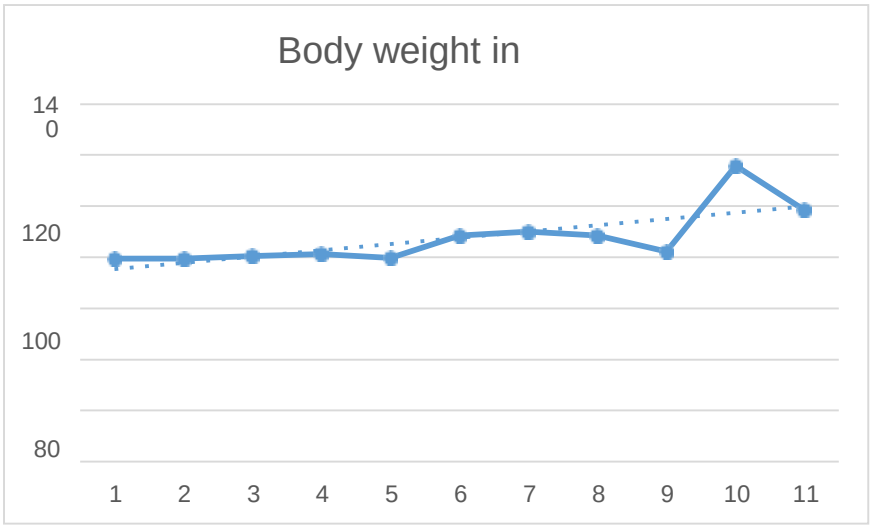


Fig no.1 Body weight of all the groups

Above table 1 and fig 1 showed the Body weight of all the groups was recorded at the commencement and termination of the experiment. Body weight gradually increased from its initial weight in all the groups except the diabetic group. Body weight gain in *T. cordifolia* treated groups was relatively less compared to control groups. Whereas the diabetic group lost the weight compared to initial body weight.

Effects of *T. cordifolia* stem extract on blood glucose levels of control and experimental groups of 2 diabetic rats were shown in Table no. 2 and Figures 2-7.

Table no. 2 Effects of *T. cordifolia* stem extract on blood glucose levels of control and experimental groups of type 2 diabetic

Duration and groups	**0Day**	**15Days**	**30Days**	**45Days**	**60Days**	**70Days**
Control I	78.6	79.8	82.3	79.7	77.5	80.9
Diabetic II	79.4	88.6	115.8	126.4	143.4	147.3
Diabetic +T.c 150 mg/kg lII	80.5	90.1	98.6	110.6	100.8	101.6
Diabetic +T.c 300 mg\kg IV	81.3	88.6	89.5	129.9	127.6	125.9

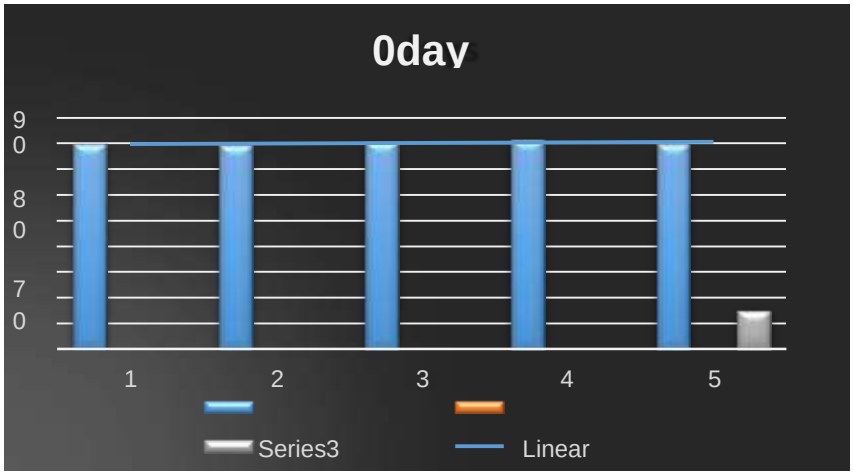


Fig No.2 Effects of *T. cordifolia* stem extract on blood glucose levels intial

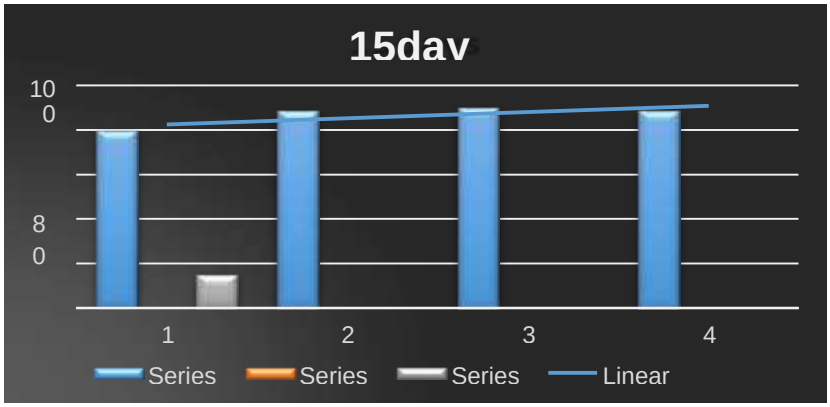


Fig No.3 Effects of *T. cordifolia* stem extract on blood glucose levels after 15 days

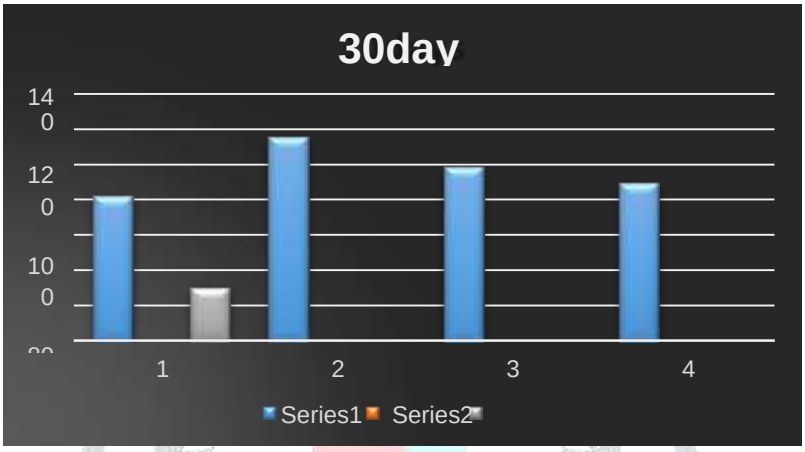
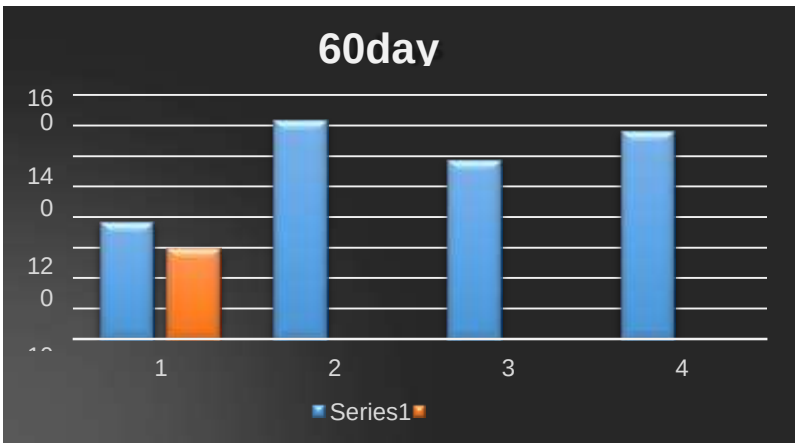


Fig No.4 Effects of *T. cordifolia* stem extract on blood glucose levels after 30 days

Fig No.5 Effects of *T. cordifolia* stem extract on blood glucose levels after 45 days



FigNo.6 Effects of *T. cordifolia* stem extract on blood glucose levels after 60 days

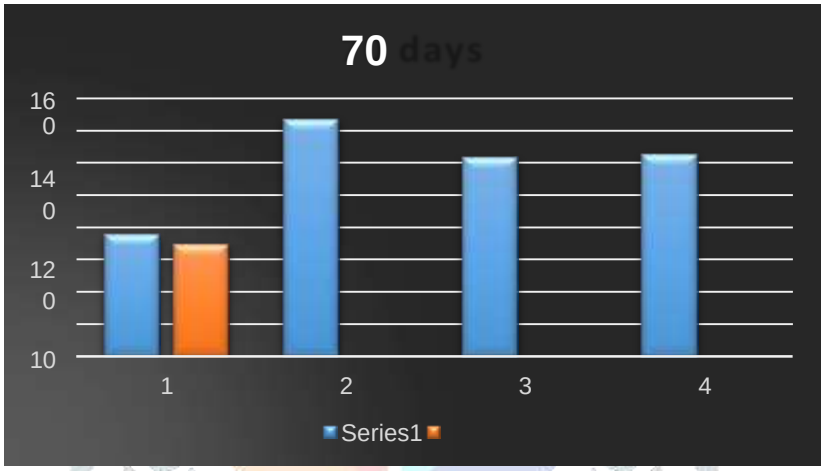


Fig No.7 Effects of *T. cordifolia* stem extract on blood glucose levels after 70 days

The above graph and tables was observed and got Blood glucose level reached a border case (potential to develop diabetes) after feeding with high fat feed for seven weeks (45 days). Hyperglycemia was observed (Seven week) by 60 day and remained throughout in diabetic group. After treatment with T. cordifolia extract blood sugar level minimized.

CONCLUSION

Aim of the present investigation was to develop an animal model mimicing human type 2 diabetes. High fat fed wistar rats after 28 days became obese and the blood glucose level reached 110 mg/dl. Such individuals in human being are designated as border cases potential to develop diabetes. In this animal model obesity causes the border case exerting stress on beta cells to produce compensatory insulin. The stress, damaged the beta cells of islets of Langerhans- resulting hyperglycaemia, thus leading to type 2 daibetes. However in human being type 2 diabetes also occurs due to resistance of insulin at receptor levels or molecular defect of insulin / receptor. Type2 daibetes developed in the present investigation only represents the earlier stated event but not the latter.

The present work was to evaluate the therapeutic efficacy of *T. cordifolia* stem extract on type 1 and type 2 diabetes induced animal model Wistar rat various biochemical parameters investigated include blood glucose, after treatment with *T. cordifolia* stem extract with 150 and 250 mg/kg/day to diabetic group elevated levels were restored to control group level. Whereas glucose level decreased in diabetic group to that of control group of for treatment with *T. cordifolia* stem extract. The studies on the islets of Langerhans suggest that theplant extract treatment to diabetic group (150 and 250 mg/kg/day) to type1 and type2 diabetic rats resulted in the recovery of damaged islets and restoring the beta cell number, hence enhanced the insulin secretion, thus establishing the homeostasis of blood glucose

The plant extract of *T. cordifolia* stem extract has improved the damaged islets of Langerhans and enhanced insulin secretion of beta cells in type 1 and type 2 diabetes. Therefore the plant extract of *T. cordifolia* has a therapeutic efficacy in alleviating type 1 diabetes and type 2 diabetes but type 2 diabetes is totally invisible.

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