

**ANTI-DIABETIC ACTIVITY OF ROOT EXTRACTS
OF TRAGIA INVOLUCRATA**

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Summary

Anti-diabetic activity of petroleum ether (PETI), chloroform (CHTI) and aqueous (AQTI) extracts of roots of *Tragia involucrata* (Linn) were evaluated in alloxan induced diabetic rats. Initially on 1st day under mild ether anaesthesia blood samples were collected by cardiac puncture from all the normal rats for estimation of blood glucose and biochemical parameters like serum cholesterol, triglycerides, HDL, LDL, Creatinine, urea and serum alkaline phosphate. After wards Alloxan (150mg/kg i.p) induced diabetic rats were given with vehicle (distilled water), standard drug tolbutamide (80mg/kg, p.o), petroleum ether (100mg/kg, p.o), Chloroform (100mg/kg,p.o) and aqueous extracts (200mg/kg, p.o) of *Tragia involucrata* respectively once daily for 21 days. Blood samples were withdrawn at prefixed time intervals like on 1st, 7th, 14th and 21st days as mentioned above and were subjected for biochemical parameters as mentioned earlier. To study the antidiabetic effect of extracts in diabetic state, whole pancreas from all the animals were removed and kept in 10% formalin solution for histopathological studies. The anti diabetic activity of these three extracts were assessed by maintenance of blood glucose and biochemical parameters estimated from serum as mentioned above along with prevention of change in body weight in diabetic conditions. Petroleum ether, chloroform and aqueous extracts have shown significant anti diabetic activity by reducing blood glucose levels in diabetic rats. Aqueous extract had better prevented the decline of body weight noted with alloxane induced diabetic rats than petroleum ether and chloroform extract treated groups. It can be concluded that *T. involucrata* had possessed significant anti diabetic activity as it lowers blood glucose levels and contained alteration in serum biochemical parameters and body weight in diabetic rats.

Keywords: *T.involucrata*, root extracts, Anti diabetic, biochemical parameters, body weight.

Introduction

Diabetes mellitus is a metabolic disorder in which the body does not produce or properly utilize insulin. The name diabetes comes from the Greek for a siphon and refers to the polyuria common in both diabetes mellitus and diabetes insipidus but with sweet taste of glycosuric urine [1] only in previous condition. It causes disturbances in carbohydrate, protein and lipid metabolism and present with complications such as retinopathy, microangiopathy and nephropathy [2]. The currently available oral antihyperglycemic agents for clinical use have characteristic profile of side effects [3, 4]. Management of diabetes with agents devoid of any side effects is still a challenge to the medical system. This has led an increased demand for natural products with antihyperglycemic potential having fewer side effects. In the world Indian traditional medicine is one of the richest medicinal systems and many herbs, plant products of more than 400 plant species have been shown to have antihyperglycemic action [5-8]. Presently several laboratories are involved in isolation of new herbal hypoglycemic agents. But some of the plants reputed in the indigenous system of medicine are not scientifically established for their activities [9]. The plant *T. involucrata* Linn (Family: Euphorbiaceae) also called as Indian stinging-nettle, is mentioned for its bitter, acrid, sweet, cooling, diaphoretic, antiperiodic, depurative and alternant activities with its roots[10]. It is administered internally against suppression of urine [11] and used as a base of an external application in leprosy [12] and for its antipyretic and wound healing properties [13]. Various chemical constituents like alkaloids, flavonoids, lipids, phenolic compounds, proteins, saponins and triterpenoids have been reported [14]. The objective of this study was to evaluate the antidiabetic activity of Petroleum ether (PETI), Chloroform (CHTI) and Aqueous (AQTI) extracts of roots of *T. involucrata* in alloxan induced diabetic rats.

Methods

Preparation of the extract:

The plant *T. involucrata* (**Plate1**) was collected from shrubberies and hedges and were authenticated by a botanist of our college. The roots were separated, dried under shade, powdered in a grinder and was extracted successively with Petroleum ether (60-80°C) for 18 hours, completely dried marc extracted with chloroform for 18 hours and similarly with distilled water for 18 hours. The extracts were dried at 45 °C until solid/semisolid mass was obtained, stored in air tight container in refrigerator below 10°C. Petroleum ether and chloroform extracts were prepared as suspensions with tween-80 and the aqueous extract was dissolved in distilled water, were used throughout experimental studies.

Tragia involucrata Linn. (*Fagonia cretica* Linn)
Euphorbiaceae.

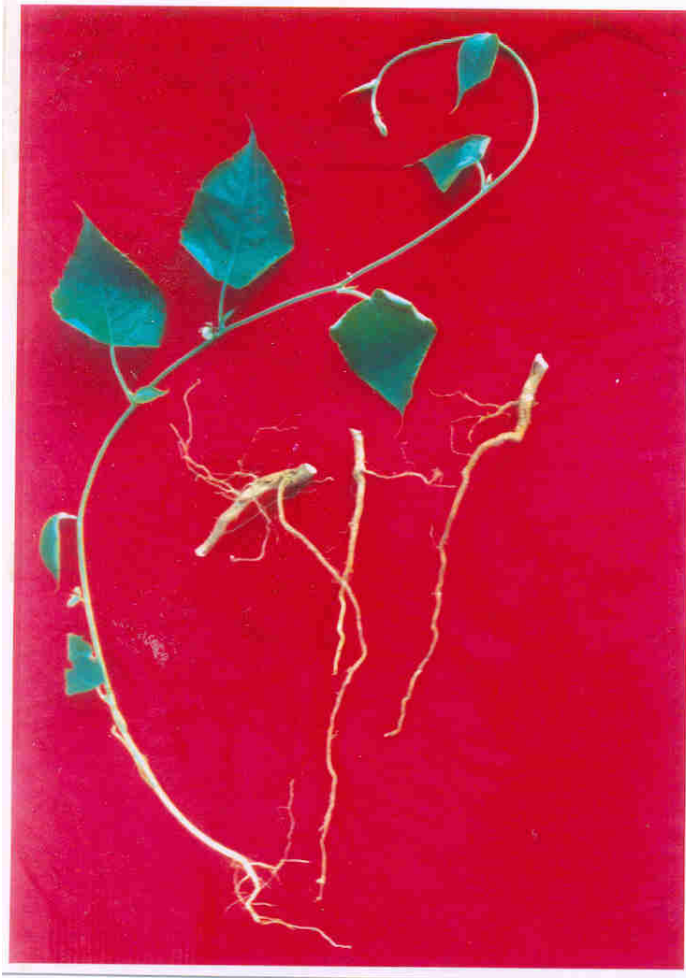


Plate-1

Preliminary phytochemical screening:

Preliminary phytochemical investigations were carried out on PETI, CHTI and AQTI extracts of *T.involucrata* by following standard methods [15, 16].

Determination of LD₅₀ of *T. involucrata*:

The acute toxicity of PETI (500mg/kg), CHTI (500mg/kg) and AQTI (1000mg/kg) of *T. involucrata* were determined by using albino mice of either sex (20-30gms) using the fixed dose (up and down method) by following OECD Guideline No: 425 [17]. 1/5th of the LD₅₀ dose of the respective extracts i.e., 100mg/kg, 100mg/kg and 200mg/kg were taken for the experimental study.

Experimental animals:

Male albino rats of Wistar strain (140-180gm) were procured from Venkateshwara Enterprises, Bangalore and were maintained under standard husbandry conditions of temperature (26 ± 2°C), relative humidity (45-55%) and 12 hours dark/light cycle. The animals were fed with standard pellet diet (Gold Mohr, Lipton India Ltd. Bangalore) and water supplied *ad libitum*. Good laboratory practice (GLP) was adopted for animal handling, and diabetes was induced in experimental animals by an intra peritoneal (150mg/kg) injection of alloxan monohydrate.

Anti-diabetic activity:

Experimental design:

Initially on 1st day under mild ether anaesthesia blood samples were collected by cardiac puncture from all the normal rats for estimation of blood glucose and biochemical parameters like serum cholesterol, triglycerides, HDL, LDL, Creatinine, urea and serum alkaline phosphate. After alloxan treatment the diabetic state was assessed by measuring fasting blood glucose concentration. After the induction of diabetes with alloxan the rats with blood glucose levels above 250mg/dl were selected for the study. The rats were divided into six groups as follows. Group I control rats given with 0.5 ml of vehicle, Group II with standard tolbutamide (80mg/kg, p.o) [18], Groups III, IV and V were treated with PETI (100mg/kg, p.o), CHTI (100mg/kg, p.o) and AQTI (200mg/kg, p.o) extracts respectively for 21 days. During this period, animals in all groups had free access to standard diet and water. Blood glucose levels were estimated on 1st, 7th, 14th and 21st day. On 21st day under mild ether anesthesia blood sample were collected from overnight fasted rats by cardiac puncture, centrifuged and serum glucose levels were estimated by oxidase/peroxidase method. Serum was also subjected for biochemical parameters estimation like serum cholesterol by CHOD-PAP method [19], triglycerides by DHBS colorimeter method [20], HDL by Phosphotungstate method [21], LDL by Friede Wald's equation from triglycerides [22], Creatinine (photometric) [23], urea [24] and serum alkaline phosphatase [25]. Whole pancreas from all the animals were removed and kept in 10% formalin solution for histopathological studies.

Effect of root extracts of *T. involucrata* on body weight in alloxan- induced diabetic rats: During the study period of 21 days the rats were weighed once in every week and the mean change in body weight were calculated.

Statistical analysis:

The results are expressed as mean \pm SEM. Comparison between the groups was made by analysis of variance (ANOVA) followed by Dunnet's 't' test, and $P < 0.05^*$, $P < 0.01^{**}$, $P < 0.001^{***}$ were considered as significant.

Results

Phytochemical investigation:

Preliminary phytochemical tests of roots of *T. involucrata* revealed the presence of tannins and flavonoids in PETI, alkaloids in CHTI and alkaloids and saponins in AQTI extracts.

Anti-diabetic activity:

At prefixed time intervals anti diabetic activity was assessed by monitoring the blood glucose levels and change in body weight in diabetic animals those were treated with PETI, CHTI, and AQTI extracts on 1st, 7th, 14th and 21st days. All the three extracts have shown a significant anti diabetic activity by reducing blood glucose levels and the effect was comparable to the standard anti-diabetic drug, tolbutamide. PETI and AQTI extracts showed a greater effect in reducing blood sugar level (table-1) than CHTI extract.

A steady decline in body weight was noticed with alloxan induced diabetic rats. AQTI extract treated group had better maintained an optimum body weight and so did with the PETI extract treated group than CHTI extract.

Discussion

The present investigation showed that in the alloxan induced diabetic rats all the extracts of *T. involucrata* have significantly reduced the blood glucose levels. Tolbutamide (80mg/kg) produced a lesser antidiabetic effect than *T. involucrata* extract. These results confirm the previously observed hypoglycemic effect produced by *T. involucrata* after the oral administration in healthy rats. Tolbutamide is a sulphonylurea derivative that produces experimental and clinical hypoglycemia in normal animals and in mild alloxan-diabetic animals. In type-2 diabetes tolbutamide increases the release of insulin by the beta cells of islets of pancreatic tissue. However, in severe alloxan-diabetic animals such as in type-1 diabetes, these animals do not have pancreatic beta cells. Thus tolbutamide does not produce hypoglycemic effect in this condition. Histopathological examination of islets of langerhans of pancreas of normal control group (**Plate 2**) showed that there were normal acini and normal cellular population with clearly brought out neuclei and cytoplasm.

Effect of root extract of *Tragia involucrata* on body weight in alloxan induced diabetic rats

Group	Treatment	Body weight at different days (grams)				
		0 hrs	1 st day	7 th day	14 th day	21 st day
A	Normal control	170±3.651	171.67±3.073 (0.98)*	166.67±2.108 (1.96)	171.67±3.073 (0.98)*	175±2.236 (2.94)
B	Diabetic control	175.00±2.236	161.67±3.073 (7.62)	175.00±2.236 (17.14)	120.00±2.582 (31.43)	96.67±2.108 (44.76)
C	Standard	171.67±3.073	161.67±1.667 (5.83)	158.33±3.073 (7.77)	156.67±2.108 (8.74)	155.00±2.236 (9.71)
D	PETI	165.00±2.236	153.33±2.108 (7.07)	148.8±1.667 (10.10)	143.33±2.108 (13.13)	140.00±3.651 (15.15)
E	CHTI	166.67±2.108	156.67± 2.108 (6.00)	136.67±2.108 (18.00)	130.00±2.582 (22.00)	118.33±4.773 (29.00)
F	AQTI	166.67±2.108	158.33±1.667 (5.00)	155.00±2.236 (7.00)	148.33±3.073 (11.00)	155.00±2.236 (7.00)
One way ANOVA						
	F		7.133	21.643	49.946	86.880
	df		30	30	30	30
	P		<0.01	<0.01	<0.01	<0.01

n=6

Significance P<0.05*, P<0.01**, P<0.001***,

A-Normal control (Tween 80), B- Diabetic control, C- Standard (Tolbutamide 80mg/kg),

D- Petroleum ether extract (100mg/kg), E- Chloroform extract (100gm/kg),

F-Aqueous extract (200gm/kg)

topathological photomicrographs of pancreas

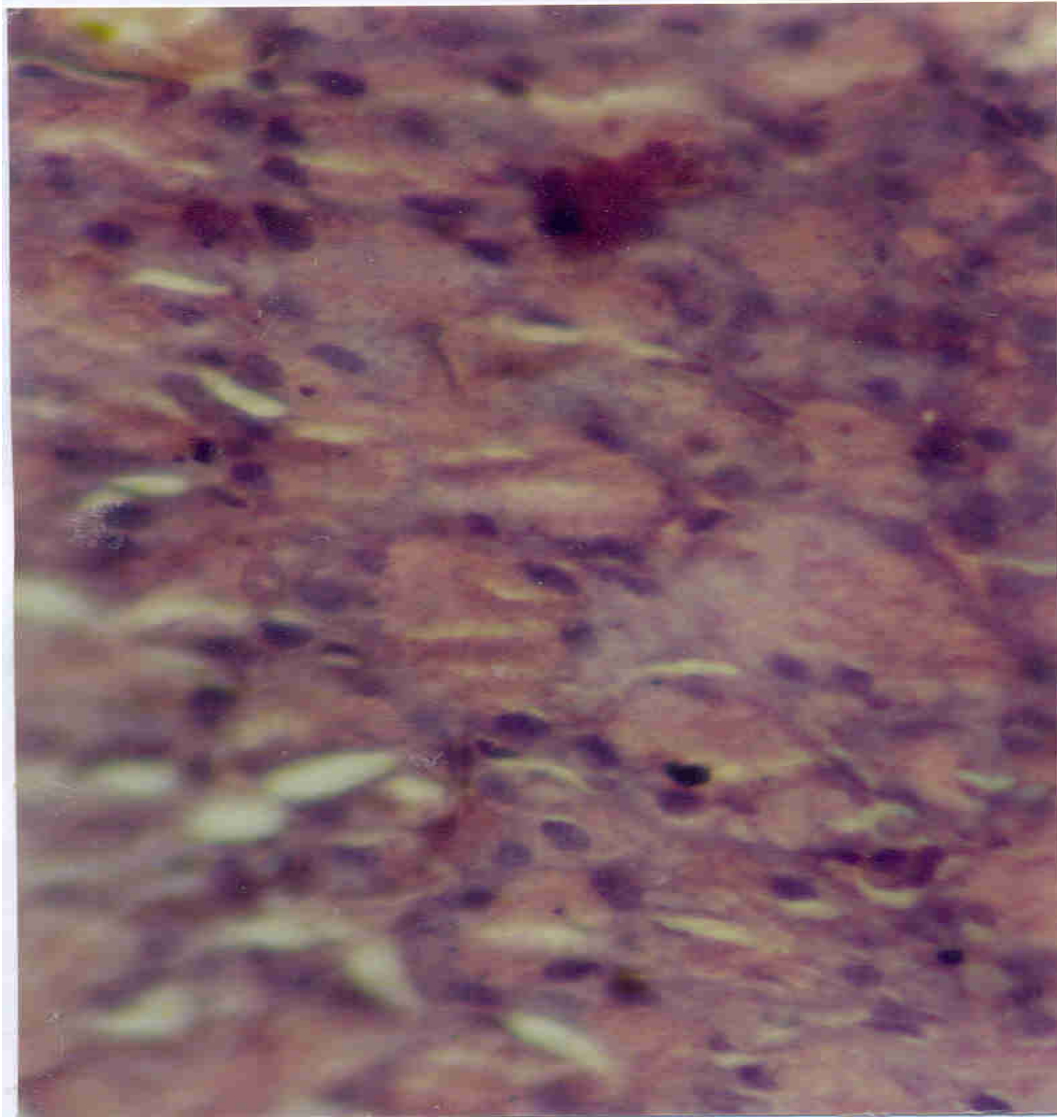


Plate-2
Normal control (40 x)

Diabetic control group (**Plate 3**) showed that dimensions of islet were considerably reduced with an extensive damage of islets cell, degenerative vascular and lytic changes in the islet of langerhans.



Tolbutamide treated group showed total restoration of cellular population including beta cells of langerhans with a marked hyperplasia and enlargement of islets (**Plate 4**).

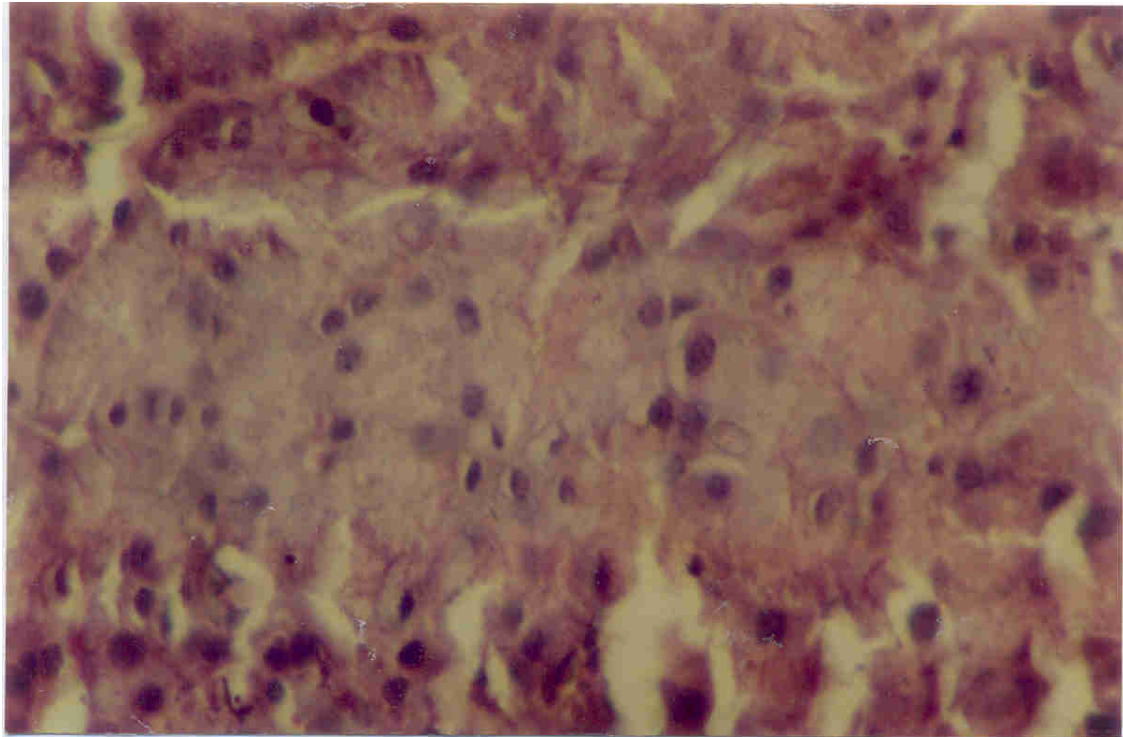


Plate-4
Tolbutamide treated (40 x)

PETI extract treated group showed restoration of cellular population with some damage to the beta cells, marked hyperplasia and enlargement of islet being revealed (**Plate 5**).



CHTI extract treated group showed that cellularity has been restored although few beta cells revealed vacuolated appearance (**Plate 7**).

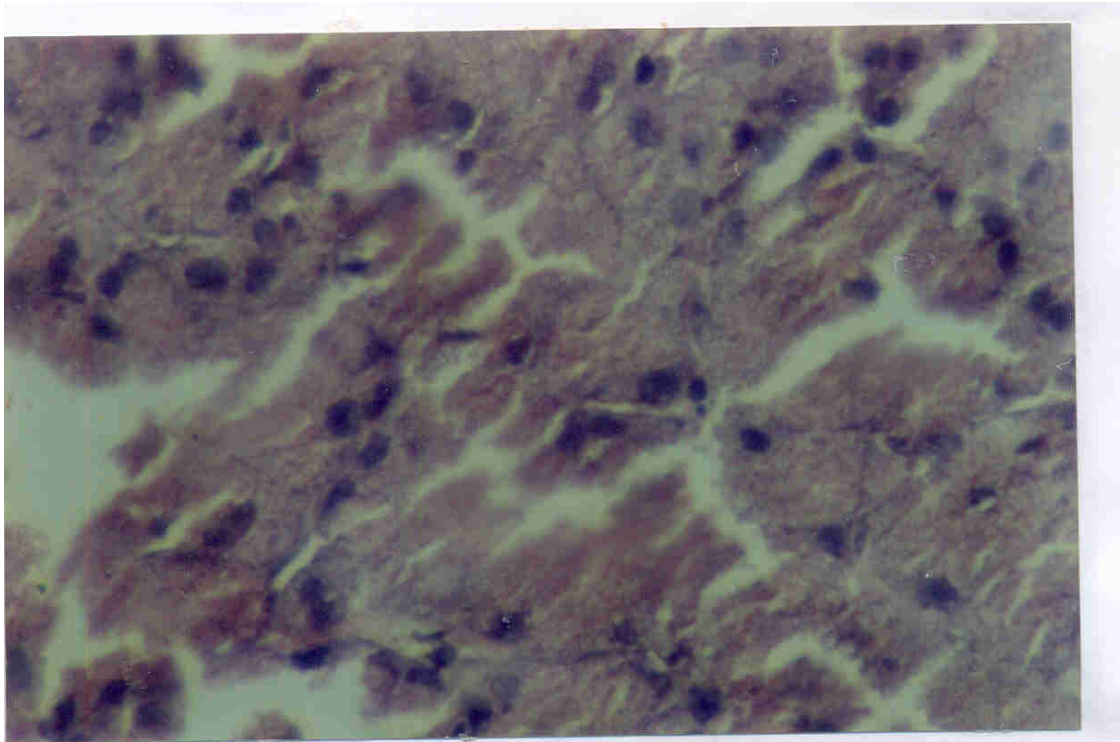


Plate-7
Chloroform extract treated (40 x)

AQTI extract treated group showed the restoration of normal architecture of pancreatic islet with evidence of hyperplasia and enlargement of islets (**Plate 9**).

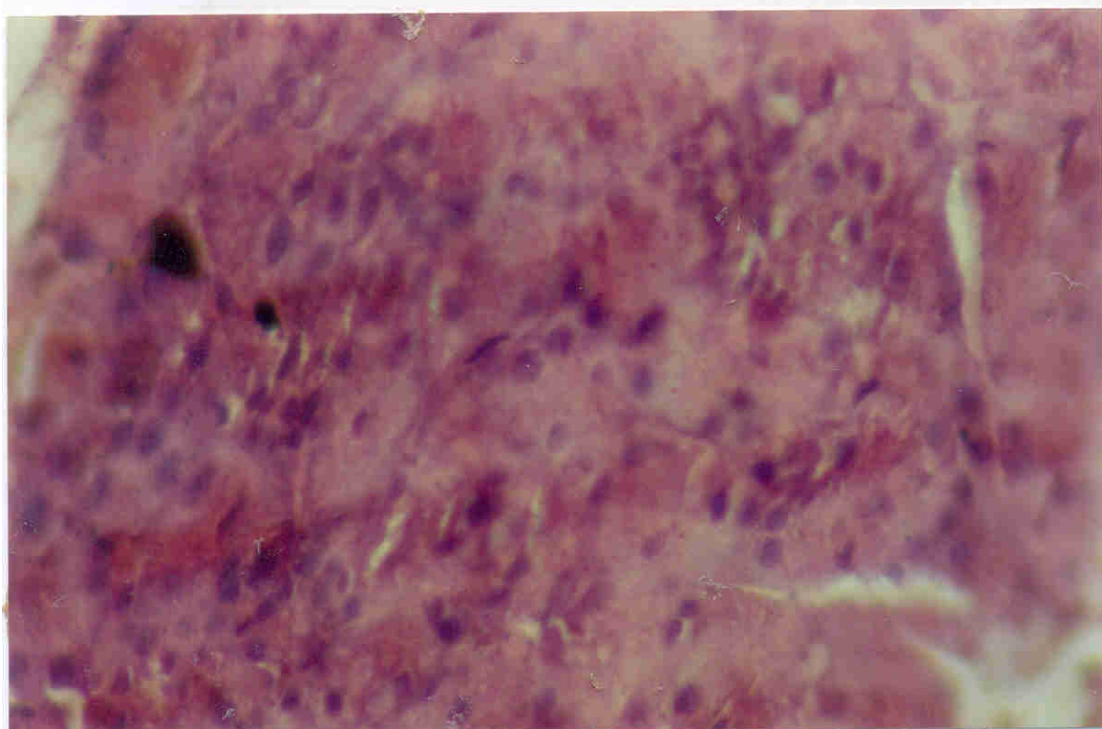


Plate-9
Aqueous extract treated (40 x)

The possible anti diabetic activity of the extracts might be due to stimulation of residual pancreatic insulin or by increasing peripheral utilization of glucose. Glycosides and flavanoids, tannins, organic sulphur compounds, catechol and alkaloids are active ingredients of hypoglycemic plants [26]. Flavanoids are reported to regenerate the damaged pancreatic beta cells [27] and glycosides stimulate the secretion of insulin in beta cells of pancreas [28]. Our preliminary phytochemical test revealed the presence of tannins, flavanoids, alkaloids and saponins in these extracts which might be accounted for anti diabetic activity.

In conclusion, the anti diabetic activity observed with our study was Standard >aqueous > petroleum ether >chloroform extract.

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Anti-diabetic activity of root extracts of *Tragia involucrata* in alloxan induced diabetic rats

Group	Treatment	BLOOD GLUCOSE LEVELS					BIOCHEMICAL PARAMETERS IN SERUM						
		0 hrs	1 st day	7 th day	14 th day	21 st day	Cholesterol	Triglycerides	HDL*	LDL	Creatinine	urea	Alkaline Phosphates
A	Normal control	78.5 ± 0.563	79.17 ± 0.477	78.67 ± 0.614	78.5 ± 0.428	78.83 ± 0.307	143.33 ± 3.827	89.5 ± 2.217	39.2 ± 1.470	92.7 ± 3.273	0.49 ± 0.0546	26.2 ± 1.014	120.3 ± 4.379
B	Diabetic control	175.00 ± 2.236	161.67 ± 3.073	145.00 ± 2.236	120.00 ± 2.582	96.67 ± 2.108	263.70 ± 3.593	202.3 ± 7.464	32.7 ± 0.8433	195.2 ± 5.741	1.41 ± 0.0532	63 ± 2.380	355.8 ± 6.416
C	Standard	79.67 ± 0.494	250.5 ± 2.579	205 ± 2.324 ***	139.33 ± .929***	91.17 ± .778***	160.0 ± 2.966	110.7 ± 3.242	52.0 ± 2.145	106.3 ± 5.031	0.55 ± 0.0629	30 ± 1.366	125.2 ± 5.218
			** (4.38)	(25.6)	(51.65)	(69.56)	(39.32)	(45.18)	(59.00)	(45.43)	(60.49)	(52.18)	(64.78)
D	PETI	76.83 ± 0.872	249.17 ± 2.469	217.83 ± 1.579***	164.5 ± 2.029***	121.33 ± .892***	191.30 ± 4.716	143.5 ± 2.487	38.80 ± 0.7923	125.7 ± 4.602	0.67 ± 0.0181	32.7 ± 1.202	143.2 ± 3.478
			*** (4.90)	(20.96)	(42.89)	(59.51)	(27.39)	(28.65)	(19.05)	(35.50)	(52.22)	(47.62)	(59.70)
E	CHTI	76.83 ± 1.014	253.67 ± 1.558**	231 ± 2.569***	194 ± 3.055	154.67 ± 2.004	229.0 ± 4.712	179.0 ± 4.211	34.0 ± 0.9309	152.8 ± 6.549	0.80 ± 0.0398	35.8 ± 2.120	205.2 ± 4.922
			(4.13)	(16.17)	(32.69)	*** (48.36)	(13.17)	(11.25)	(4.09)	(21.38)	(42.40)	(42.26)	(42.28)
F	AQTI	77.33 ± 0.955	256 ± 1.653	216.67 ± 1.944***	152.33 ± 2.186***	113.67 ± 2.603	174.7 ± 2.753	121.2 ± 1.939	46.2 ± 2.272	113.5 ± 3.862	0.54 ± 0.0542	27.8 ± 1.352	134.7 ± 3.765
			(2.29)	(21.41)	(47.13)	(62.03)	(33.76)	(39.7)	(41.63)	(41.57)	(61.05)	(55.68)	(62.05)
One way ANOVA		F	1496.00	1220.30	973.66	1460.20	138.25	111.45	23.148	56.821	48.818	69.047	360.92
		df	30	30	30	30	30	30	30	30	30	30	30
		P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

n=6, Significance P<0.05*, P<0.01**, P<0.001***,

A-Normal control (Tween 80), B- Diabetic control, C- Standard (Tolbutamide 80mg/kg), D- Petroleum ether extract (100mg/kg)

E- Chloroform extract (100gm/kg), F-Aqueous extract (200gm/kg)