INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN: 2320-2831

IJPAR |Vol.11 | Issue 1 | Jan-Mar -2022
Journal Home page: www.ijpar.com

Research article *Open Access*

Desmodium triflorum leaf aqueous extract on streptozotocin induced Diabetic rat

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ABSTRACT

The hypoglycemic effect of aqueous extract from leaves of Desmodium triflorum (200 & 400 mg/kg) (AEDT) was evaluated by Streptozotocin-induced diabetic rats. Animals were induced for diabetes with Streptozotocin (60 mg/kg of body weight-) and treated orally with two different doses of desmodium triflorum. Glibenclamide used as standard drug. The extract showed significant ($p<0.01$) anti-hyperglycemic as compared to diabetic control. The AEDT 400 mg/kg show beneficial effects on blood glucose like as standard also shows significant level of secondary metabolites. Thus both dose could serve as good oral hypoglycemic agents and seems to be promising for the development of phytomedicines for diabetes mellitus.

Keywords: Desmodium triflorum, Hypoglycemic, Streptozotocin, Glibenclamide

INTRODUCTION

Diabetes is a serious metabolic disorder with micro and macrovascular complication that results in significant morbidity and mortality3. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries [1]. Modern medicines like Biguanides, Sulphonylureas and Thiozolidinediones are available for the treatment of diabetes. But they also have undesired effects associated with their uses [2]. Alternative medicines particularly herbal medicines are available for the treatment of diabetes. Common advantages of herbal medicines are effectiveness, safety, affordability and acceptability [3]. Medicinal

plants and their products have been used in the Indian traditional system of medicine and have shown experimental or clinical anti-diabetic activity [4, 5]. Medicinal Plants are a rich source of natural products. Medicinal plants and their products have been widely used for treatment of diabetic populace all around the world with less known scientific basis of their functioning [6,7]. Hence, natural products from medicinal plants need to be investigated by scientific methods for their anti-diabetic activity. The Survey of literature reveals that the medicinal plant of Desmodium triflorum belongs to the family Fabaceae is used in the leaves are galactagogue and ground with cow's milk, they are given daily in the morning. They are also given to children to treat diarrhea due to indigestion and also in convulsions. Expressed juice from a well macerated plant, is applied to abscesses and wounds that do not heal readity. The main actions include anti-spasmodic, sympathomimetic, central nervous system stimulation, curare-mimetic activity and diuretic. Anthelmintic activity has also been demonstrated in D. triflorum. A decoction of D. triflorum is commonly used to treat diarrhea and dysentery in Indonesia, Malaysia the Philippines, Thailand, as well as China India and Sri Lanka. In the Philippines, a decoction is also used as a mouthwash and as an expectorant. In Thailand, the whole plant is used as an antipyretice and to quench thurst. In Indonesia, Malaysia, the Philippines, Laos and India the crushed plant or a poultice of the leaves is externally applied on wounds, ulcers, and for skin problems in general, apparently for its antiseptic properties. It is also used as forage, as a green manure and ground cover. Plant Part Type of extract Pharmacological activity Treatment procedure on animals Finding activity/Trend/ Effective Dose Observed mechanism Aerial parts Flavonoid and alkaloid fractions Antioxidant and anti-inflammatory 10 mg/kg, i.p. in rats Flavonoid fraction exhibited antiinflammatory activity better than alkaloid fraction and indomethacin. Flavonoid fraction exhibited better superoxide dismutase, glutathione peroxidase and catalase activity - superior antioxidant activity. Presence of polyphenols such as caffeic acid and chlorogenic acid, which are reported antioxidants, in the flavonoid fraction [8-10]. The literature showed AEDT used for number of ailments by traditionally and scientifically. The present study, we reported hypoglycemic potentials of Desmodium triflorum in Streptozotocin diabetic rat model.

MATERIAL AND METHODS

Preparation of extracts of leaves of *Desmodium triflorum*

The collected leaves were shade dried completely and ground into powder with mechanical grinder. The powder was passed through sieve no. 60 to get uniform powdered. Solvent used for Extraction Ethanol & Aqueous. The 1 Kg of dried powder of *Desmodium triflorum* leaves was defatted with n-hexane. The defatted powder material (marc) thus obtained was extracted with sufficient quantity of Ethanol and distilled water.

Maceration process

Maceration process involves the separation of medicinally active portions of the crude drugs. The drug material is taken in a stopper container and immersed in the bulk of the solvents in the ratio of 1:2 (Drug &Solvent) and allowed to stand for 7 days in a room temperature with frequent shaking of every 30 min up to 6 hours on each day. The solvent was removed by filtration by using thin masculine cloth, distillation under reduced pressure and evaporation. The resulting semisolid mass was vacuum dried and percentage yield was calculated.

Estimation of secondary metabolites

The ethanol and aqueous extracts of leaves of *Desmodium triflorum* are subjected to estimation of total phenolic, tannin and flavonoid by following method.

Determination of total phenolic contents in *Desmodium triflorum* extracts [11]

The concentration of total phenol in plant extracts was determined using spectrophotometric method. The extracts in the concentration of 1 g/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml methanol solution of extracts, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% NaHCO3. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of NaHCO3. The samples were thereafter incubated in a thermostat at 45^{o}C for 45 min. The absorbance was determined using spectrophotometer at 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same

procedure was repeated for the standard solution of Gallic acid (10- 100mg/ml) and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of Gallic acid equivalent (mg of GA/g of extract).

Estimation total tannins in *Desmodium triflorum* extract [12]

The total content of tannin in extracts per gram was determined by folin denis method (100g of sodium sulphate+20g of phosphomalybdic acid +50 ml of phosphoric acid and 750ml of distilled water was refluxed or boiled for 2 hrs and make up the volume 1000 ml with distilled water). The spectroscopy estimation of tannin is based on the measurement of blue colour formed by reduction of phosphor tungsto molybdic acid by tannin like compound in alkaline medium. 1 ml of extracts and standard solution of tannic acid (10-100 mg/ml) was made up to 7.5 ml with distilled water. Then 0.5 ml of Folin Denis reagent and 1 ml of Na_2CO_3 solution was added. The volume was made up to 10 ml with distilled water and absorbance was measured at 700 nm. Total tannic acid content was expressed mg equivalent of tannic acid per gram of extracts.

Determination of total flavonoids contents in *Desmodium triflorum* extracts [13]

The total flavonoids content of each plant extract was estimated as per Zhishen *et al* . In- brief, each sample (1.0ml) was mixed with 4ml of distilled water and subsequently with 0.30 ml of a $NaNO_2$ solution (10%). After 5 min, 0.30 ml of an $AlCl_3$ solution (10%) w a s added followed by 2.0 ml of NaOH solution (1%). Immediately, after thorough mixing the absorbance was measured at 510 nm versus the blank. Standard curve of rutin was prepared (10-100 mg/ml) and the results are expressed as rutin equivalents (mg rutin/gm dried extract).

Extract selection for anti- diabetic rats

Based on the literature, the plant / extracts possess flavonoid, tannin and phenolic compounds might have Immunomodulatory, wound healing property and avoiding excess animal usage, so we have selected the extract having the high content of flavonoid, tannin and Phenolic compound such as Aqueous extract of *Desmodium triflorum*. The aqueous extract selected also based on the yield.

Experimental animals

All the experiments were carried out using Swiss Albino mice (25-30 g) and Wistar rats (150-200 g). The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2^0C and relative humidity of 30–70%. A 12 hrs day: 12 hrs night cycle was followed. All animals were allowed free access to water and fed. Ethical clearance was obtained from Institutional Animal Ethical Committee of NRI College of pharmacy, Agiripalli, Andhra Pradesh Reg.No:11/PO/a/16/IAEC.

Acute toxicity[14]

The acute toxicity study was carried out with aqueous extract of *Desmodium triflorum* as per OECD 423 Guidelines. Wister albino mice with weight ranging (25-30g) were taken for the experiment. The animals were made into a group of 3 each, dose of *Desmodium triflorum* aqueous extract were given according to the body weight (mg/kg), starting dose of 5 mg /kg was given to the first individual animal, no death was occurred and higher doses were given to next group of animals. The animals were observed for a further 14 days for any signs for delayed toxicity.

Table 1: Acute toxicity study of aqueous extract of leaf *of Desmodium triflorum* based on OECD 423 guidelines

S. No	Number of animals	Dose in mg/kg	Report
1	3	5mg/kg	No death
2	3	50mg/kg	No death
3	3	500mg/kg	No death
4	3	2000mg/kg	No death

Oral glucose tolerance test [15]

Fasted rats were divided into five groups of six rats in each. Group I served as control, received 2ml distilled water and Group II received only glucose. Group III received standard drug glibenclamide as an aqueous suspension at a dose of 5mg/kg b. wt. Groups IV & V received the AEDT extract at a dose of 200 & 400 mg/kg b. wt. as an aqueous extract of *Desmodium Triflorum.* After 30 min of extracts administration, the rats of all groups were orally treated with 2 g/kg of glucose except Group I. Blood samples were collected from the rat tail vein just prior to glucose administration and at 30, 90 and 120 min after glucose loading. Blood glucose level was measured immediately by using digital glucometer (one touch select, Johnson & Johnson, USA).

Induction of Diabetes

STZ was freshly prepared by dissolving in citrate buffer (0.01M, PH-4.5) and kept on ice prior to practice. The overnight fasted rats were made diabetes with a single intraperitoneal injection of STZ (60 mg/kg). After 4hrs STZ administration 5% glucose was administered orally in drinking water for a day to overcome the early hypoglycemic phase. Rats were allowed to stabilize for three days. On the third day (72hrs) blood samples were drawn to estimate the blood glucose concentration to confirm the development of diabetes. Rats with plasma glucose estimated by using digital glucometer (accu-chek, Roche Diabetes Care India) and above 250 mg/dl were considered as diabetic and used in the study. The animal confirmed diabetes were only used for anti-diabetic, immunomodulatory and wound healing activity

Streptozotocin Induced Anti-diabetic activity [16]

Experimental design

In the experiment a total of 30 overnight fasted rats were used. The 24 rats were rendered diabetic by Streptozotocin (60mg/kg, ip). The animals divided into five groups of six rats each.

Group I - Normal control received distilled water
Group II -served as Diabetic control [Streptozotocin (60mg/kg, ip)]
Group III- served as standard treated with 5 mg/kg of Glibenclamide for 11 days orally
Group IV & V - Treated with 200 & 400 mg/kg of aqueous extract of *Desmodium Triflorum* for 11 days orally

Blood was collected by the retro-orbital puncture under light ether anesthesia on 0, 3, 6, 9 & 11th day for estimation blood glucose level by using digital glucometer (one touch select, Johnson & Johnson, USA).

STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) followed by Dunnett's method of multiple comparisons was employed using Graph pad Instat 5.0 software. $p<0.05$, $p<0.01$ & $p<0.001$ was considered to be statistically significant.

RESULTS

Preliminary phyto-chemical studies of *desmodium triflorum* extracts

The preliminary phytochemical analysis of different extracts of *Desmodium triflorum* shows presence of steroid, flavonoids, glycosides, tannin, alkaloids, phenolic compound, proteins and carbohydrate.

Table 2: Preliminary phytochemical screening of *Desmodium triflorum*.

S.No	Constituents	Tests	Ethanol	Aqueous
1	Alkaloids	Mayer's test	+	+
		Dragondraff's test	+	+
		Hager's test	+	+
		Wagner's test	+	+
2	Sterols	Burchard test	-	-
		Salkowski's	-	-
3	Carbohydrates	Molisch's test	+	+
		Fehling's test	+	+
		Benedict's test	+	+
		Barfoed's test	+	+
4	Glycosides	Legal test	-	+

		Kellerkiallani test	-	-
		Borntrager's test	-	+
5	Fixed oils & Fats	Spot test	-	-
		Saponification test	-	-
6	Phenolic Compounds	Ferric chloride	+	+
7	Proteins & amino acids	Biuret test	+	+
		Ninhydrin test	-	+
		Millon's test	-	+
		Xanthoproteic test	-	+
		Cysteine test	-	+
		Tryptophan test	-	+
8	Terpenoids & Saponins	Foam test	-	-
		Haemolysis test	-	-
9	Tannins	Gelatin test	+	+
		Fecl3 test	+	-
		Lead acetate test	+	+
10	Gums & mucilage	Mucilage test	-	+
		Hydrolytic test	-	-
11	Flavonoids	Shinoda test	-	+
		Conc.H2SO4	-	+
		lead acetate	+	+

Where + =present, - =absent

Estimation of tannin, flavanoid and phenolic compound content in *desmodium triflorum*

Total phenolic content and flavonoids using different concentrations of Gallic acid, tannic acid and rutin respectively. The total phenolic, flavonoids and tannin content in extracts of *Desmodium triflorum* have been presented in table & Fig. Observation shows that the total phenol & Flavanoid content in aqueous extract shows significant (P<0.001) different from ethanol extract.

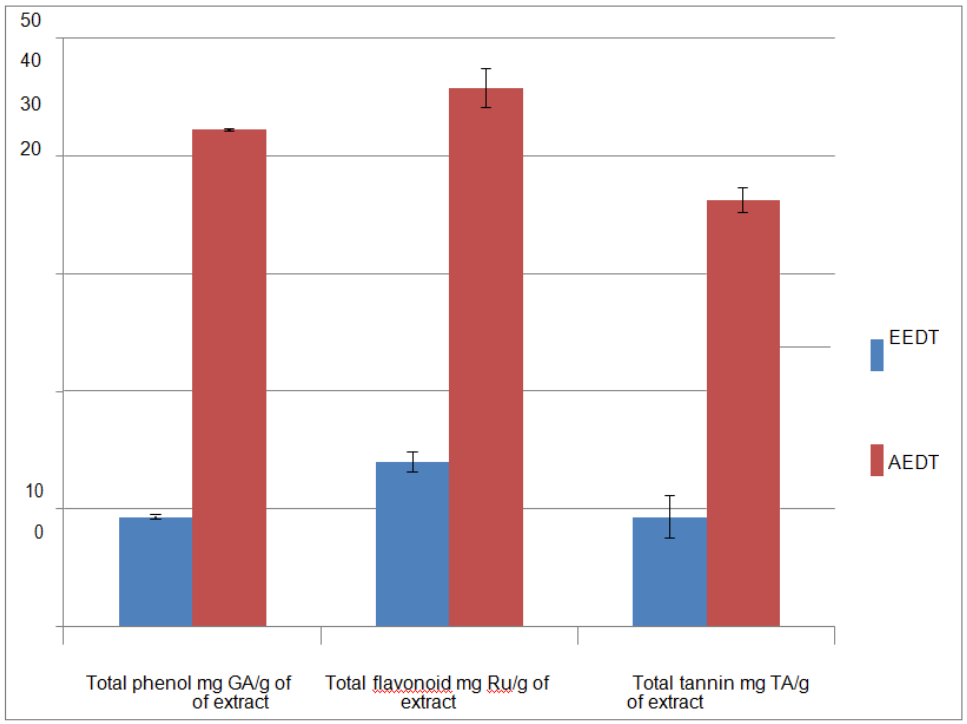


Fig 1: Total phenol, flavonoid and tannin content of different extracts of leaves of *Desmodium triflorum*.

ACUTE ORAL TOXICITY STUDY

Table 3: Observation parameters in acute toxicities of aqueous extract of *Desmodium triflorum*

PARAMETERS	OBSERVATION
Tremors	Not observed
Convulsions	Not observed
Salivation	Normal
Sleep	Slight sedation
Diarrhoea	Feces Normal
Lethargy	Observed laziness
Skin and Fur	Normal
Eyes and Mucous membrane	Normal
Respiratory	Normal
Circulatory	Normal
Autonomic and Central nervous system	Depression observed
Somatomotor activity	Less motor activity

There was no mortality observed up to 2000mg/kg and studies were carried out with 1/10th of LD50 of extracts as 200 mg/kg and double the dose.

Effect of *desmodiumtriflorum extract* on oral glucose tolerance test

The effects of extract of *Desmodium triflorum* (200&400 mg/kg) on glucose tolerance test are shown in table & figure. The administration of extract improved the glucose tolerance in the fasted normal rats. At 30 min after glucose administration the peak value of blood glucose level increased rapidly and then subsequently decreased at 90 and 120 minutes. Extract showed significant hypoglycemic (P< 0.001) effect after 90 minutes of treatment like Standard compared to control.

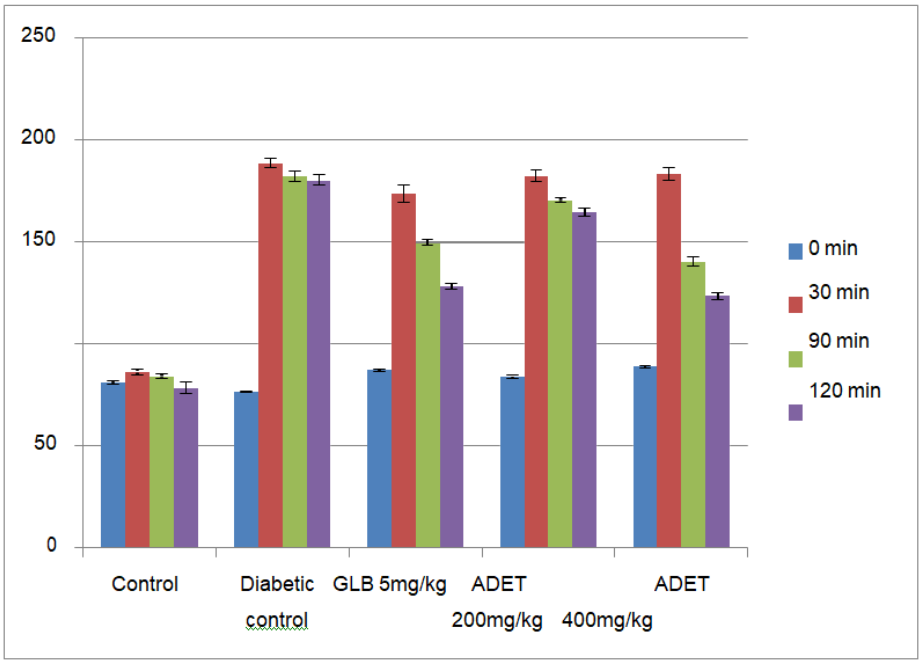
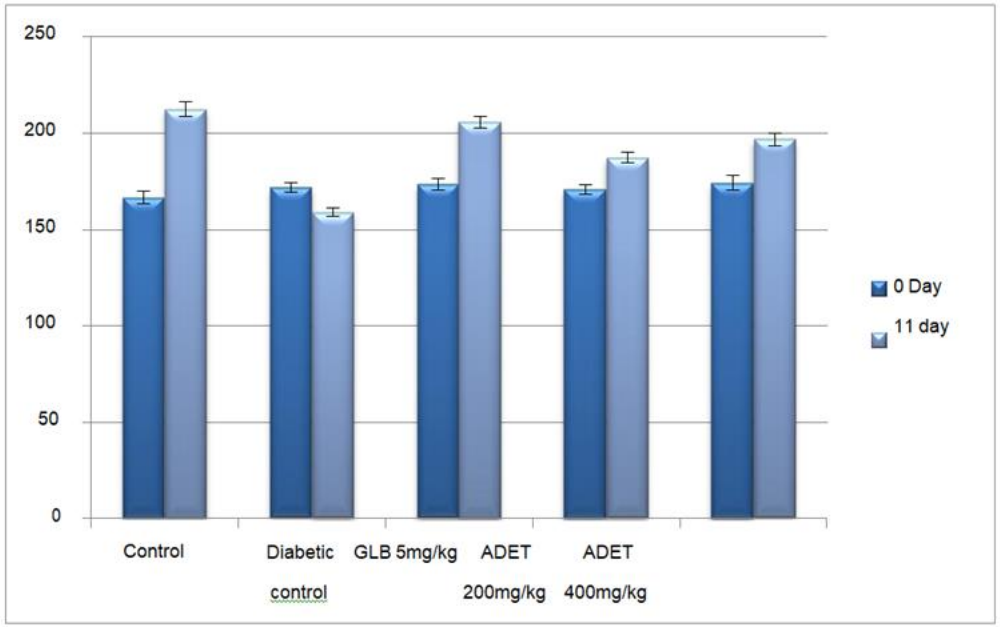


Fig 2: Effects of *Desmodium triflorum* extract on oral glucose tolerance in rats

Body weight and urine glucose level

The table shows the body weight of the normal and treated groups significantly differ from diabetic control on 11th day. Also the same urine glucose level of normal and treated groups also significantly differs from diabetic control on 11th day shown in Table.



The values are mean±SEM, n=6 when treated group compared with diabetic control **p<0.01

Fig 3: Effect of *Desmodium triflorum* on body weight

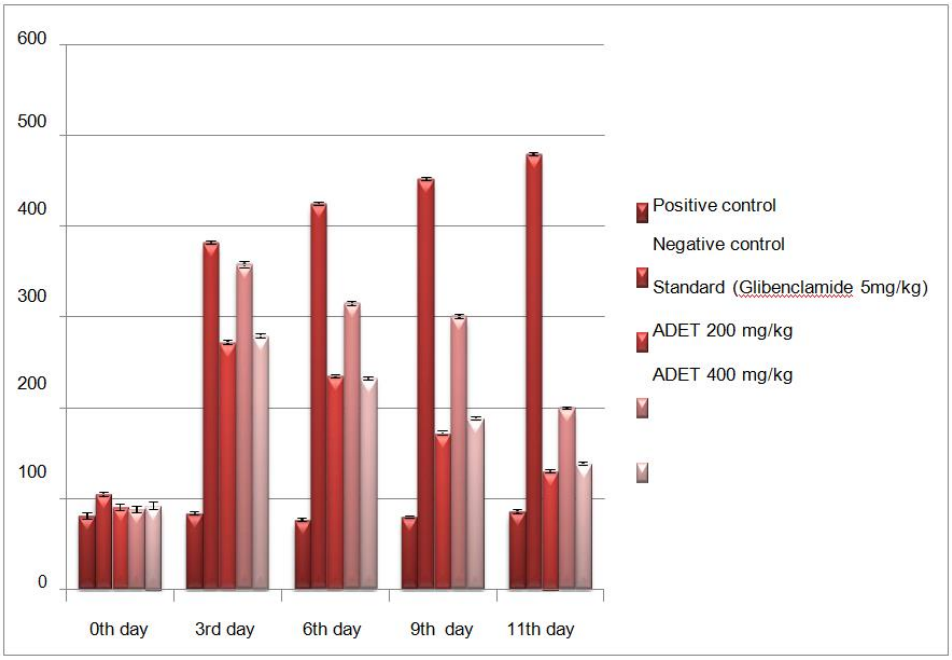
Table 4: Effect of leaves of *Desmodium triflorum* on urine sugar level in Streptozotocin induced diabetic rats.

Groups	Treatment	Urine sugar on 11th day
Positive control	2 ml of Distilled water	-
Negative control	Streptozotocin 60 mg/kg	+++
Standard	glibenclamide (5mg/kg)	-
AEDT	200 mg/kg	+
AEDT	400 mg/kg	-

(Trace = +, significant= +++, Nil = -)

Effect of leaves of *Desmodium triflorum* extract on blood glucose level

The standard (Glibenclamide (5mg/kg), aqueous extract 200 & 400 mg/kg treated groups, significantly decrease in blood glucose level (130.0±13.39, 200±10.2 & 138.20±13.87) on 11th day compared to negative control (479.32±10.82). (Figure: Table No:). Thus, the AEDT 400 mg/kg was found to be more significant ($p<0.001$) like standard drug in lowering blood glucose level compare to diabetic control and non-significant to normal control.



The values are mean ± SEM, n=6 when compared with diabetic control ***p<0.001

Fig 4 : Effect of AEDT leaves of *Desmodium triflorum* on blood glucose level on Streptozotocin induced diabetic rats.

DISCUSSION

In the present study, the extract of *Desmodium triflorum* leaves (200 and 400 mg/kg body weight) was investigated for antidiabetic activity on STZ diabetic rats. Significant declines of body weight gain in STZ-diabetic rats were noted after 11 days. Similar observations were noted in many experimental diabetes researches. An increase in body weight implies that anabolic effects have overridden the catabolic ones. No variation means protection against weight loss. Decrease in body weight would mean that catabolism has persisted. The destruction of β-cells and disorder of insulin secretion in the diabetic state causes physio-metabolic abnormalities such as a decrease in body weight gain and increase in food and water intake and urine volume. The diabetic rats induced by STZ also showed these changes [17]. STZ induced diabetes is characterized by severe weight loss [18]. The decrease in body weight in diabetic rats might be the result of protein wasting due to unavailability of carbohydrate as an energy source. Increased food consumption and decreased body weight observed in diabetic rats in comparison to normal rats indicates a polyphagic condition and weight loss due to excessive breakdown of tissue proteins. In the present study, the levels of serum glucose were statistically decreased in diabetic rats of group extract treated group.

CONCLUSION

The present study showed that the treatment of diabetic rats with Desmodium triflorum leaves extract improved the normoglycemia in diabetic rat. From the present study, it is obviously that the role of AEDT leaves extract on hyperglycemia may be attributed to The inhibition of oligosaccharides and polysaccharides digestion with lowering secretion of their specific hydrolytic enzymes, The diminution of intestinal glucose and other monosaccharides absorption rate, The enhancement of glycogenesis and decline of glycogenolysis and gluconeogenesis processes. Accordingly, it can be concluded that AEDT leaves extract has a beneficial influence on Diabetic and its complications. Finally, additional experimentation is required to elucidate the influences of AEDT leaves extract and its constituents as promising therapeutic agents for Diabetic Mellitus and its complications.

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