Investigation of Anti-diabetic activity of Leaves of Trianthema porctulacastrum Linn., Leonotis nepetaefolia (L.) R.Br. and Diplocyclos palmatus (L.) Jeffrey

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Abstract
The traditional system of medicine is so ingrained in our culture and about 80% of the Indian population depends on this indigenous system and traditional medicine for relief. With such a huge section of an ever increasing population relying on herbal remedies, it is imperative that the plant products which have been in use for such a long time be scientifically supported for their efficacy. Diabetes a metabolic disorders is most frequent occurring disease in India. The present paper was designed to investigate anti-diabetic activity of ethanolic extract of leaves of *Trianthema porctulacastrum* Linn., *Leonotis nepetaefolia* (L.) R.Br. and *Diplocyclos palmatus* (L.) Jeffrey in alloxan induced. Results indicate that all the ethanolic extract of leaves was showed significant activity however, of *Trianthema porctulacastrum* Linn. showed more potent anti-diabetic activity than other two when compared with standard drug.

Keywords: Medicinal plants, Anti-diabetic activity, Alloxan,

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Introduction
Diabetes is a long-term condition of the metabolism of carbohydrates, fats, and proteins marked by elevated fasting and postprandial blood sugar levels. According to estimates, the prevalence of diabetes will rise from 4% in 1995 to 5.4% by 2025. According to WHO, developing nations will shoulder the majority of the burden. Studies carried out in India over the past ten years have shown that not only is the prevalence of diabetes high, but it is also rising quickly among urban residents. In India, there are thought to be 33 million persons who have diabetes. By 2025, this figure is probably going to rise to 57.2 million. [1-2]

Due to their natural origins and lack of side effects, herbal medicines have experienced an exponential surge in popularity over the past several years in both developed and developing nations. Medicinal plants, minerals, and organic materials constitute the basis for many of the ancient medicines still in use today. In herbal formulations utilised in Indian traditional healthcare systems, a variety of therapeutic plants known as rasayana are present. In Indian medical systems, the majority of practitioners create and administer their own prescriptions. The 21,000 plants that are used as medicines worldwide are recorded by the World Health Organization (WHO). 150 of these 2500 species, all of which are found in India, are utilised economically on a regular basis. [3-4] So, far no any systematic study was carried out in investigate the anti-diabetic activity of leaves of selected plants therefore, the present work was undertaken.

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Material and Methods

Collection of herbs and their authentication

The leaves of *Trianthema portulacastrum* Linn., *Leonotis nepetaefolia* (L.) R.Br. and *Diplocyclos palmatus* (L.) Jeffrey was collected in the months of July-September 2021 from the various local sites of Malwa region of Madhya Pradesh and identified & authenticated by Dr. S. N. Dwivedi, Retd. Prof. and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, (M.P.) and was deposited in our Laboratory. Voucher specimen No. J/Bot./TPL-32; J/Bot./LNL-33 & J/Bot.DPL-34 was allocated.

Preparation of extract

250 gm of dried leaves of *Trianthema portulacastrum* Linn., *Leonotis nepetaefolia* (L.) R.Br. and *Diplocyclos palmatus* (L.) Jeffrey were coarsely powdered and loaded in soxhlet apparatus using ethanol as a solvent to obtain the extract. After completion of extraction the extract was concentrated and was kept in dessicator for further use. [5]

Acute Toxicity Studies of Extract

Organization for Economic co-operation and Development (OECD) regulates guideline for oral acute toxicity study. It is an international organization which works with the aim of reducing both the number of animals and the level of pain associated with acute toxicity testing. [6]

Procurement of experimental animals

The mice were used for acute toxicity study as per OECD guidelines 423. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization.

Experimental animals

The Wister strains of male albino rats weighing between 100 and 150g were obtained for the present study, from Govt. Veterinary Hospital, Mhow, Madhya Pradesh. The animals were housed in larger spacious cages and they were fed with commercial pelleted rat chow marketed by Hindustan Lever Ltd., Bangalore, India, under the trade name Gold Mohur Rat Feed and had free access to water ad labium. The animals were well acclimatized to standard environmental conditions of temperature and 12h light dark cycles throughout the experimental period. The animals used in the present study were approved by the Institutional Animal Ethical Committee.

Preparation of Alloxan Monohydrates

Alloxan was prepared by weighing 1 gm of alloxan and dissolving in 20ml of water for injection. Alloxan at this calculated dose is said to have a concentration of 50mg/ml.

Anti-diabetic Activity [7-8]

Different groups of each six rats were used in the present investigation. The basal concentration of blood glucose level of all the animals was recorded and 6 animals were separated to serve as normal control. The remaining animals received a single injection of Alloxan monohydrate in water for injection at a dose of 150-mg/kg bodyweight given by intra-peritoneal route. After 4 days of Alloxan administration, the blood glucose was estimated and animals with blood glucose levels in the range 280 mg/dl and 380 mg/dl were selected and divided into groups. Group 1:- Untreated control (Normal saline water); Group 2:- Diabetic control (Alloxan 150 mg/kg); Group 3:- Diabetic+ Glibenclaminde (10mg/kg); Group 4:- Diabetic +EETPL (500 mg); Group 5:- Diabetic + EELNL (500 mg) and Group 6:- Diabetic + EEDPL (500 mg)

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Statistical Analysis

Data were analyzed by comparing values for different treatment groups with the values for individual controls. The significant differences among values were analyzed using analysis of variance (one-way ANOVA) in latest computer software programme. All the obtained results are expressed as X (Mean) ±SEM, n=6. (One way ANOVA followed by Bonferroni multiple comparison test).

Results and Discussion

The ethanolic extract of leaves of *Trianthema portulacastrum* Linn., *Leonotis nepetaefolia* (L.) R.Br. and *Diplocyclos palmatus* (L.) Jeffrey were screened for acute toxicity study by OECD guideline no. 423 for determination of $LD_{50.}$ The results showed that the extract was belonging to category-5(unclassified). Hence, LD_{50} was 5000 mg/kg, therefore, ED_{50} was 500 mg/kg. Therefore dose of 500 mg were selected for present investigation. The results were presented in table 1. In the present investigation the data of the blood glucose level of rats treated with Alloxan (150mg/kg body weight) produced diabetes within 72 hours. After 72 hours of Alloxan administered the blood glucose levels of rats were observed. It was observed in that significant lowering of sugar in ethanolic extract of all the extracts i.e., leaves of *Trianthema portulacastrum* Linn., *Leonotis nepetaefolia* (L.) R.Br. and *Diplocyclos palmatus* (L.) Jeffrey. The administration of ethanolic extract at a dose of 500 mg/kg body weight showed significant anti-diabetic activity at 21st day which was evident from the 1st day on wards as compared to standard. The decreasing blood glucose levels are comparable with that of 10 mg/kg of Glibenclamide. The blood glucose levels of the anti-diabetic activity of alloxan induced diabetic rats were shown in table 2. It represents that decrease in blood glucose levels. The Glibenclamide (10 mg/kg body weight) shows significant effect on compare to the initial and more significant effect on the 7th Day compare to the initial.

Table 1: Determination of LD_{50} and ED_{50} of Ethanolic Extract of Leaves of *Trianthema portulacastrum* Linn., *Leonotis nepetaefolia* (L.) R.Br. and *Diplocyclos palmatus* (L.) Jeffrey

S/No.	No. of Animals	Extract Dose (mg/kg)	No. of death of animals		
			EETPL	EELNL	EEDPL
1.	3	5	0	0	0
2.	3	50	0	0	0
3.	3	300	0	0	0
4.	3	2000	0	0	0
5.	3	5000	0	0	0

Table 2: Effect of Administration of Feeding the Ethanolic Extract of Selected Herb on Serum Glucose Estimation in Normal and Diabetic Rats

Group	Serum glucose (mg/dL)			
	0 day	7th day	14th day	21st day
Control	83.16±0.04	84.12±0.11	85.11±0.04	86.23±0.09
Diabetic control	295.11±0.11	366.89±0.07##	418.29±0.18###	407.09±0.11###
Standard (10mg/kg)	283.17±0.21	202.21±1.23**	160.39±1.21***	110.21±1.03***

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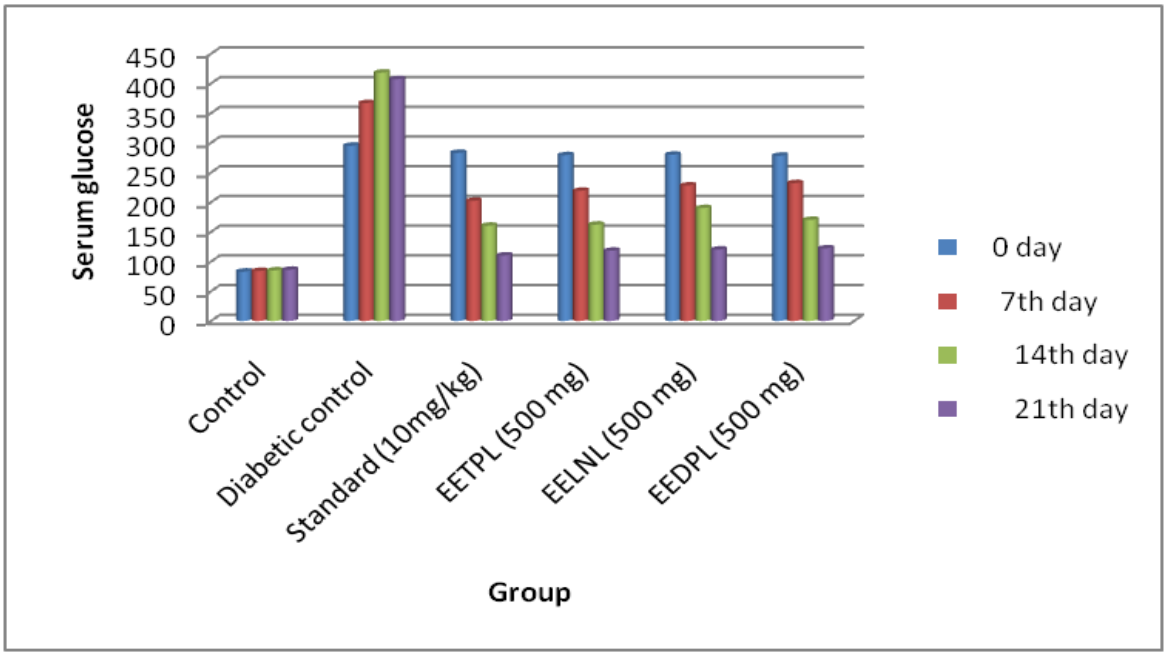
EETPL (500 mg)	279.11±0.03	219.21±1.11**	162.21±1.11***	118.21±1.21***
EELNL (500 mg)	280.01±0.09	228.11±0.12**	190.02±1.22***	120.26±1.43***
EEDPL (500 mg)	278.21±0.23	232.11±0.23**	170.21±1.22***	122.32±1.29***

All values are expressed as mean ± S.E.M (n=6), ***P<0.001 as compared diabetic control (normal saline), **P<0.01 as compared diabetic control (normal saline), $^{###}$P<0.001 as compared to Control. One-way ANOVA followed by Bonferroni multiple comparison test.



Graph 1: E Serum Glucose Estimation in Normal and Diabetic Rats

Conclusion

From the results it was concluded that ethanolic extract of leaves was showed significant activity however, of *Trianthema porctulacastrum* Linn. showed more potent anti-diabetic activity than other two when compared with standard drug.

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