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Original Research Paper

STUDY OF ANTIDIABETIC ACTIVITY OF *TYPHONIUM TRILOBATUM* IN GLUCOSE LOADED & ALLOXAN INDUCED HYPERGLYCEMIC AND NORMOGLYCEMIC RATS

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ABSTRACT

Objective: The objective of the present study aims to study the anti-diabetic activity of methanolic extracts of ethyl acetate and chloroform fraction of leaves of the selected plant *Typhonium trilobatum*. **Methods:** The hypoglycemic activity/antidiabetic activity of ethyl acetate and chloroform fraction of *Typhonium trilobatum* in single dose and multi dose treatment has been studied in normoglycemic animals and in diabetic animals. **Results:** The extracts of ethyl acetate and chloroform fraction of *Typhonium trilobatum* show anti-diabetic activity. **Conclusion:** From the results it was conformed that ethyl acetate fraction of plant shows higher anti-diabetic activity than chloroform fraction.

Keywords: *Typhonium trilobatum,* Antidiabetic activity, Alloxan.

INTRODUCTION

Diabetes Mellitus

The term diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms like thirst, polyurea, blurring of vision, and weight loss.[1] This is affecting nearly 25% of the population.[2] Clinically diabetes patients are characterized by marked increase in blood sugar level followed by normal or mild hyperlipidemia along with long term vascular and neurological complication. The management of diabetes mellitus is considered as global problem whose successful treatment is yet to be discovered. The modern drugs like insulin , oral hypoglycemic agents , control the blood sugar level as long as they are regularly administered.[3,4] In today's scenario the herbal product are more preferred than the synthetic products as they less toxic and less side effects when compare with synthetic products. Extracts of various plants viz. methanolic extract of root bark[5], ethanol insoluble extract of the leaves[6] aqueous extract of the leaves of shoot cultures[7] and the leaves[8] were reported to possess anti-diabetic effect. Therefore the herbal plants of *Typhonium trilobatum*[9] selected for the study. In traditional practice, the leaves of some of the species of Typhonium genus are used in DM. So the leaves of Typhonium genus compelled us to study the effect of *Typhonium trilobatum*[6] leaves on DM. Typhonium trilobatum is a genus in the Araceae family endemic to tropical Asia, the South Pacific, and Australia. It consists of approximately 50 species that are typically found growing in wooded areas. It has been valued in

Ayurveda and Unani systems of medicine for possessing variety of therapeutic properties. According to Ayurveda, the rhizome is used with effect for treating vomiting, cough, asthma, excessive expectoration, pyogenic sore throat, headache, gastric ulcer, abscess, snake bite. This study was designed to explore the healing effects of topically applied *Typhonium trilobatum* extracts in rat intraoral rout.[10, 11]

MATERIALS AND METHODS

Plant Collection

Large number of leaves of *Typhonium trilobatum* appears in the month of July and August. Leaves were collected from the Interior Villages of West Bengal. Before their use they were carefully identified by Prof. Sushil Ku. Mallick of S.V.M Autonomous College, Jagatsingpur.

Preparation of the Powder

The leaves were dried over a polythene cover in shade drying method with the help of a fan at 21^0 C room temperature and pulverized using a mechanical grinder. The powder is further passed through a fine mesh sieve to get a fine powder.

Preparation of the Extract and Its Fraction

The powdered material was extracted with methanol by reflux condenser method. The liquid extracts were concentrated under vacuum to yield dry extracts and preserved in a desiccators till further experiments & used for fraction with chloroform (CME-Chloroform Methanolic Extract) and ethyl acetate (EAME-Ethyl Acetate Methanolic Extract) by the use of separating funnel.

Preparation of the Drug Solution

Preparation of the Solution of the Fractions

The semi-solid fractions of *Typhonium trilobatum* are dissolved in sufficient quantity of solvents (tween and normal saline) and then were used for administration.

Glibenclamide

Glibenclamide 2.5 mg/kg body .wt was dissolved in sufficient quantity of solvent (normal saline) and used in treatment.

Alloxan

Alloxan is a cyclic urea analogue and is composed of 1-methyl-1-nitroso urea and glucose and have the unique property of producing chronic experimental diabetes by specific cytotoxin action of β-cells of the islets of langerhans degeneration. Alloxan 120mg/kg body .wt. was dissolved in the normal saline and used to induce diabetes in rats.[13]

Selection of Animals

Healthy adult albino rats of Wister strain weighing 150-200 gms and adult albino mice of Swiss strain weighing 20-25 gms were selected for toxicological and anti-diabetic studies respectively with the approval from the Institutional Animal Ethics Committee. CPCSEA number: 955/A/06/CPCSEA (CPCSEA stands for Committee for the Purpose of Control & Supervision of Experiments on Animals, India)

Maintenance of Animals

The animal house was well ventilated and animals had 12 ±1 hr. day and night schedule with temperature between 11-20 $\pm 2^0$C. The animals were housed in large spacious hygienic cages during the course of experimental period. The animals were feed with rat pallets feed supplied by M/s Hindustan Liver Ltd., Bangalore, India and Aquaguard filtered water. The place where the experiments were conducted kept very hygienic by cleaning with antiseptic solutions.

Models for Anti-Diabetic Screening

Hypoglycemic activity/anti-diabetic activity of various fraction of methanolic extract of *Typhonium trilobatum* in single dose treatment.

- In normoglycemic animals
- In diabetic animals

Hypoglycemic activity/anti-diabetic activity of various fraction of methanolic extract of *Typhonium trilobatum* multi dose treatment.

- In normoglycemic animals
- In diabetic animals

EXPERIMENTAL SETUP

Hypoglycemic Activity Of various fraction of *Typhonium trilobatum* in single dose treatment *In Normoglycemic Animals*

To check whether the various fraction of *Typhonium trilobatum* is acting as an insulin secretagogue (stimulation of insulin secretion

from the pancreatic β-cell) the study was carried out as mentioned below.

Table 1: Overnight fasted animals were grouped in 6 groups of 6 animals each groups

Groups	Animals used	Sex	Treatment
Gr.I	Albino rats	M	Served as control (normal saline).once daily
Gr.II	Albino rats	M	Served as standard (glibenclamide 2.5 mg/kg)in p.o once
Gr.III	Albino rats	M	EAME 150 mg/kg (p.o/i.p) dose -1 once daily.
Gr.IV	Albino rats	M	EAME 300 mg/kg (p.o/i.p) dose -2 once daily
Gr.V	Albino rats	M	CME 150 mg/kg (p.o/i.p) dose -3 once daily
Gr.VI	Albino rats	M	CME 300 mg/kg (p.o/i.p) dose -4 once daily

Blood glucose levels were measured in all 6 groups in different time interval like 0, 1,2,4,6 hrs.

Alloxan Induced Diabetic Animal

To check whether the various fraction of *Typhonium trilobatum* has anti diabetic activity. The study was carried out was mentioned below.

Table2: Overnight fasted animals were grouped in 6 groups of 6 animals each groups

Groups	Animals used	Sex	Treatment
Gr.I	Albino rats	M	Served as control (normal saline).once daily
Gr.II	Albino rats	M	served as standard (glibenclamide 2.5 mg/kg)in p.o once daily
Gr.III	Albino rats	M	EAME 150 mg/kg (p.o/i.p) dose -1 once daily
Gr.IV	Albino rats	M	EAME 300 mg/kg (p.o/i.p) dose -2 once daily
Gr.V	Albino rats	M	CME 150 mg/kg (p.o/i.p) dose -3 once daily
Gr.VI	Albino rats	M	CME 300 mg/kg (p.o/i.p) dose -4 once daily

Blood glucose levels were measured in all 6 groups in different time interval like 1, 2,3,4,6 hrs. (All these rats are treated with Alloxan 120 mg/kg i.p prior to 72 hrs.)

Glucose Loaded Hyperglycemic Animals

It is a standard procedure used to monitor blood glucose level over a period of time following glucose load. To check whether the various fraction of *Typhonium trilobatum* has hypoglycemic activity as mentioned below.

Table 3: Overnight fasted animals were grouped in 6 groups of 6 animals and in each group glucose (1gm/kg) was loaded to all the groups except Gr.I

Group	Animal used	Sex	Treatment
Gr.I	Albino rats	M	Served as control(normal saline).once daily
Gr.II	Albino rats	M	Served as control(normal saline).once daily
Gr.III	Albino rats	M	EAME 150 mg/kg (p.o/i.p) dose -1 once daily
Gr.IV	Albino rats	M	EAME 300 mg/kg (p.o/i.p) dose -2 once daily
Gr.V	Albino rats	M	CME 150 mg/kg (p.o/i.p) dose -3 once daily
Gr.VI	Albino rats	M	CME 300 mg/kg (p.o/i.p) dose -4 once daily

Blood glucose levels were measured in all 6 groups in different time interval like 1, 2, 4 hr.

Anti-Diabetic or Hypoglycemic study of various fractions of *Typhonium trilobatum* in multi dose treatment.

In Normoglycemic Animals

To check whether the various fraction of *Typhonium trilobatum* has hypoglycemic activity on the multiple dose treatment is shown below.

Table 4: Overnight fasted animals were grouped in 6 groups of 6 animals in each group

Group	Animal used	Sex	Treatment
Gr.I	Albino rat	M	Served as control (normal saline).once daily
Gr.II	Albino rat	M	Served as standard(Glibenclamide 2.5mg/kg)in p.o once daily
Gr.III	Albino rat	M	EAME 150 mg/kg (p.o/i.p) dose-1 once daily
Gr.IV	Albino rat	M	EAME 300 mg/kg (p.o/i.p) dose-2 once daily
Gr.V	Albino rat	M	CME 150 mg/kg (p.o/i.p)dose-3 once daily
Gr.VI	Albino rat	M	CME 300 mg/kg (p.o/i.p)dose-4 once daily

Blood glucose levels & body wt. were measured on 0^{th}, 7th, 14th, 21st day.

In Diabetic Animals

To check whether the various fraction of *Typhonium trilobatum* has anti-diabetic activity on the multiple dose treatment in Alloxan induced animals; the study was carried was mentioned below.

Table 5: Overnight fasted animals were grouped into 6 groups of 6 animals in each group

Group	Animal used	Sex	Treatment
Gr.I	Albino rat	M	Served as control(normal saline).once daily
Gr.II	Albino rat	M	Served as standard(Glibenclamide 2.5mg/kg)in p.o. once daily
Gr.III	Albino rat	M	EAME 150 mg/kg(p.o/i.p)dose 1. once daily
Gr.IV	Albino rat	M	EAME 300 mg/kgt (p.o/i.p)dose 2. once daily
Gr.V	Albino rat	M	CME 150 mg/kg(p.o/i.p)dose 3. once daily
Gr.VI	Albino rat	M	CME 300 mg/kg (p.o/i.p)dose 4. once daily

Blood glucose levels and body weight were measured on 0^{th}, 3rd, 7th, 14th and 21^{st} day

Toxicity Studies

Acute Oral Toxicity Studies

The acute toxicity study is aimed to establish the therapeutic index i.e, the ratio between the pharmacologically effective dose and the lethal dose and also to perform the primary screening. Various fractions of *Typhonium trilobatum* was administered once orally at 5 dose levels (500, 1000,1500,2000,3000 gm/kg) to group of 10 mice of both the sexes about equal in number which have been fasting overnight (about 18 hrs). The treated mice were observed continuously for 2 hr. and then occasionally for further for four hrs and finally overnight mortality recorded. During the course of study the behaviors of the mice were carefully observed and fall of time, reduction of spontaneous activity also determined.

Sub Acute Toxicity Studies

The purpose of this study is to determine the maximum tolerated dose and daily dose for 3-4 weeks to indicate the nature of toxic reaction of the drug. In present study, the influences of *Typhonium trilobatum* fractions on haematological parameters, a pathological change on 21 days dosing in Alloxan induced diabetic rats was carried out. The diabetic rats were grouped into 6 of 6 animals each. Group 1 animals received solvent , Group III,IV,V,VI was received ethyl acetate and chloroform fraction at two dose level (150mg/kg and 300mg/kg) p.o , once daily for 21 days. At the end of the experiment period the blood was collected, serum separated and subjected to hematological and biochemical examination.

Induction of Diabetes

In the present study a single dose of Alloxan in normal saline 120mg/kg body weight was administered intraperitonially. Diabetes developed gradually was assessed after a week and an experiment was carried out to determine the blood sugar levels. Animals with blood sugar levels 200-250 mg/dl were chosen on 7^{th} day and considered as anti-diabetic screening.

Blood Collection

A small amount of blood collected without sacrificing the animals by orbital sinus puncture. The rats were made semi conscious with ether using the sterile capillary tube , puncture the orbital sinus at the inner canthus of the eye, by rotating the capillary tube with sufficient but not excessive pressure ,two or three times. As bleeding starts, the animal was held closed to the centrifuge tube and blood was collected. Applying pressure on the inner canthus for a short while stopped the bleeding. The collected blood was used to determine the biological parameters.

Determination Blood Glucose Levels

A small amount of blood collected without sacrificing the animals by orbital sinus puncture or by snipping off the tip of the tail. The rats were made semi- conscious with ether using the sterile blunt needle ; puncture the orbital sinus at the inner canthus of the eye , by rotating the needle with sufficient but not excessive pressure , two or three times as described in sub-acute toxicity study. As bleeding starts, the animal was held close to the haemogluco test strip and allows the drop of the blood to fall on the strip. The bleeding was stopped by applying pressure on the inner canthus for a short while. When the instrument gives a beep sound after 1min, the test strip was inserted. Then as bleeding starts, the animal was held close to the haemogluco test strip and allows the drop of the blood to fall on the strip. Then the instrument was allowed to react for one minute. Then the blood glucose levels were displayed on the screen was recorded.

Preparation of Serum

The collection blood was kept at room temperature, refrigerated and centrifuged, for 20 min at 2000 rpm to separate serum.

RESULTS AND DISCUSSIONS

Preliminary Phytochemical screening of extracts of *Typhonium trilobatum*

Test for Chemical Groups	Ethyl Acetate Fraction
Alkaloids	+
Glycosides	-
Reducing sugars	-
Gums	+
Flavonoids	+
Tannins	-
Saponin	-
Seroids/terpenoids	+
Proteins	-

Preliminary Phytochemical screening revealed that methanolic extract of *Typhonium trilobatum* contained alkaloids, steroids, terpenoids, flavonoids and polyphenolics compound. The presence of flavonoids and steroids are known to be bioactive for management of diabetes.[14] It is well known that certain flavonoids exhibit hypoglycemic activity.[15] and also known for their ability of beta cell regeneration of pancreas.[16] The steroids have also shown to decrease blood sugar in experimental animal models.[17] Thus the significant antidiabetic effect of methanolic extracts of *Typhonium trilobatum* may be due to the presence of more than one antihyperglycaemic principle and their synergistic properties.

Table 7: Hypoglycemic activity of various fractions of *Typhonium trilobatum* in single dose treatment in glucose loaded hyperglycemic rats in oral route

Group	0 hr.	Initial	1 hr	2 hr	4 hr	% Age decrease at the end of 8 hr
Gr.I	104.33±6.20	127.66±5.58	123.33±4.14	136.83±5.32	103.16±9.61	1.43
Gr.II	104.66±6.23	145.83±6.24	120.16±9.07	90.83±5.31c	55.83±5.06b	46.65
Gr.III	99.83±5.70	138.83±6.40	113.33±7.26	92.66±5.96c	72.33±6.92a	27.54
Gr.IV	107.33±6.25	145.83±3.96	101.66±6.28	87.16±4.36c	68.83±6.73a	35.87
Gr.V	93.16±7.28	132.16±8.88	121.16±6.75	105.66±7.40b	92.16±6.50	1.07
Gr.VI	99.83±7.60	139.16±9.78	118.33±6.00	102.83±6.85b	88.16±9.37	11.68
F- Values	0.58	4.06 **	1.38	9.33 **	5.33 **	

Values are expressed in MEAN±S.E.M of six animals. One -way ANOVA followed by Dunnet's t-test. {F-values denotes statistical significance at (p<0.05*,p<0.01**)} , { t-value denotes (p<0.05a, p<0.01b, 0.001c) in comparision to group.1}

Table 8: Hypoglycemic activity of various fractions of *Typhonium trilobatum* in single dose treatment in normoglycemic rats in oral route

	0 hr.	Initial	1 hr	2 hr	4 hr	% Age decrease at the end of 8 hr
Gr.I	104.66±8.21	104.83±8.17	102.66±7.32	106.16±7.93	103.16±6.64	1.43
Gr.II	103.33±7.21	90.16±5.59	84.66±6.78	79.33±7.73a	53.3±5.27c	48.38
Gr.III	94.16±5.06	92.33±7.31	86.66±7.71	81.66±7.81	72.83±6.75b	22.65
Gr.IV	116.16±6.47	87.33±8.47	71.16±8.85a	65.66±8.56b	59.33±8.04[b]	48.92
Gr.V	113.33±6.36	92.66±8.83	89.33±9.37	75.66±9.35a	68.66±7.30b	34.15
GrVI	95.16±5.45	87.66±7.66	84.16±9.25	73.33±6.91a	62.66±6.93b	39.41
F- Values	1.90	0.69	1.49	2.929**	6.57**	

Values are expressed in MEAN±S.E.M of six animals. One -way ANOVA followed by Dunnet's t- Test. {F-values denotes statistical significance at (p<0.05*, p<0.01**)}, {t-value denotes (p<0.05a, p<0.01b, 0.001c) in comparison to group.1}

Effect of Various Fractions of *Typhonium trilobatum* on Alloxan Induced Diabetic Rats

The EAME at a dose level of 150 mg/kg & 300 mg/kg significantly decreases the blood sugar level at a significance of (p<0.01) at the end of 1 hr. and (p<0.001) at the end of 2 hr , 3 hr , 4 hr , and 6 hr. While the CME at the dose level of 150 mg/kg significantly decrease the blood glucose level at a significance of (p<0.01) at the end of 1hr and (p<0.001) at the end of 2 hr , 3 hr , 4 hr & 6 hr. when compared with solvent control. The blood glucose lowering capacity of EAME of T.T. is comparable with the standard drug Glibenclamide (2.5mg/kg) body wt. Among the fractions the EAME at a dose level of 300 mg/kg through oral route possess highest percent decrease of blood sugar level.

Table 9: Effect of various fractions of *Typhonium trilobatum* on Alloxan induced diabetic rats

Group	0 hr.	Initial	1 hr	2 hr	4 hr	% Age decrease at the end of 8 hr
Gr.I	290.5±7.41	280.0±4.2	295.3±11.1	278.0±11.0	268.5±10.2	6.54
Gr.II	270.3±6.52	250.1±11.5b	200.5±7.6c	150.5±9.0c	90.0±6.9c	75.77
Gr.III	280.3±10.64	260.0±9.3b	240.1±4.9c	210.5±8.6c	150.5±7.5c	55.51
Gr.IV	293.5±12.46	277.1±8.8b	261.5±7.3c	101.0±7.1c	98.1±3.5c	68.55
Gr.V	261.3±11.25	239.5±7.3b	218.3±6.8c	180.0±6.0c	165.3±2.5c	38.73
GrVI	287.16±12.87	261.6±7.5	247.0±6.2	175.6±5.1c	158.0±2.1c	50.3
F- Values	8.87 **	53.14 **	488.48 **	404.87 **	309.25**	

Values are expressed in MEAN±S.E.M of six animals. One -way ANOVA followed by Dunnet's test. {F-values denotes statistical significance at (p<0.05*,p<0.01**)} , { t-value denotes (p<0.05a, p<0.01b, 0.001c) in comparison to group.1}

Hypoglycemic Activity of the Various Fractions of *Typhonium trilobatum* in Multi-Dose Treatment in Normoglycemic Rats in Oral Dose

The purpose of the study is to establish the therapeutic value of the test fractions of T.T. in long term use. The data showed in this model demonstrated that there was a decrease in the blood sugar level in the extent of 47.70%, 12.67%, 21.40%, and 25.22% in the case of both the fractions at the dose level of 150 mg/kg & 300 mg/kg respectively on the 21st day of the treatment. While the standard drug on the same day bears 50.93% reduction at the same time tested dose level & the standard drug is $p<0.001$ on the 21st day. The observed data results of the experiments suggested that the methanolic extract (ethyl acetate fraction at 300 mg/kg) of T.T. able to maintain the hypoglycemic effect up to 21st day & no behavioral changes are observed during the treatment periods.

Table 10: Hypoglycemic Activity of the Various Fractions of *Typhonium trilobatum* in Multi-Dose Treatment in Normoglycemic Rats in Oral Dose

Group	0th day	7th day	14th day	21st day	% Age decrease at the end of 8th hr.
Gr.I	104.66±3.60	104.83±3.57	104.16±3.97	105.16±4.60	
Gr.II	106.66±5.72	86.66±6.46	68.83±9.57^{b}	52.33±8.83b	50.93
Gr.III	94.66±7.68	91.16±6.71	86.83±7.76	82.66±7.53	12.67
Gr.IV	116.33±4.86	87.66±6.98	72.66±6.98	60.83±8.70a	47.70
Gr.V	113.66±5.70	100.66±7.9	92.83±6.35	89.33±4.80	21.40
GrVI	95.83±7.12	87.66±7.52	83.66±8.75	71.66±10.30a	25.22
F- Values	2.24	1.33	3.04 *	6.23 **	

Values are expressed in MEAN±S.E.M of six animals. One -way ANOVA followed by Dunnet's test. {F-values denotes statistical significance at ($p<0.05$*,$p<0.01$**)} , { t-value denotes ($p<0.05$a, $p<0.01$b, 0.001c) in comparision to group.1}

Anti-diabetic Activity of Various Fractions of *Typhonium trilobatum* in Multi-Dose Treatment in Alloxan Induced Hyperglycemic Rats in Oral Dose

The purpose of the study is to confirm the anti-diabetic effect of the test extract on longer duration of treatment. In this model the EAME registered 55.79%, 59.21% and CME registered 46.08%, 54.14% of decrease of blood glucose level at the tested dose level of 150 & 300 mg/kg respectively on the 21st day of the treatment. While the standard drug showed 60.87% reduction on the same day. The EAME at 150 mg/kg and 300 mg/kg bears a significant of $p<0.05$ to $p<0.01$ at the 7th day and $p<0.001$ on the 14th day & 21st day treatment. Whereas CME at 150 mg/kg & 300 mg/kg bears a significance of $p<0.01$, $p<0.05$ on the 7th day & $p<0.001$ on the 14th &21st day treatment. While the standard drug showed the significance of $p<0.001$ which started from 7th day onwards when compared with the solvent control. The study further support the anti-diabetic effect of the test extract whose effectiveness persist up to 21 days and the blood sugar level decrease gradually during the observed days. Which presumed that both the fractions, contains some anti-diabetic active principle responsible for this effect. It is therefore, conceivable that hypoglycemic principle in the fractions exert a direct effect in anti-diabetic rat probably by a mechanism similar to insulin. The experiment revealed that EAME & CME of T.T. at both dose level significantly ($p<0.001$) decrease the glucose level on hyperglycemic animal. The glucose lowering activity observed in the diabetic animal may due to the stimulation of the β-cells in the pancreatic islets

Table 11: Anti-diabetic Activity of Various Fractions of *Typhonium trilobatum* in Multi-Dose Treatment in Alloxan Induced Hyperglycemic Rats in Oral Dose

Group	0th day	7th day	14th day	21st day	% Age decrease at the end of 8th hr.
Gr.I	291.83±6.24	278.33±7.26	265.83±7.79	244.16±8.60	231.66±7.14
Gr.II	275.16±5.19	255.16±9.57	188.83±4.26c	152.66±6.17c	107.66±8.08c
Gr.III	284.66±9.99	263.16±11.40a	238.33±5.27a	166.33±7.49c	125.83±89.98c
Gr.IV	290.16±5.29	251.66±9.18	228.83±8.08b	136.66±7.62c	118.33±9.00c
Gr.V	265.83±5.38a	248.16±4.86	221.33±10.89b	164.83±5.87c	143.33±6.28c
GrVI	289.33±6.67	268.16±7.75	234.16±8.50a	178.33±6.79c	132.66±7.22c
F- Values	2.36	1.73	10.37 **	27.09 **	

Values are expressed in MEAN±S.E.M of six animals. One -way ANOVA followed by Dunnet's test. {F-values denotes statistical significance at (p<0.05*,p<0.01**)} , { t-value denotes (p<0.05a, p<0.01b, 0.001c) in comparision to group.1}

Determination of Hematological Parameters

Haemoglobin concentration in the blood sample was estimated as per the standard procedure. The Hb concentration in the blood was expressed as

Table 12: Determination of Hematological Parameters

Group	RBC	WBC	Hb (g/dl)	Clotting time(min)	% Neutrofil	% Esonophil	% Lymphocytes	% monocyte
Gr.1	4.83±1.07	6.83±1.22	12.66±0.88	1.06±0.31	27.83±3.87	1.90±0.49	69.16±8.20	2.83±0.47
Gr.II	5.1±0.6	7.1±1.24	11.84±1.4	1.05±0.07	32.67±1.5	1.55±0.07	68.67±3.3	1.9±0.2
Gr.III	3.66±0.42	5.83±0.94	8.33±1.17	1.35±0.39	24.16±4.36	2.66±0.91	72.66±7.10	3.66±0.66
Gr.IV	4.16±1.07	6.66±0.76	11.83±0.70	1.23±0.24	26.83±5.01	2.16±0.47	70.33±7.82	3.33±0.76
Gr.V	3.16±0.79	5.66±0.98	8.33±1.45	1.30±0.41	23.66±4.60	2.83±0.60	72.33±7.33	3.83±0.90
Gr.VI	3.83±1.01	6.16±1.24	11.83±1.88	1.28±0.42	25.16±4.79	2.50±0.67	70.83±9.16	3.50±0.76
F- Values	0.78	0.57	0.81	0.09	0.26	2.49	0.03	0.57

The hematological parameters exhibited in table showed that the animals treated with standard drug Glibenclamide and test dose levels bears normal value in RBC count and Hb count. Whereas the clotting time slightly elevated than normal. However, the diabetic rats treated with solvents showed a decrease value of RBC, WBC and Hb content when compared with normal. Therefore, it might be suggested that the test fractions has no significant effect on the haematological parameters and is evident for the safely used the EAME of *Typhonium trilobatum* leaves for a longer duration time

CONCLUSION

The ethyl acetate fraction (EAME) of leaf extract of *Typhonium trilobatum* (EAME) results maximum yield value than chloroform fraction (CME). The ethyl acetate fraction showed maximum control in blood sugar hyperglycemic Wister rats than other fraction. The ethyl acetate fraction reduces the blood sugar level to a maximum extent in both normoglycemic and hyperglycemic model through the oral route. Toxicological study reveals that the EAME were safe and does not alter normal physiological and behavioral effect even at a higher dose level of 3000 mg/kg body weight. Administration of EAME significantly reduces the elevated glucose level in alloxan induced diabetic rats confirms the anti-diabetic activity. This also reduces the normal glucose level, which reveals the hypoglycemic property. The results of the present investigation indicate that the EAME may have a place in the therapy of DM as an anti-diabetic or hypoglycemic agent. The hypoglycemic or anti-diabetic effect of the EAME may be due to the influence of glycogensis, glycogenolysis metabolic activity property of one or more of its constituents. Thus it is concluded that the EAME

of leaves of *Typhonium trilobatum* is beneficial in lowering the blood sugar concentration and its management of other diabetic complications without any doubt.

FUTURE STUDIES

In future, the active principle responsible for the effect to be isolated and further pharmacological investigation to be carried out to elucidate the mechanism of hypoglycemic or anti-diabetic effect of EAME and CME of *Typhonium trilobatum*. More Pre-clinical as well as Clinical studies are required to establish whether the administration of EAME and CME of *Typhonium trilobatum* can potentiate the anti-diabetic and hypoglycemic effect of conventional anti-diabetic and /or hypoglycemic agents. There is a requirement to study the anti-oxidant property and glucose uptake potential of the EAME and CME of *Typhonium trilobatum* for obtaining additional information relating to the mechanism of action.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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