e-ISSN: 0975-5160, p-ISSN: 2820-2651

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2025; 15(2); 34-43

Original Research Article

Phytochemical and Pharmacological Evaluation of Crinum Latifolium in Streptozotocin Induced Diabetes in Wistar Rats

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Received: 18-3-2025 / Revised: 21-4-2025 / Accepted: 26-5-2025
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Conflict of interest: Nil

Abstract:

Background: Leaves and their bioactive constituents are used for the treatment of diabetes mellitus throughout the world; especially in countries where access to the conventional treatment of diabetes mellitus is inadequate. There have been several reports on the hypoglycemic activity of Crinum latifolium (CL). Hence, the present study was undertaken to investigate the anti-diabetic effect of CL leaves extract alone and in combination with an oral hypoglycemic drug, Glibenclamide on Streptozotocin induced diabetic rat models.

Methods: Streptozotocin (45mg/kg, I.P) was used to induce diabetes in albino rats weighing 200- 250 g. The fasted diabetic rats were divided in to 6 groups of 6 animals each. Group I served as the normal control, receiving only the vehicle. Group II Streptozotocin (45mg/kg, I.P) was used to induce diabetes. Group III was treated with standard drug Glibenclamide (10mg/kg) administration. Groups IV, V and VI were pre-treated with ethanolic extract of Crinum latifolium at doses of 100mg/kg, 200mg/kg, and 400mg/kg respectively. Biochemical parameter Such as serum was analyzed for total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL).

Result: The fasting blood glucose levels will be determined on day 0, 7, 14, 21 and 28th day by using glucometer. Data were statistically analyzed by t-Test followed by Dunnet's multiple comparison test. All the test groups showed significant ($P < 0.05$) decreased fasting blood glucose levels in Streptozotocin induced diabetic rats treated with the aqueous extract of CL. However, the Combination of CL extract with Glibenclamide produced a significant ($P<0.05$) decrease in blood glucose level which is higher than that produced by Glibenclamide alone. The decrease in blood glucose levels is probably due to reduction in the intestinal absorption of glucose.

Conclusion: CL possesses significant hypoglycemic activity probably due to presence of flavonoids, tannins, steroids and trepenoids and the aqueous extract of leaves contain higher levels of phenol and flavonoids which exhibit greater antioxidant activity by lowering the diabetic complication.

Keywords: Type 2 Diabetes mellitus; Crinum latifolium, Antioxidant, Antidiabetic, TLC.

Introduction

Medicinal herbs are significant source of pharmaceutical drugs. Latest trends have shown increasing demand of phytodrugs Herbal medicines are the drugs or preparations made from plants or parts of plants (leaves, root, bark, seeds and flowers) valued for its medicinal, aromatic or savory qualities to treat the symptoms of wide range of problems from depression to cold and flu. [1] It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. [2] Some medicinal herbs have proven antidiabetic activity potential. Glibenclamide, also known as glyburide, is an antidiabetic drug in a class of medications known as sulfonylureas, closely related to sulfonamide antibiotics is a popular remedy for pancreatic diseases. Diabetes mellitus is metabolic disorder caused due to disturbed glucose metabolism and in these blood sugar level increases because of deficiency in the production of insulin by the pancreas. [3] It is characterized by polydipsia, polyphagia, hyperglycemia, polyurea and due to disturbance in carbohydrate, protein and lipid metabolism associated with absolute or relative deficiency in insulin secretion or insulin action. [4,5]

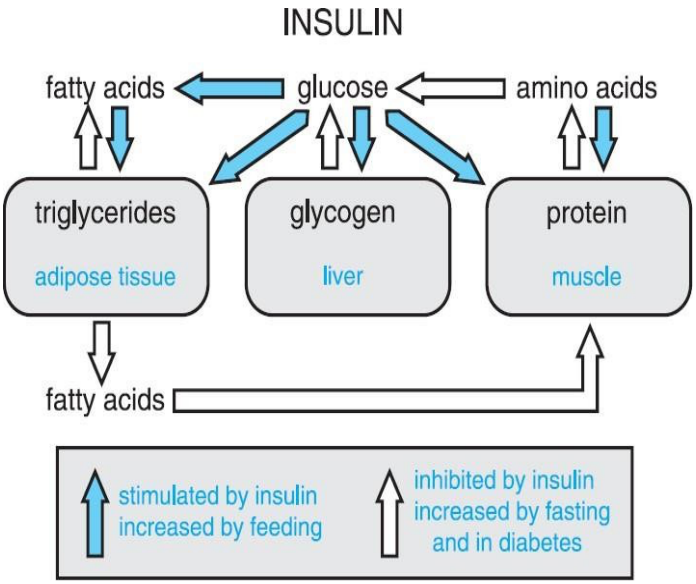

Figure 1:

Plant Profile

Figure 2:

Crinum latifolium [6-9]

Synonyms: Crinum longistylum Herb.

Common Name:

Pink Striped, Trumpet Lily, Milk and Wine Lily, Ceylon Swamp lily, Sukhdarshan (Sudarshan)

- **Kingdom-** Plantae
- **Clade-** Angiosperms
- **Order-** Asparagales
- **Family-** Amaryllidaceae
- **Genus-** Crinum
- **Species-** latifolium

Chemical Constituents: The extract of leaves of Crinum latifolium contains Carbohydrates, Alkaloids, Glycosides, Saponins, Phytosterols, Phenols, Tannins, Flavonoids, Proteins and amino acids, Fats & fixed oils Gum and mucilage's the aqueous extract or juice of leaves of Crinum latifolium also contains alkaloids. Examples: crinamine, crinamidine, crinafoline, and crinafolidine.

Crinum latifolium Uses:

- Antimicrobial activity
- Anti – inflammatory activity
- Thrombolytic nature
- Antitumor activity
- Anthelmintic activity
- Anticancer activity
- Antioxidant activity

Methodology:

Collection and authentication of plant material: In the present study, the leaves of Cranium latifolium were collected from local area of Jaipur, Rajasthan.

Drying of crude drugs material: Leaves of Cranium latifolium were cut in to small pieces and dried under the shade. After the complete drying leaves pieces were grinded to convert in to coarse powder for further studies. [10]

Preparation of plant extract: The leaves were dried under shade, powdered with a mechanical grinder and passed through a 40-mesh sieve. The successive solvent cold extraction method used to obtain various extracts including petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts. The solvents were removed from the extracts under reduced pressure by using a rotatotry vaccum evaporator. The percentage yields of extracts were noted. [11]

Physicochemical evaluation: Physicochemical studies was carried out using Ash values (Total ash value, Acid-insoluble ash value, Water soluble ash value), Extractive values (Alcohol soluble extractives, Water-soluble extractives), Loss on drying. Particle size of the microspheres was determine using optical microscopy. The eye piece micrometer was calibrated with the help of a stage micrometer. Phytochemical screening was perform using the process of successive solvent cold extraction method of leaves of Crinum latifolium, obtaining the extracts to use for to confirm the presence of various phytochemical using various identification tests. [12-15]

Experimental Design: In the experiment, a total of 36 albino rats were used. The rats were divided into 6 groups, comprising of 6 rats in each group. Experimental design for preliminary antidiabetic activity of various crude extracts of Cranium latifolliu. [16]

- Groups Treatment I Normal control albino rats received distilled water (NC)
- Groups II Diabetic control albino rats (DC)
- Groups III Albino rats received standard drug glibenclamide 10 mg/kg b. wt (Standard 10)
- Groups IV Albino rats received ethanol extract 100 mg/kg b. wt
- Groups V Albino rats received ethyl acetate soluble fraction of ethanolic extracts of Cranium latifollium200 mg/kg b. wt
- Groups VI Albino rats received ethyl acetate insoluble fraction of ethanolic extracts of Cranium latifollium400 mg/kg b. wt

Blood samples of all groups of rats were collected from tail vein before and 2, 4, 6, 8 and 24 hr after treatment. Blood glucose levels were determined in each group of rats. Blood glucose levels were measured by using Gluco-meter. The results were compared with that of the standard drug.

Acute oral toxicity (Acute toxic class method) [17]: The method enables a judgment with respect to classifying the test substances to one of the series of toxicity classes defined by fixed LD50 cut off values.

Acute toxicity describes the adverse effects of a substance which result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 hours). To be described as acute toxicity, the adverse effects should occur within 14 days of the administration of the substance. Acute toxicity is distinguished from chronic toxicity, which describes the adverse health effects from repeated exposures, often at lower levels, to a substance over a longer time period (months or years).

The acute oral toxicity studies of extracts were carried out as per the guidelines of Organization for Economic Co-operation Development (OECD) guidelines, draft guidelines 423 adopted on 17th December 2001 received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Biochemical parameter: [18] Such as serum was analyzed for total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL).

Statistical Analysis: [19,20] The result of the study were subjected to one way analysis of variance followed by student's t-test for multiple comparisons. Values with $P < 0.05$ were considered significant.

Result & Discussion

Table 1: Physiochemical Properties

Organoleptic characters	Cranium latifollium
Colour	Grey to brown
Odour	Characteristic
Taste	Astringent
Surface characteristics	Glossy and leathery
Shape	Cylindrical

Glossy and leathery Shape Cylindrical The color, odour, taste and shape of Cranium latifollium are shown in this table.

Determination of foreign matters of Cranium latifollium

Table 2: Foreign matters

Weight of powder sample (g)	Type of foreign matter	Weight of foreign matter (g)	Percentage foreign matter (% w/w)
100 g	Plant matter	0.00 g	Nil
	Animal matter	0.00 g	Nil
	Mineral matter	0.00 g	Nil

- It was found that there were no visible signs of plant, animal and mineral foreign matter.
- It indicates that powder of Cranium latifollium found to be free from foreign matters contamination.

Phytochemical Test:

Table 3: Qualitative chemical tests of different extracts of Cranium latifollium

Tests	Petroleum ether Extract	Ethanol extract	Acetone soluble ethanolic extract	Acetone insoluble ethanolic extract
Steroid		+	-	+
Triterponoids	-	+	+	-
Glycosides	-	+	+	+
Carbohydrates	-	+	-	+
Alkaloids	-	+	+	+
Flavonoids	-	+	+	+
Tannins	-	+	-	+
Proteins and amino acid	-	+	-	+
Lipids	+	-	-	-

Petroleum ether extract leaves showed presence of steroid, acetone soluble fraction showed the presence of triterponoids, glycoside, alkaloid and flavonoid whereas ethanolic extract and acetone insoluble fractions leaves showed the presence of various phytochemical groups alkaloids, flavonoids, phytosterols, glycosides, tannins and proteins.

Chromatographic Studies:

Table 4: Thin layer chromatographic studies of ethanolic extract of Cranium latifollium

Solvent systems	Spraying reagents
Flavonoids	
Acetone: water (7: 3) n-Hexane: acetone (3: 7) Chloroform: acetone (3 : 7)	10 % Alcoholic $FeCl_3$ 20 % Alcoholic $FeCl_3$ 20 % Alcoholic lead acetate
Glycosides	
Acetone: methanol: water (100 :13.5: 10) Toluene: acetone: formic acid (2.5: 1: 1)	Anisaldehyde sulphuric acid 10% Alcoholic KOH

Flavonoids:

- Stationary phase: Silica gel G Mobile phase: n-Hexane: acetone
- Proportion: 3: 7
- Detection: 10 % Alcoholic $FeCl_3$

Table 5: T.L.C plate of Cranium latifollium extracts under UV and visible light for flavonoids

Fractions	Numbers of spot	Color of spot		Rf Value
		Under UV light	Under visible light	
Cranium latifollium	1	-	Purpule	0.3

Figure 3: TLC of flavonoids Glycosides

- Stationary phase: Silica gel G
- Mobile phase: Acetone: methanol: water Proportion: 100: 13.5: 10
- Detection: 10 % Alcoholic KOH

Table 6: T.L.C plate of Cranium latifollium ethanolic extract under UV and visible light for glycosides

Fractions	Numbers of spot	Color of spot		Rf Value
		Under UV light	Under visible light	
Cranium latifollium	1	-	Pink	0.79

Figure 4: TLC of glycosides

Acute toxicity Studies:

Table 7: Acute toxicity study of ethanol extracts of Cranium latifollium

S. no.	Dose (mg / kg b. wt)	Numbers of animals	Observation
1	5	3	All animals survived
2	50	3	All animals survived
3	200	3	All animals survived
4	300	3	All animals survived
5	500	3	All animals survived
6	1000	3	All animals survived
7	1000	3	All animals survived
8	2000	3	All animals survived
9	5000	3	All animals survived

Table 8: Acute toxicity study of acetone soluble fraction of ethanolic extracts of Cranium latifollium

S. no.	Dose (mg / kg b. wt)	Numbers of animals	Observation
1	5	3	All animals survived
2	50	3	All animals survived
3	200	3	All animals survived
4	300	3	All animals survived
5	500	3	All animals survived
6	1000	3	All animals survived
7	1000	3	All animals survived
8	2000	3	All animals survived
9	5000	3	All animals survived

Mortality was not observed in any groups of rats up to the dose level of 5000 mg/kg body weight. As per OECD guideline, these extracts come under the category -5 Hence, these are safe. The LD_{50} as per OECD guideline, this extract under category was found to be 2500 mg/kg. The LD_{50} of these extract should be effective 1/4 to 1/20. Therefore, dose of ethanolic extract, acetone soluble fraction of ethanolic extract, acetone insoluble fraction of ethanolic extract of Cranium latifollium for hypoglycemic activity was found to be 200, 400 mg/kg body wt.

Preliminary Pharmacological activity:

Table 9: Effect of different extract of Cranium latifollium on blood glucose level in streptozotocin induced diabetic rats

Group	Treatment	Blood glucose level mg/dl					
		0hr	2hrs	4hrs	6hrs	8hrs	24hrs
I	NC	68.16 ±3.44	68.66 ±3.62	69.16 ±3.74	71.83 ±4.44	72.13 ±3.44	72.83 ±3.44
II	DC	379.83 ±8.04	384.66 ±8.61	388.33 ±9.84	392.16 ±9.83	396.83 ±9.48	398.00 ±9.06
III	Standard 10	395.00 ±6.83	257.33 ±6.80**	220.16 ±7.47**	157.50 ±6.76**	229.00 ±7.23**	322.66 ±6.47**
IV	EE 100	402.56 ±4.61	381.56 ±5.68	359.65 ±5.84	305.06 ±4.31*	345.61 ±5.39*	385.82 ±6.01^{ns}
V	ASEE 200	403.02 ±4.33	390.76 ±4.68	380.75 ±6.53	365.06 ±6.33*	375.61 ±5.39*	388.82 ±5.02^{ns}
VI	AIEE 400	392.00 ±5.41	356.00 ±6.15	274.16 ±7.47*	215.16 ±6.74**	268.83 ±5.32**	358.50 ±6.38*

n=6, *p<0.05- significant, **p<0.01-more significant v/s diabetic control, SEM= standard error mean, SD = standard deviation, n= number of animals

NC - Normal control, DC - Diabetic control, Standard 10 - Glibenclamide10 mg/kg b.wt, EE 200 - Ethanol extract 200mg/kg b.wt, ASEE 200- Acetone soluble fraction of ethanolic extract 200 mg/kg b.wt., AIEE 200- Acetone insoluble fraction of ethanolic extract 200 mg/kg b.wt.

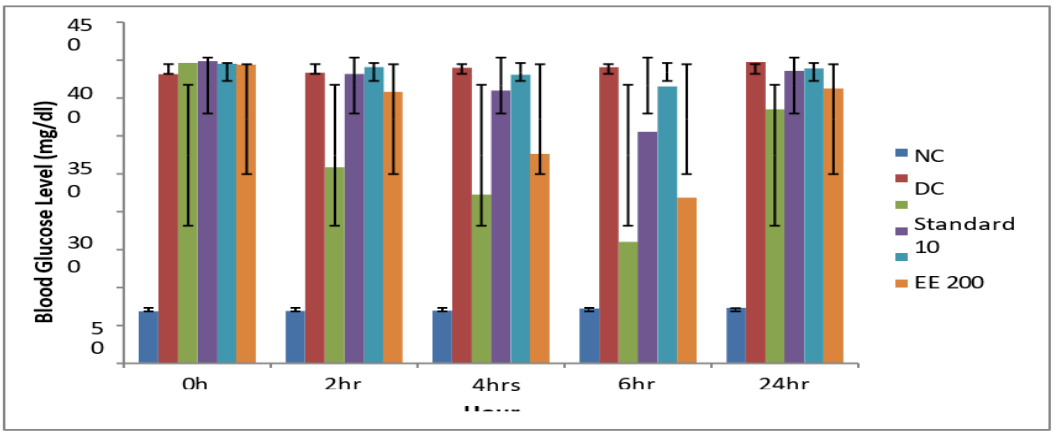

Figure 5: The antihyperglycemic activity of Cranium latifollium of various extracts were studied against standard drug glibenclamide.

Table 10: Percentage (%) reduction of blood glucose level in streptozotocin induced diabetic rats on different extract of Cranium latifollium

Group	Treatment	Basal Value 0 hr	Percentage (%) reduction of blood glucose level mg/dl				
			2hrs	4hrs	6hrs	8hrs	24hrs
I	NC	68.16± 3.44	---	---	---	---	---
II	DC	379.83± 8.04	----	----	----	----	---
III	Standard 10	395.00± 9.83	34.85	44.26	60.12	42.02	18.31
IV	AIEE 100	401.50± 8.33	6.43	20.42	42.34	30.34	9.25
V	AIEE 200	392.00± 8.41	9.18	30.06	45.11	32.42	8.54
VI	AIEE 400	392.83± 8.07	25.58	42.33	54.52	37.59	14.44

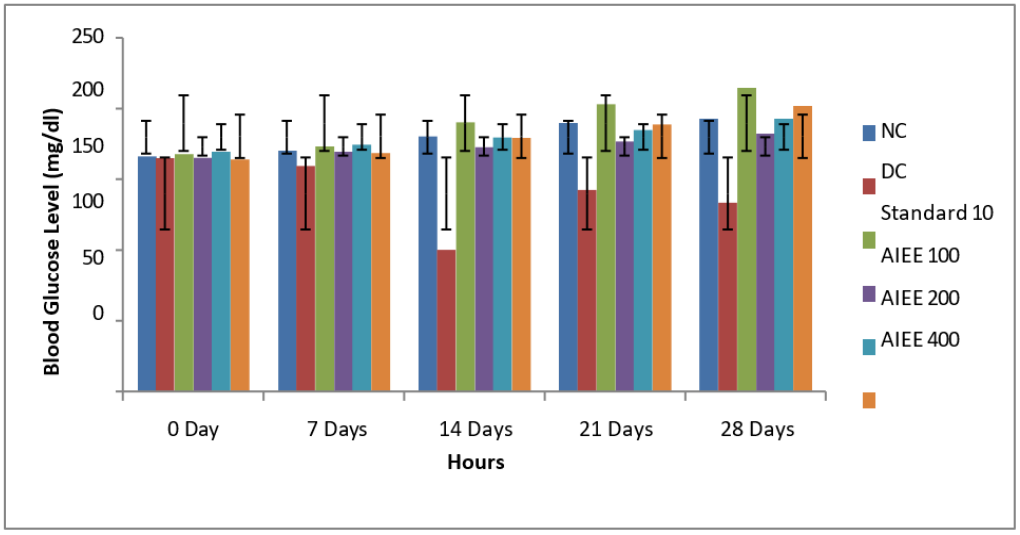

Figure 6: Effect of normal control, diabetic control, and glibenclamide 10 mg/kg b.wt and acetone insoluble fraction of ethanolic extract 100, 200, 400 mg/kg b.wt of Cranium latifollium on blood glucose level for acute study.

Results were determined by one-way analysis of variance (ANOVA non parametric) with student's t-test with P<0.05 considered significant.

Body Weight:

Table 11: Effect of different extract of Cranium latifollium on body weight in streptozotocin induced diabetic rats:

Group	Treatment	Average body weight (g) +SEM				
		0 Day	7 Days	14 Days	21 Days	28 Days
I	NC	166.16 ±3.30	170.06 ±3.31	180.01 ±4.09	189.58 ±3.72	192.67 ±5.24
II	DC	164.50 ±4.34	159.08 ±4.21	100.09 ±6.35	142.30 ±3.80	133.26 ±3.24
III	Standard 10	167.58 ±3.60	173.12 ±4.63*	190.27 ±4.30**	202.96 ±5.62**	214.56 ±3.48**
IV	AIEE 100	165.15 ±4.22	169.40 ±5.26	172.64 ±4.95*	176.63 ±5.68*	182.14 ±3.24*
V	AIEE 200	169.14 ±4.90	174.13 ±6.90*	179.16 ±4.13*	184.50 ±4.68**	192.48 ±2.20*
VI	AIEE 400	163.82 ±3.18	168.43 ±4.93**	179.12 ±4.08**	188.60 ±4.16**	201.77 ±2.01**

n=6, *p<0.05- significant, **p<0.01-more significant v/s diabetic control, SEM= standard error mean, SD = standard deviation, n= number of animals

NC - Normal control, DC - Diabetic control, Standard 10- Glibenclamide10 mg/kg b.wt , AIEE 100- Acetone insoluble fraction of ethanolic extract 100 mg/kg b.wt , AIEE 200- Acetone insoluble fraction of ethanolic extract 200mg/kg b.wt , AIEE 400- Acetone insoluble fraction of ethanolic extract 400mg/kg b.wt.

Table 12: Percentage (%) increase on body weight in streptozotocin induced diabetic rats of different extract of Cranium latifollium

Group	Treatment	Basal Value 0 hr	Percentage (%) increase of body weight			
			7 Days	14 Days	21 Days	28 Days
I	NC	166.16 ± 3.30	----	----	----	----
II	DC	164.50 ± 4.34	----	----	----	----
III	Standard 10	167.58 ± 3.60	03.30	13.53	21.11	28.03
IV	AIEE 100	165.15 ± 4.22	02.57	04.53	6.951	10.28
V	AIEE 200	169.14 ± 4.90	02.96	05.92	09.08	13.79
VI	AIEE 400	163.82 ± 3.18	02.81	09.33	15.14	22.16

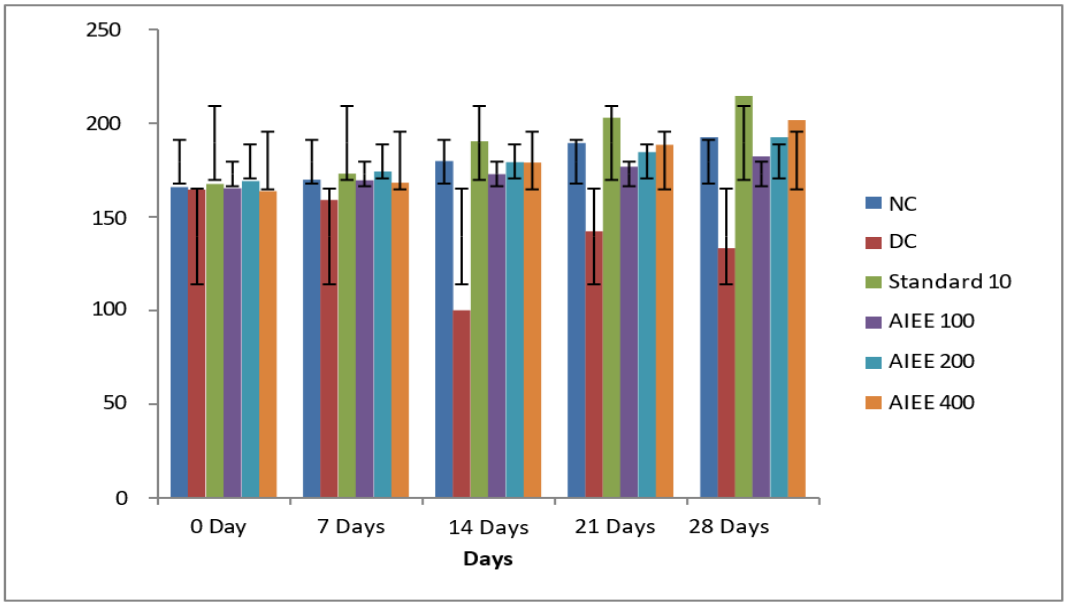

Figure 6:

Effect of normal control, diabetic control, and glibenclamide 10 mg/kg b.wt and acetone insoluble fraction of ethanolic extract 100, 200, 400 mg/kg b.wt of Cranium latifollium on blood glucose level on body weight. b. wt after 28 days produce more significant increase body weight as compared to 100, 200 mg/kg b.wt dose. Results were determined by one-way analysis of variance (ANOVA non parametric) with student's t-test with $P<0.05$ considered significant.

Biochemical Parameter:

Table 13: Effect of different extract of Cranium latifollium on serum lipid profile in streptozotocin induced diabetic rats

Group	Treatment	Serum lipid profile levels mg/dl				
		TG	TC	HDL	LDL	VLDL
I	NC	80.10 ±0.41	78.26 ±1.67	26.28 ±0.73	40.18 ±1.18	16.07 ±0.24
II	DC	152.17 ±1.47	146.28 ±1.87	14.14 ±0.48	90.10 ±1.01	30.09 ±0.42
III	Standard 10	93.84 ±1.19**	88.10 ±1.21**	23.07 ±0.78**	62.15 ±0.63**	18.92 ±0.59**
IV	AIEE 100	120.09 ±1.16 ns	111.16 ±1.55ns	17.00 ±1.55ns	79.08 ±1.09ns	25.26 ±0.38*
V	AIEE 200	113.19 ±1.17*	106.28 ±1.31*	18.62 ±0.47**	71.24 ±0.60*	24.27 ±0.35**
VI	AIEE 400	99.13 ±1.13**	96.16 ±1.87**	20.54 ±0.26**	68.19 ±0.72**	20.25 ±0.57**

n=6, *p<0.05- significant, **p<0.01-more significant v/s diabetic control, SEM= standard error mean, SD = standard deviation, n= number of animals

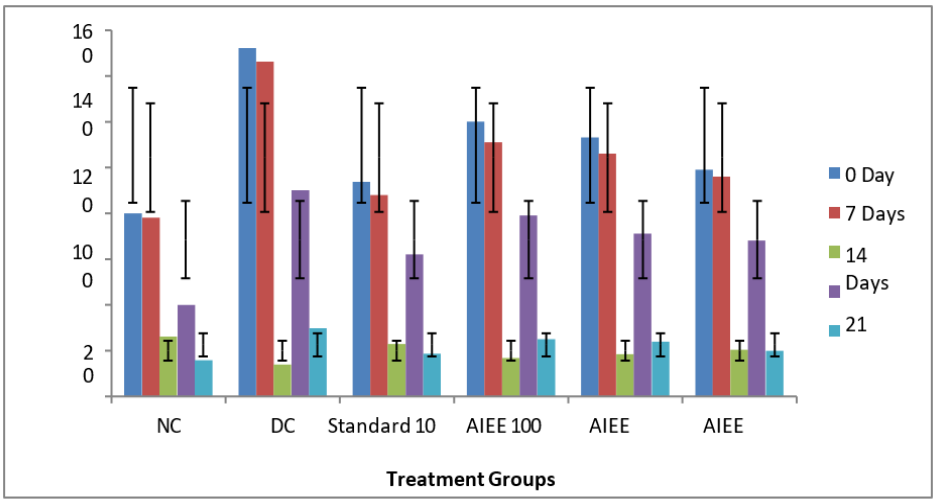

Figure 7: Percentage (%) reduction after 28 days treatment of different extract of Cranium latifollium on serum lipid profile in streptozotocin induced diabetic rats.

Results were determined by one-way analysis of variance (ANOVA non parametric) with student's t-test with $P<0.05$ considered significant.

Conclusion

In conclusion the finding of the study providing compelling evidence that the acetone-insoluble fraction of ethanolic extract of Cranium latifolium demonstrates promising antidiabetic activity and is safe at high doses (400mg/Kg b.wt). It significantly improves blood glucose levels and lipid profiles, suggesting its potential as a natural therapeutic agent for diabetes management. The Phytochemical screening of the extracts revealed the presence of carbohydrates, glycoside, steroids, flavonoids, alkaloids, tannins, amino acids and some of major constituents in the plant Cranium latifollium. At 400 mg/kg b.wt, the acetone-insoluble fraction significantly reduced blood glucose level, comparable to glibenclamide and normalized lipid profile (↓TG, TC, LDL, VLDL; ↑HDL) in diabetic rats, similar to the standard drug.

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