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EVALUATION OF ANTIDIABETIC ACTIVITY OF ROOTS OF TINOSPORA CORDIFOLIA ON EXPERIMENTAL ANIMALS

Anurag Gaur*, Praveen Kumar, Dr. Shamim Ahmad, Jiyaul Hak, Dr. Nasiruddin Ahmad Farooqi

Translam Institute of Pharmaceutical Education and Research Meerut, U.P.

***Corresponding Author: Anurag Gaur**

Translam Institute of Pharmaceutical Education and Research Meerut, U.P.

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ABSRACTS

The present study was an attempt to investigate the effect of *T. cordifolia root* extract on streptozotocin induced diabetes in Wistar rats. Two gropus of streptozotocin-induced diabetic rats were orally treated with *T. cordifolia root* extract (150 and 300mg/kg) respectively. The blood glucose level, body weight, Glycosylated hemoglobin, liver glycogen, lipid profile, Antioxidant status were measured at the end of the study i.e. after 40 days of treatment. *T. cordifolia root* extract were found to be significant ($p < 0.05$) in reducing the blood glucose level, glycosylated hemoglobin, lipid profile, whereas both the treatments increased body weight, liver glycogen content and antioxidant status when compared to the diabetic control. It has been concluded that *T. cordifolia root* extract, in addition to the antidiabetic activity, also possess antihyperlipidemic and antioxidant activities in the streptozotocininduced diabetic model.

KEYWORDS: Two gropus of streptozotocin-induced diabetic rats were orally treated with *T. cordifolia root* extract (150 and 300mg/kg) respectively.

INTRODUCTION

Diabetes is a chronic disorder. It may be characterized by hyperglycaemia. These may help in insulin secretion defects and both insulin action. Due to development of insulin resistance the inadequate insulin secretion and tissues dimension may lead to abnormalities of fats, carbohydrate and metabolism of protein. These may lead to change or may increases the concentration of blood glucose level. These may damage many systems of the body like blood vessels, nerves. Diabetes is one of the most leading causes of morbidity and mortality in all over the world. According to the survey it was concluded that 0.5 to 3% of person was surfer from these diseases. Now a days its reaches to more than 7 %. Around 200 to 300 million people are affected and it should be double or triple in next few years.[1,2]

MATERIALS AND METHODS

Preparation of the plant material

The plant material is collected from botanical garden of our university. With the help of a botanist, it was identified as *T. cordifolia.* Sample is been preserved and documented in the herbarium. A small pieces of plant root were washed. Then it will be dried in room temperature. By the use of electric mixer these roots are converted into the powder form. experiment is carryout to study the effects of ethanolic roots extract of *T. cordifolia.* Around 60g of powder is been weighed and soaked into 600ml of 90% ethanol solution at room temperature. For occasionally shaking this preparation is leave for overnight. Whatman filter paper is use for filtration of extraction. By using Soxhlet evaporation method for the filtration and it should be done until drying and dried to obtained 5g of dried extract.

Experimental protocol for type 1 diabetes

This experiment is done for the investigation and determination of effect of ethanolic root extract of *T. cordifolia* on the STZ induced diabetic rats. Animals are weighed around 150 to 190g. animals are feed by the laboratory food and ad libitum water is been provided thought out the experiment. Animals were grouped into 6 for 6 weeks of age. Each group consist of 10 animals. Group (I) control, Control group with 150 mg/kg/day *T. cordifolia* root extract treatment (II), Group (III) Diabetic control, Group (IV) Diabetic treated with 150 mg/kg/day, Group (V) Diabetic treated with 250 mg/kg/day *T. cordifolia* root extract and Group (VI) diabetic treated with 300 mg/kg/day *T. cordifolia* root extract.

Animals of groups IV, V and VI were given a single injection of streptozotocin (STZ-50 mg/kg) with citrate buffer (pH 4.5). Animals with Group I, II and III injected with buffer alone. After 72 to 75 hr of injection, blood were taken from the tail of conscious rats and by the use of glucometer glucose were estimated. This process is repeated every week until autopsy. After 10 to 11 days of STZ injection animals of group II and III

received 150 mg/kg/day and group VI received 300 mg/kg /day *T. cordifolia* root extract which were given orally for minimum 6 weeks. By the using of intubations tube these doses were given daily. Body of each rat is weighed in every group. After completion of 6 weeks animals were ready for autopsy and make the animals fasted overnight. Autopsy is been done by the use of light ether anaesthesia. 5 % EDTA vials is used for the collection of blood which were taken out from superior and inferior vena cava punctures. This is been used for the further experiment (biochemical parameters measurement).

Experimental protocol for type 2 diabetes

irrespective sexes of animals were weigh which is ranges from 80 to 100g. these animals are separated into five group for 6 weeks of ages. Each group consist of 10 animals. Group (I) control, Control group with 150 mg/kg/day *T. cordifolia* root extract treatment (II), Group (III) Diabetic control, Group (IV) Diabetic treated with 150 mg/kg/day, Group (V) Diabetic treated with 250 mg/kg/day *T. cordifolia* root extract. A high fat diet is been given to the animals of groups III, IV and V. animals were use for 100 days in which this may induces the obesity, insulin resistance and shows pre-diabetic state. Group I animals may give normal diets. On 65-day, A single dose of injection of STZ-15 mg/kg is been given to the animal group III, IV and V with citrate buffer (pH 4.5). An oral glucose of 3 g/kg body weight until 75 days. Animal with group I and II is been injected with buffer alone. After 72 to 75 hr of injection, blood were taken from the tail of conscious rats and by the use of glucometer glucose were estimated. Diabetes is been formed in the animals with the blood glucose level ranging above 140 mg/dl. On 76th day of animals were treated with normal diet. Group III animals were treated with 150 mg/kg b.w/day and group IV and V animals were treated with 250 mg/kg/day *T. cordifolia* root extract from 76th to 98th day. At the time of autopsy animals were fasting and were given light ether anaesthesia. 5 % EDTA vials is used for the collection of blood which were taken out from superior and inferior vena cava punctures. This is been used for the further experiment (biochemical parameters measurement). Estimation of insulin is done on the basis of plasma.

Statistical analysis

ANOVA is use for the statistical analysis. student's "t" test, body weight, relative pancreas weight, biochemicals parameters, islet size and P-cell count of all the groups were measured. Duncan's new multiple range test (DMRT)was also use for this analysis. Maximum significant level was fixed at 0.05.

RESULTS AND DISCUSSIONS

Table 1: Effects of *T. cordifolia* root extract on body weight of control and experimental groups of type.

Body weight Groups	Normal	Final
Control I	148±7.12	182±13.57
Control + *T. cordifolia* 150 mg/kg/day II	143±3.73	184±5.04
Diabetic III	147±4.65	158.2±12.7
Diabetic + *T. cordifolia* 150 mg/kg/day IV	154.5±3.05	165.51±9.3
Diabetic T. *cordifolia* 250 mg/kg/day V	156.5±3.75	167.55±8.5
Diabetic T. *cordifolia* 300 mg/kg/day VI	152.48±2.48	169.45±5.48
ANOVA F-value (df= 4,35)	0.321 P<0.05	17.147 P<0.05

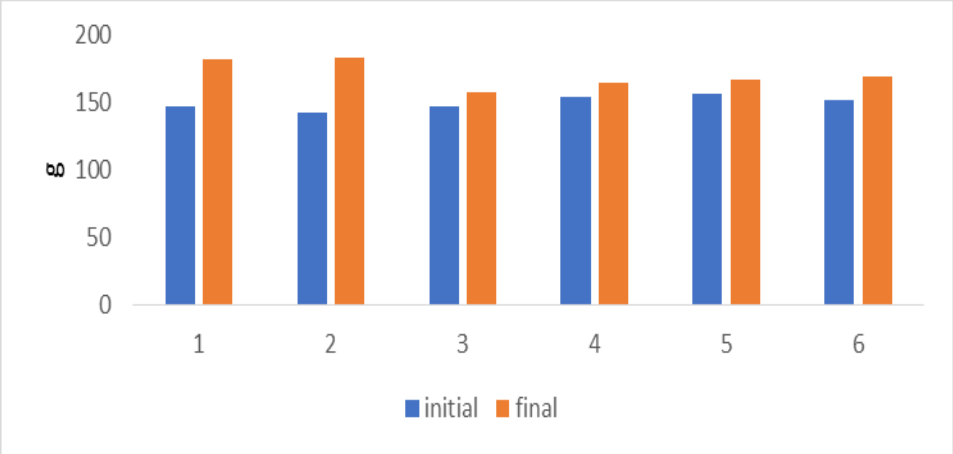

Figure 1: Effects of *T. cordifolia* root extract on body weight (g) of control and experimental groups of type 1 diabetic rats.

Table 2: Effects of T. cordifolia root extract on blood glucose levels (mg/dl) of control and experimental groups of type 1 diabetic rats.

duration groups	0 days	1st day	10th day	20th day	30th day	40th day
Control I	85.4±3.12	79.84±4.57	87±2.45	87±2.47	87±4.5	89±1.9
Control + *T. cordifolia* 150 mg/kg/day II	72.4±4.73	81.45±5.47	75.1±3.15	68.14±3.48	65±4.5	68±2.8
Diabetic III	72.48±6.59	500±15.74	380±45.28	385±21.78	371.45±17.48	387±8.47
Diabetic + *T. cordifolia* 150 mg/kg/day IV	73.4±4.05	380±26.3	298±17.45	288±16.58	185±19.47	135±7.15
Diabetic T. *cordifolia* 250 mg/kg/day V	75.5±4.75	390.47±14.5	328±19.45	219.4±5.48	168±13.47	145±5.4
Diabetic T. *cordifolia* 300 mg/kg/day VI	77.51±5.48	395.14±15.48	339±18.45	220±4.85	166±13.75	99±3.54
ANOVA F-value (df= 4,35)	2.47 P<0.05	115.45 P<0.05	39.7 P<0.05	57.15 P<0.05	138.47 P<0.05	58.45 P<0.05

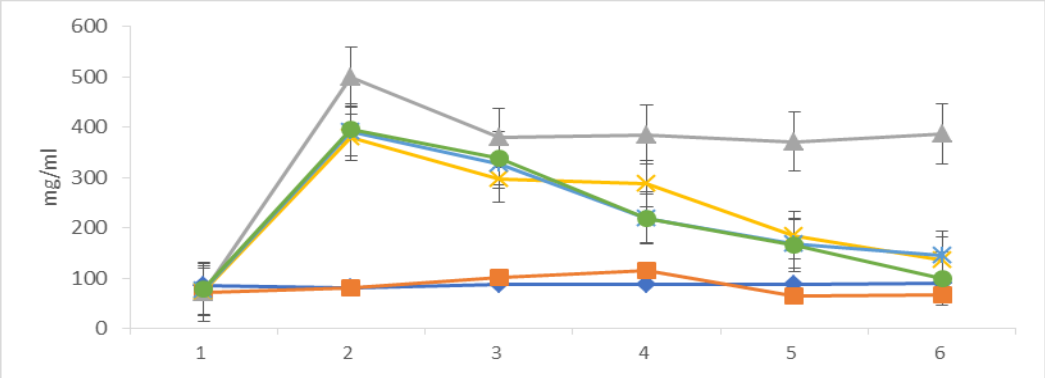

Figure 2: Effects of T. cordifolia root extract on blood glucose levels (mg/dl) of control and experimental groups of type 1 diabetic rats.

Table 3: Effects of T. *cordifolia* root extract on glycosylated haemoglobin (HbAlc%) levels of control and experimental groups of type 1 diabetic rats.

Parameter groups	Glycosylated Haemoglobin (%)
Control I	6.18±0.15
Control + *T. cordifolia* 150 mg/kg/day II	4.87±3.73
Diabetic III	15.18 ± 0.40
Diabetic + *T. cordifolia* 150 mg/kg/day IV	6.48 ± 0.48
Diabetic T. *cordifolia* 250 mg/kg/day V	7.27 ± 0.68
Diabetic T. *cordifolia* 300 mg/kg/day VI	8.27 ± 0.85
ANOVA F-value (df= 4,35)	165.47 P<0.05

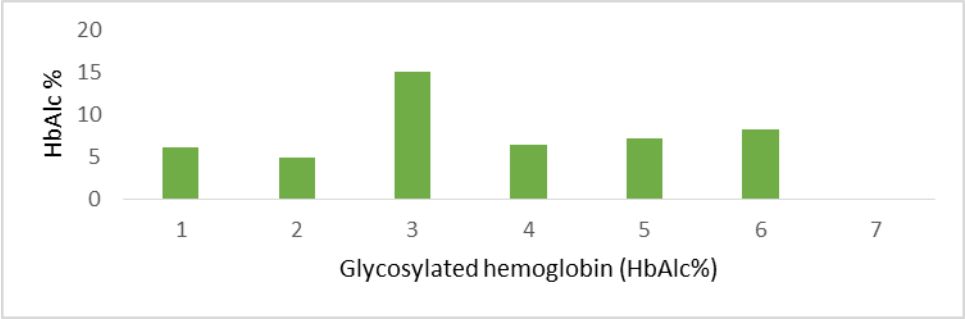

Figure 3: Effects of T. *cordifolia* root extract on glycosylated haemoglobin (HbAlc%) levels of control and experimental groups of type 1 diabetic rats.

Table 4: Effects of T. *cordifolia* root extract on triglyceride levels of control and experimental groups of type 1 diabetic rats.

Parameter groups	triglyceride (mg/dL)
Control I	54 ± 0.89
Control + *T. cordifolia* 150 mg/kg/day II	53.6 ± 2.12
Diabetic III	85.6 ± 2.5
Diabetic + *T. cordifolia* 150 mg/kg/day IV	76 ± 1.5
Diabetic T. *cordifolia* 250 mg/kg/day V	68.45 ± 4.5
Diabetic T. *cordifolia* 300 mg/kg/day VI	70.71 ± 5.4
ANOVA F-value (df= 4,35)	105.47 P<0.05

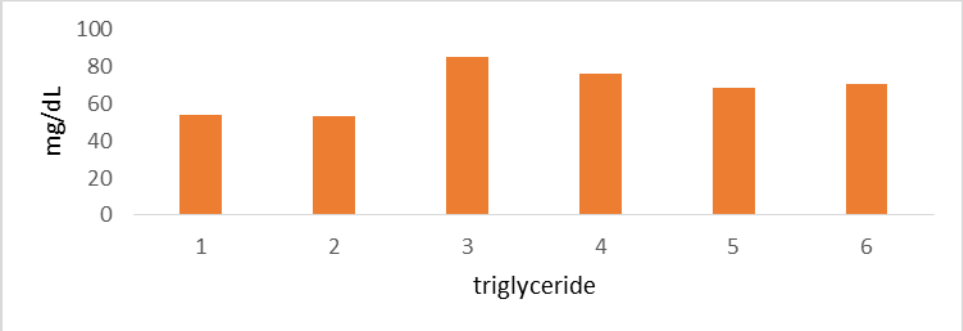

Table 5: effect of T. cordifolia root extract on body weight (g) of control and experiment group of type 2 diabetic.

duration / groups	0 Days	35 days	45 Days	60 days	75 days	80 days	90 Days	100 days
Control I	85.5±2.74	91.5±4.14	126.5±4.58	158±2.94	209±1.25	218±1.28	218±2.48	238±5.18
Diabetic II	80.8±3.14	109±2.47	154±4.56	210±3.18	335±1.54	319±3.25	305±2.09	275±3.19
Diabetic + *T. cordifolia* 150mg/kg/dayIII	78.4±3.14	110.4±3.48	157±5.18	258±1.75	333±2.14	313±2.45	318±3.18	299±4.19
Diabetic + *T. cordifolia* 250mg/kg/day IV	81.6±3.14	122.4±1.64	160±6.01	248±3.48	245±1.28	328±1.25	321±5.15	310±3.49
Diabetic + *T. cordifolia* 300mg/kg/day V	82.7±3.54	130.8±1.75	167±5.18	250±1.52	230±2.48	329±2.15	329±4.15	315±4.19
ANOVA F value (df= 3, 28)	0.784 P<0.05	14.78 P<0.05	18.55 P<0.05	8.48 P<0.05	65.48 P<0.05	48.15 P<0.05	28.14 P<0.05	28.15 P<0.05

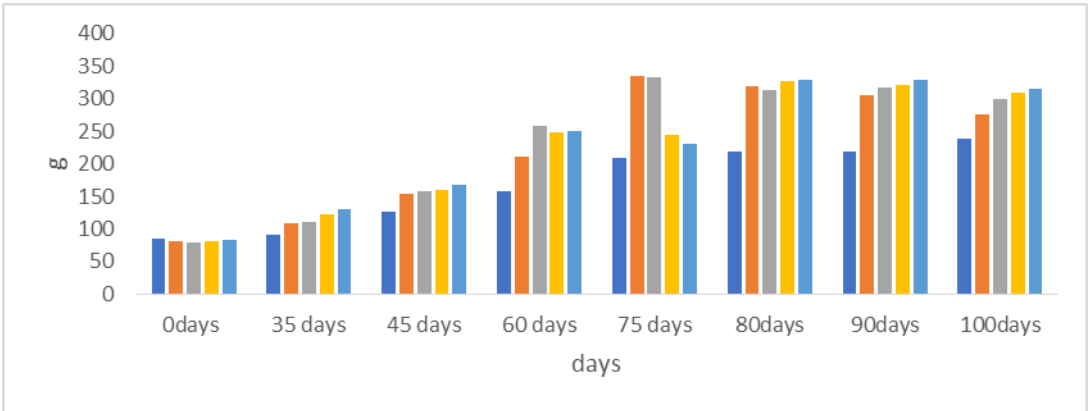

Figure 5: Effects of *T. cordifolia* root extract on body weight (g) of control and experimental groups of type 2 diabetic rats.

Table 6: Effects of *T. cordifolia* root extract on blood glucose level of control and experimental groups of type 2 diabetic rats.

duration / groups	0 days	35 days	45 days	60 days	75days	80days	90days	100 days
Control I	80.5±3.74	92.5±3.14	83±3.58	85±5.94	88±2.25	83±3.28	82±4.48	85±4.18
Diabetic II	81.8±4.14	90±3.47	125±5.56	219±4.18	248±2.54	235±4.25	240±3.09	249±4.19
Diabetic + *T. cordifolia* 150mg/kg/day III	81.4±4.14	88±4.48	148±4.18	235±3.75	250±3.14	149±3.45	119±4.18	115±5.19
Diabetic + *T. cordifolia* 250mg/kg/day IV	82.6±4.14	89±2.64	110±5.01	215±4.48	245±2.28	119±2.25	118±6.15	119±4.49
Diabetic + *T. cordifolia* 300mg/kg/day V	83.7±2.54	92±2.75	115±4.18	217±2.52	234±4.48	128±4.15	120±5.15	110±6.19
ANOVA F value (df= 3, 28)	0.245 P<0.05	0.558 P<0.05	7.48 P<0.05	29.48 P<0.05	45.48 P<0.05	38.15 P<0.05	160.14 P<0.05	35.36 P<0.05

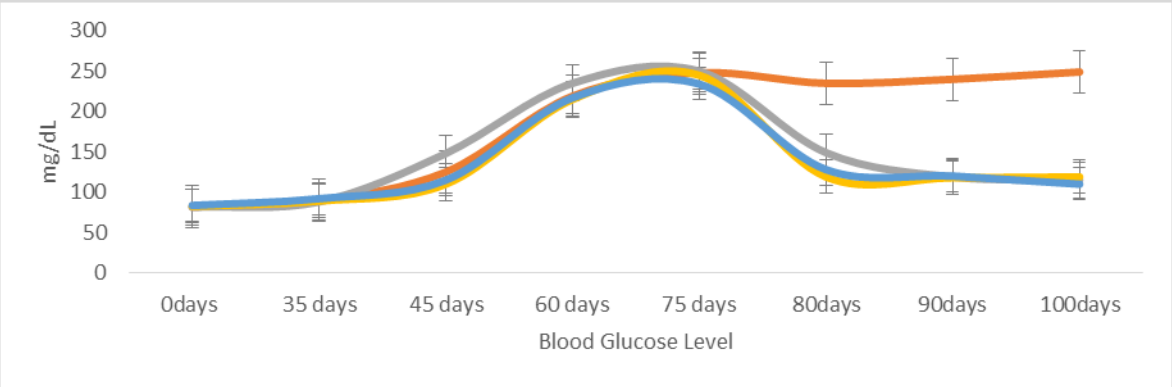

Figure 6: Effects of *T. cordifolia* root extract on blood glucose level of control and experimental groups of type 2 diabetic rats.

Table. 7: Effects of *T. cordifolia* stem extract on LDL of control and experimental groups of type 2 diabetic rats.

Parameter / groups	Glycosylated Haemoglobin (HbAlc)
Control I	5.06 ± 0.063
Diabetic II	7.45±0.248
Diabetic + *T. cordifolia* 150 mg/kg/day III	7.4 ± 0.274
Diabetic T. *cordifolia* 250 mg/kg/day IV	8.4 ± 0.254
Diabetic T. *cordifolia* 300 mg/kg/day V	6.08 ± 0.285
ANOVA F-value (df= 4,35)	49.18 P<0.05

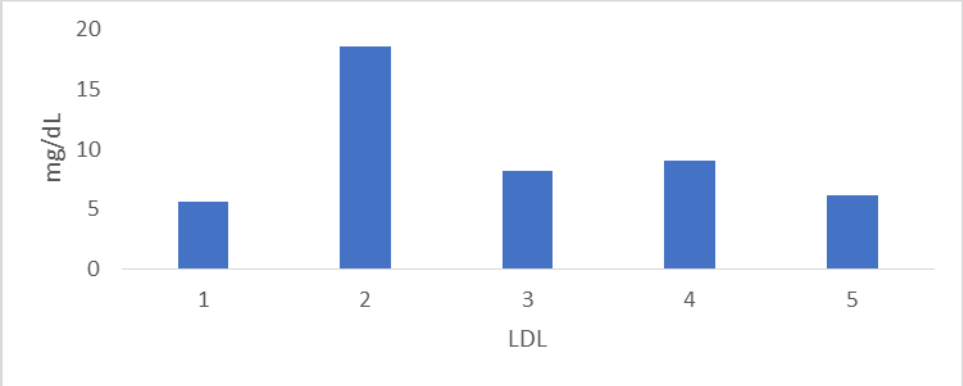

Figure 7: Effects of *T. cordifolia* stem extract on LDL of control and experimental groups of type 2 diabetic rats.

Table 8: Effects of *T. cordifolia* root extract on HDL of control and experimental groups in type 2 diabetic rats.

Parameter / groups	HDL (mg/dL)
Control I	29.08 ± 2.46
Diabetic II	16.5 ± 2.15
Diabetic + *T. cordifolia* 150 mg/kg/day III	26.02 ± 3.05
Diabetic T. *cordifolia* 250 mg/kg/day IV	22.02 ± 6.05
Diabetic T. *cordifolia* 300 mg/kg/day V	23.71 ± 6.45
ANOVA F-value (df= 4,35)	25.48 P<0.05

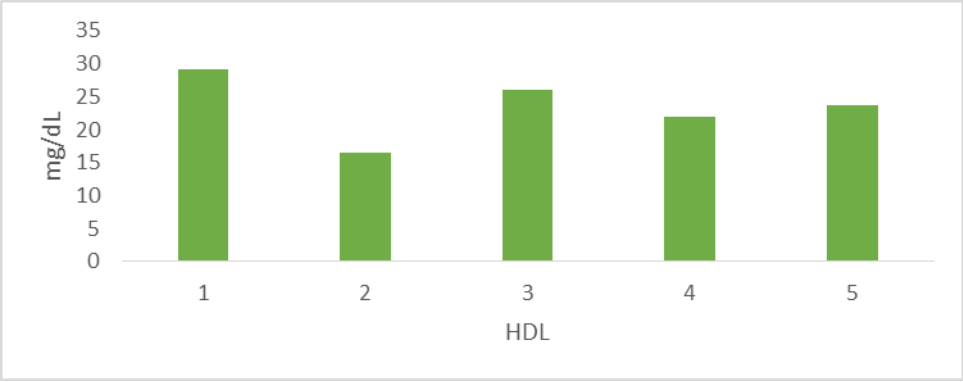

Figure 8: Effects of *T. cordifolia* root extract on HDL of control and experimental groups in Type 2 diabetic rats.

CONCLUSION

The main aim of the present study is to develop an animal model for traditional medicine (T. cordifolia) in anti-diabetic activity. Animals become obese by high fat fed and the blood glucose level reached 129 mg/dl. Animal model may cause obesity and causes stress this may result into hyperglycaemia. 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, triglyceride, cholesterol, LDL, VLDL, HDL, glycosylated hemoglobin percentage (HbAlc %) and blood urea. Except HDL, levels of blood glucose, triglyceride, cholesterol, LDL, VLDL, glycosylated haemoglobin percentage and blood urea were elevated in diabetic group. After treatment with T. cordifolia root extract with 150,250 and 300 mg/kg/day to diabetic group. Whereas HDL level decreased in diabetic group and the level of HDL was restored to that of control group of for treatment with T. cordifolia root extract. The studies on the islets of Langerhans suggest that the plant extract treatment to diabetic group (150,250 and 300 mg/kg/day) to type 1 and type 2 diabetic rats resulted in the recovery of damaged islets and restoring the β cells number, hence enhanced the insulin secretion. The plant extract of T. cordifolia root extract has improved the damaged islets of Langerhans and enhanced insulin secretion of β 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. Though the T. cordifolia stem extract has therapeutic efficacy, the

actual chemical compound which will alleviate diabetes is further to be investigated.

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