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ANTI-DIABETIC ACTIVITY OF METHANOLIC EXTRACT OF DESMOSTACHYA BIPINNATA IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT
The present study was aimed to evaluate the anti-diabetic activity (potential) of Desmostachya bipinnata on streptozocin induced experimental diabetes in rats. Desmostachya bipinnata(linn) is a sacrificial grass, which has pharmacological activities like anti-oxidant, anti-inflammatory, diuretic, anti-diarrhoeal, anti-ulcerogenic. The extract many chemical constituents i.e., flavonoids, phenolic compounds like tannins and carbohydrates. Oral administration of methanolic extract of Desmostachya bipinnata (200 and 400 mg/kg/bodyweight/rat/day) for 15 days significantly reduced the elevated levels of blood glucose and in diabetic rats. Electronic microscopic studies showed significant morphological changes in mitochondria and endoplasmic reticulum of β cells of STZ induced diabetic rats. Also a decrease in the number of secretory granules of beta cells was observed in the STZ induced diabetic rats. Effect on weight gain and fluid intake has revealed the increase in weight gain and fluid intake. The extract produced a significant ($P<0.05$) uptake of glucose by isolated rat hemi-diaphragm but less effective to that of the reference drug, Glibenclamide. Glucose uptake by isolated rat hemidiaphram method revealed the increase in glucose uptake in standard and extract treated groups. The pathological abnormalities are normalized after treatment with Desmostachya bipinnata extract, the efficacy of Desmostachya bipinnata was capable with Glibenclamide, a well-known hypoglycemic drug. so it can be concluded that the methanolic extract of DB may be effective as anti-diabetic.

KEYWORDS: diabetes, D. bipinnata, methonolic extract of D. bipinnata, Electron microscope, Glucose by isolated rat hemidiaphram.

INTRODUCTION
The pharmacotherapy of diabetes has recently undergone unprecedented expansion. Diabetes mellitus is a disease in which homeostasis of carbohydrate, protein and lipid metabolism is improperly regulated by hormone insulin resulting in elevation of fasting and postprandial blood glucose level.[1] The major complications associated with diabetes include retinopathy, neuropathy, nephropathy, atherosclerotic coronary artery disease and peripheral atherosclerotic vascular disease. Besides hyperglycemia, several other factors like hyperlipidemia and enhanced oxidative stress play a major role in diabetic pathogenesis.[2] There are an estimated 143 million people in the world with diabetes mellitus and this number will probably double by the year 2030.[3] Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. In recent times, there has been a renewed interest in the plant remedies.[4] Desmostachyabipinnata is a species of D. bipinnata(L), commonly known as 'Darbha' in Telugu, belonging to the family Poaceae, is drought and salt-tolerant C4grass of desert or semi-desert conditions with a deep, strong rhizome, making it an excellent sand-binder. The plant contain flavonoids, phenolic compounds like tannins and carbohydrates also. The plant selected for this present work is locally available in the East Godavari district and has been used for long a time in local folklore medicine for the treatment of diabetics.

MATERIALS AND METHODS
Plant Material and Preparation of Extracts
The plant material was collected at pitapuram area of East Godavari (Andhra Pradesh). The plant authentication was done by Dr. A.Srinivasa Rao, Dept. of Botany, P.R Degree College, Kakinada, East Godavari District, Andhra Pradesh, India & the voucher was preserved. The plant material was thoroughly cleaned, shade dried at room temp. for 23 days & then pulverized

to a coarse powder and shifted. 95% ethanol was added to coarsely powered (2kg) plant material & extracted by using soxhlet apparatus. The extract was concentrated by distillation under reduced pressure & evaporated to dryness.

Animals

Healthy adult albino rats of wistar strains weighing 150-250gm of either sex were used in this study. The animals were kept properly in polypropylene cages under standard laboratory conditions (12/12hr light/dark cycle at 25±50c). The rats were fed a commercial diet & water ad libitum & were divided into 5 groups. The experimental protocol was approved by the Institutional Animal Ethical Committee (Approval no: 12/2016/CPCSEA).

Acute Toxicity Studies

Wistar albino rats 160-180 g of either sex were used for acute toxicity studies to determine a safe dose as per acute toxic class method of organization of econamic co-operation and development(OECD) 423 guidelines (E cobicon) 1997.[5] Animals were fasted for 3 hours prior to the experiment and were observed continuously for 3 hours for general behavioral, neurological, autonomic profiles, mortality and then every 30 minutes for the next three hours and finally throughout the study,up to 14th day of oral administration of extract (dose range 250-500 mg/kg).

Acute toxicity study was performed according to OECD guideline 423. Animals were fasted prior to dosing, food but not water should be withheld overnight. Following the period of fasting, the animals were weighed and extract was administered.[5] Three animals are used for each step. The dose level of whole plant extract to be used as the starting dose is selected from one of the dose levels 1/10th and 1/5thof 2000mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dose animals. After administration of test sample, the animals were observed continuously for first 4 hours for behavioral changes and at the end of 24 hr formortality rate if any.

Induction of Hyperglycemia

The rats were fasted overnight and the diabetes was induced by a single i.p injection of a freshly prepared solution of streptazocin (STZ) 50 mg /kg body weight in 0.1 M normal saline [6]. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. Control rats were injected normal saline alone. After 48 hours, the development of diabetes having glycosuria and hyperglycemia (blood glucose range 250mg/ d L) were considered as diabetic and used for the drug treatment. The extract in aqueous solution was administered orally through an oral feeding tube (gavage) at a concentration of 200 and 400 mg/body weight/day for 15 days.

Experimental deign

Animals were divided into five groups, six animals in each. Group I is Normal control rats treated with 25 % normal saline. Group II is Diabetic control rats treated with normal saline. Group III is standard group treated with Glibenclamide. (Reference drug).Group IV is Test 1 Diabetic + Effective dose of plant extract (200mg/kg body weight) in 25% Tween 20 in distilled water. Group V: Diabetic + Effective dose of plant extract (400mg/kg body weight) in 25% Tween 20 in distilled water. Blood glucose levels will be checked at weekly intervals i.e., on 0 or initial, 1 day, 7th, 15th, days of the experiment. The determination of blood glucose levels is done by tail tipping method using Accuchek-sensor glucometer.

Statistical Analysis:- The statistical significance was measured by using one way analysis of variance (ANOVA) & followed by Dunnett's comparison test. All the data are presented as mean ± SEM & $p < 0.05$ was considered as significant.

RESULTS

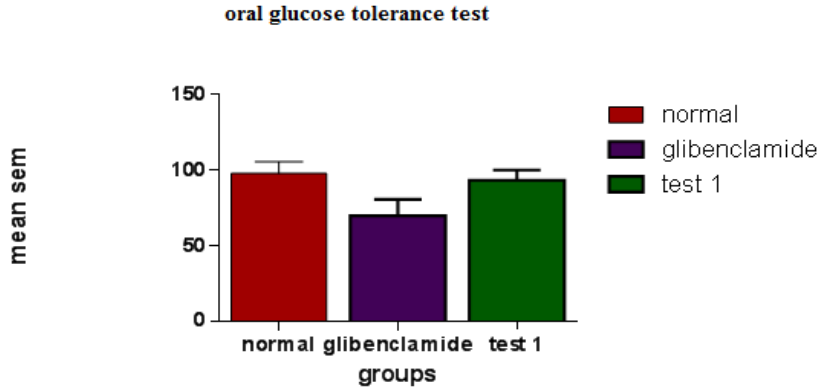
Oral Glucose Tolerance Test (OGTT)

The blood glucose concentrations of the animals were measured at the beginning of the study. Then the rats were orally treated with 3 g/kg body weight glucose solution after 30 minutes of the product and drug treatment. The measurements were repeated after 30, 90 and 150 minutes after the glucose load.

Table 1: blood glucose levels.

Group	Dose	0 Minitue	30 Minutes	90 minutes	150 munities
Normal control	5%tween 80 1ml/kg	81.00±2.7	115.80±7.04	106.40±4.7	89.20±1.83
Glibenclamide	4mg/kg	82.8±2.42	75.80±3.79**	64.00±5.21	58.80±2.08*
Extract	400mg/kg	83.00±5.29	97.80±1.50*	97.80±3.80	97.60±5.94

(The values are expressed as mean + or – SEM,compared with Dunnet's multiple comparision test).$p<0.05$ was considered as significant.

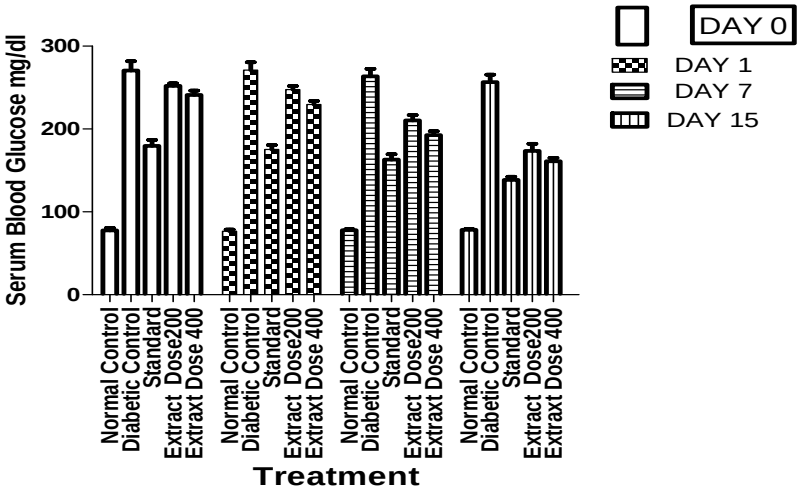


Graph 1: oral glucose tolerance test.

Table 2: Effect of methanolic extract of whole plant of desmostachya bipinnata extract on Streptazocin induced diabetic rats on blood glucose levels.

Day	Normal control	Diabetic control	Standard	Extract test1 (200mg/kg body weight)	Extract test 2 (400 mg/kg body weight)
0	77.66±2.83	270.5±11.26	179.5±7.34***	252.16±3.00	241±5.44*
1	76.66±1.92	271±9.54	175.33±5.28***	247.66±4.18	229.5±4.44**
7	77.83±1.27	263.5±9.01	163±6.54***	210.33±6.47*	192.5±4.97***
15	78.33±1.11	256.66±8.90	138.66±3.70***	173.5±8.67**	161±4.19***

(Values are expressed as mean+ or – SEM), Dunnett's multiple comparison test,*P Value < 0.05, **P Value < 0.01, *P Value < 0.001 significant).

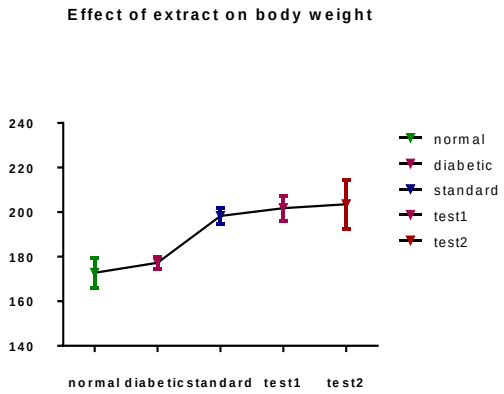


Graph 2: Treatment vs Serum Glucose Levels.

Table 3: Effect of Desmostachya Bipinnata whole plant extract on body weight and fluid intake.

Group	Body weight(g)	Body weight(g)	Fluid intake ml/day
	Before treatment	After treatment	
Untreated control	194+-1.88	220.5+-1.85	22.54+-0.34
Diabetic control	190.66+-2.33	168.5+-2.513	75.78+-0.57
Diabetic + glibenclamide(10mg/kg)	198.45+-1.74	200.5+-1.96**	53.610+0.57*
Diabetic+ methanolic extract of Desmostacha bipinnata (200mg/kg)	179.45+-1.45	198.56+-1.78*	56.45+-0.166
Diabetic+ methanolic extract of desmostachya bipinnata (400 mg/kg)	198.5+-2.12	203.56+-1.65**	42+-0.357

In normal group, a significant weight gain was observed compared to diabetic group. The extract treated groups also shown significant weight gain compared to diabetic control. All values are expressed as mean+-SEM (n=6) as compared to standard(diabetic control)
*P Value < 0.05, **P Value < 0.01, ***P Value < 0.001 significant)

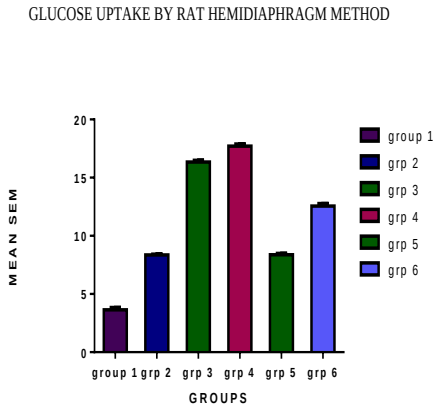


Graph 3: methanolic extract of Desmostachya Bipinnata whole plant extract on body weight.

Table 4: Effect of Desmostachya bipinnata extract on glucose uptake by rat hemidiaphram method.

S. NO.	Incubation Medium	Glucose Uptake (mg/g/30 m)
Group 1	2ml of Tyrode solution with 2% glucose	3.64±0.28
Group 2	2 ml Tyrode solution with 2 % glucose+0.62 ml insulin (0.4 IU/ml)	8.35±0.70****
Group 3	2 ml Tyrode solution with 2 % glucose+ 0.4 ml glibenclamide (1 mg/ml)	16.35±0.149***
Group 4	2 ml Tyrode solution with 2 % glucose+0.62 ml insulin (0.4 IU/ml)+0.4 ml Glibenclamide (1 mg/ml).	17.71±0.17***
Group 5	Tyrode solution with 2 % glucose+1.0 ml of Desmostachya bipinnata extracts (2 mg/ml).	8.36±0.12***
Group 6	Tyrode solution with 2 % glucose+1.0 ml of Desmostachya bipinnata extract (2 mg/ml)+0.62 ml insulin (0.4 IU/ml).	12.56±0.16**#

Results were represented as mean±SEM. Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. P values <0.05 were considered significant.
*P<0.05 as compared to control, The total volumes for all the groups were made up to 4 ml with distilled water.



Graph 4: Glucose Uptake by Rat Hemidiaphragm Method.

Histopathology Studies

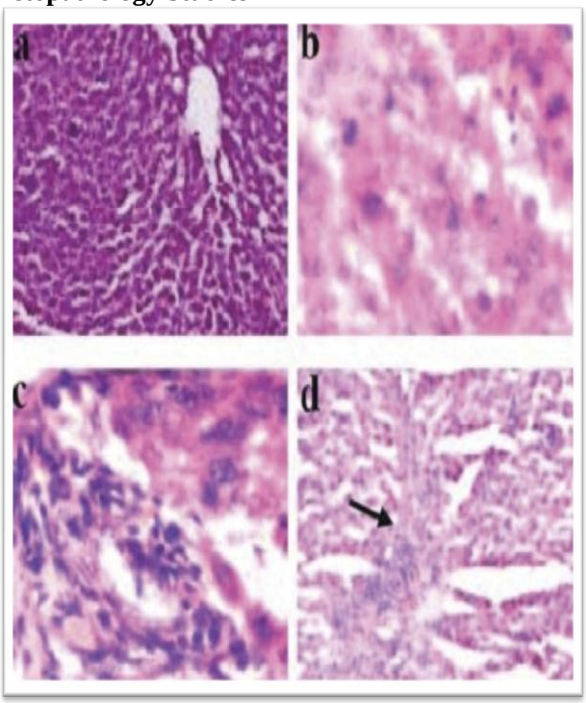
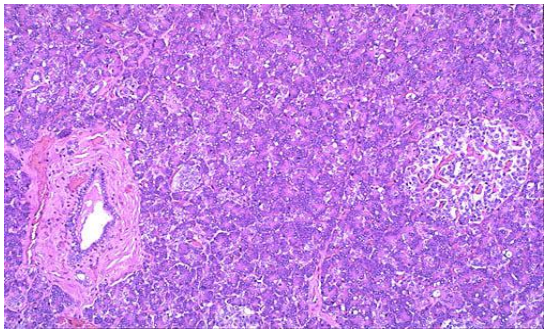


Figure 1: Hematoxylin and eosin staining of rat liver during the pathogenesis of diabetes induced hepatic.

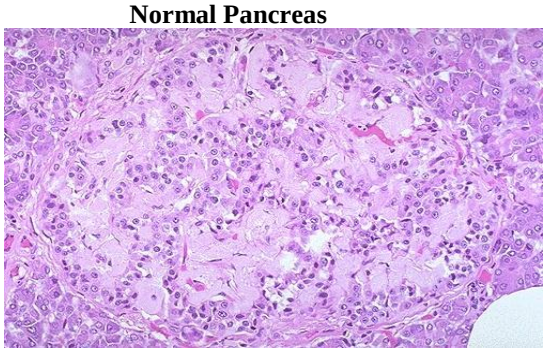
fibrosis: A control liver, B: streptazocin treated, 7th day, severe congestion and hepatic necrosis, day 15th: severe neutrophilic infiltration and fatty changes and D: marked hepatic fibrosis and deposition of collagen fibers.

Histopathology of pancreas

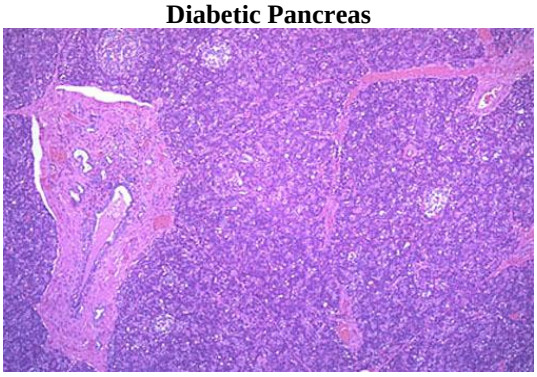
Light patches are islets of laungerhans; darker regions are enzyme producing cells in normal control pancreas. In the next slide, there appear to be fewer glandular cells and more connective tissue throughout the pancreas. It is very difficult to find out recognizable islets of laungerhans.



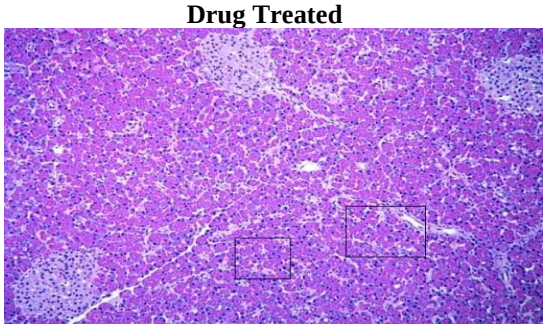
Normal Pancreas



Diabetic Pancreas



Drug Treated



Test 1 treated (DP200)

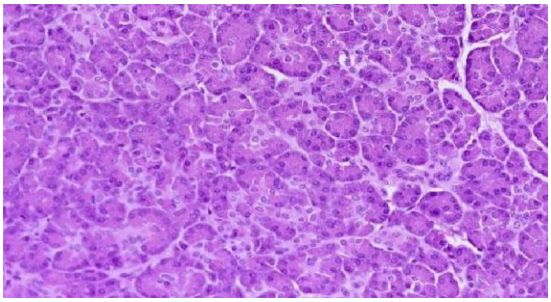


Figure: 2: Histopathology of pancreas. Test 2 (DP400) treated.

DISCUSSION

The preliminary phytochemical investigation of the extract revealed the presence of steroids, terpenoids, alkaloids, carbohydrate and phenolic compounds such as tannins, and flavonoid. These compounds are likely to be responsible for the observed antioxidant and anti-diabetic activity of this extract either single or in synergy with one another.

Streptozocin (STZ) is commonly used for experimental induction of type-1 Diabetes Mellitus, which causes selective pancreatic beta cell toxicity mediated through the release of nitric oxide (NO). This results in rapid reduction in pancreatic islet pyridine nucleotide concentration and subsequent beta cell necrosis. The action of STZ on mitochondria generates Superoxide (SOD) anions, which leads to diabetic complications. Based on above perspectives, in the present study, the oxidative stress has been assessed in rats made diabetic by STZ. Sulfonylureas, such as Glibenclamide, are often used as a standard anti-diabetic drug in STZ induced diabetes to compare the efficacy of variety of anti-hyperglycemic compounds.

Acute toxicity studies on wistar albino rats showed no mortality at a dose of 200 mg/kg, during a time period of 14 days. During the study, no noticeable responses were seen in the rats. This helps to predict that it does not contain any type of toxicity and is safe

In acute toxicity study there was no behavioral changeup to 4 hours and no mortality was observed up to the end of 48 hours even at the maximum tested dose of 1000mg/kg per oral. As a result an effective dose of 200 and 400mg/kg body weight is taken.

The extract was evaluated for in-vivo anti-diabetic activity using Streptozotocin at a dose of 50mg/kg body weight. In the present study it was found that there is a marked elevation in the blood glucose level after administration of streptozotocin. This is in accordance with the reports published by various authors where the increase in glucose levels has been attributed to the destruction of beta-cells by Streptozotocin. Damage to the beta-cells is associated with the liberation of stored insulin after which the insulin synthesis is stopped leading to a persistent diabetic state. Since insulin no longer be available, glucose absorption is impaired leading to hyperglycemia. There was a significant rise in

blood glucose level in group II, group III and group IV after Streptozotocin administration compared to normal group I. After the treatment of animals with the methanolic whole plant extract of Desmostachya bipinnata for 15 days, it was observed that the elevated blood glucose levels decreased significantly. These results have been tabulated and represented by means of a graph using ANOVA followed by Dunnett's multiple comparision test.

In our study, there was a significant elevation in blood glucose level in diabetic control group as compared with normal animals. The methonolic extract of Desmostachya bipinnata –treated group exhibited significant reduction in fasting glucose levels as compared to the diabetic control group. Over production of glucose by means of excessive hepatic glycogenesis is one of the fundamental basis of hyperglycemia in diabetes mellitus. The decrease in body weight with diabetes mellitus has been attributed to the gluconeogenesis i.e. catabolism of proteins and fats, which is associated with the characteristic loss of body weight due to increased muscle wasting and loss of tissue proteins. In the present study, diabetic rats treated with extract, showed an increase in body weight as compared to diabetic control, which may be due to its protective effect in controlling muscle wasting i.e.; reversal of gluconeogenesis. The ability of D.bipinnata extract in effectively controlling the increase in blood glucose levels in the diabetic group of rats (table 1) and significant increase in body weight (table 2) may be attributed to its anti-hyperglycemic activity.

As in case of Weight gain and increased fluid intake, after the treatment with methanolic extract of Desmostachya bipinnata 200 mg/kg b.wt and 400 mg/kg b.wt in diabetic induced rats, the body weight was found to be increased from 198±1.94 to 179.145±1.76and from 198.16±1.78to 233±1.476 respectively. Fluid intake was also found to increase up to 56 ml/day and 42 ml/day for the groups treated with the methanolic extract at the dose of 200 mg/kg b.wt and 400 mg/kg b.wt respectively when compared to untreated group (22ml/day). Similar effect was observed in the diabetic group treated with Glibenclamide (10mg/kg b.wt). In this group the body weight was found to be increased from 216.66±1.745 to 236.33±1.96 and fluid intake was also found tobe increased up to 53 ml/day when compared to the untreated control groups

Histopathology studies showed that there were a reduced number of beta cells in diabetic animals. There was no change in the number of cells on treatment with extract or glibenclamide, which suggest that there is no regeneration of beta cells or no insulinogenic property. The histopathology reveals that the site of action may be extra pancreatic and not the regeneration of beta cells and the decrease in blood glucose levels may be attributed to the stimulation of glucose uptake by peripheral tissues and decrease in gluconeogenesis. Hence, the anti-hyperglycemic effect may be probably brought about by an extra pancreatic mechanism.

CONCLUSION

Desmostachya bipinnata is widely used herb in folk and Ayurvedic system of medicine for various properties. The whole plant extract has been shown to have significant anti-diabetic activity. The methanolic extract of D. bipinnata was evaluated for in-vivo anti-diabetic activity against Streptozocin induced diabetic rats. The anti-hyperglycemic effect of methonolic extract of whole part of Desmostachya bipinnata Linn is mediated through the peripheral mechanisms and theeffect may be attributed to the components such as flavonoids, tannins, triterpinoids, and antioxidant properties present in the extract. Since Streptozotocin destroys the beta-cells the mechanism of drugs acting by enhancing the release of Insulin is generally not suggested in Streptozocin diabetic models. Therefore D. bipinnata may act by either inhibiting the glucose absorption from the gut or by enhancing the transport of glucose from the blood. Our study supports the traditional usage of the whole plant of D.bipinnata by Ayurvedic physicians for the control of diabetes. Hence it might help in preventing the complications of diabetes and serves as a good adjuvant in the present armamentarium of anti-diabetic drugs

REFERENCES

1. Choudhary N, Jha A, Sharma S, Goyal S, Dwivedi J. Anti-diabetic potential of chloroform extract of flowers of Calotropis gigantea: An in vitro and in vivo study. International Journal of Green Pharmacy, 2011; 5(4): 296-301.
2. Karunakar Hegde, Jaisal KK, Anti-diabetic Potential of Ethanolic Extract of Holarrhena antidysenterica Linn Leaves. International Journal of Pharma Sciences and Research, 2014; 5: 429-435.
3. Amar B Singh, J P Chaturvedi, T Narender and Arvind K Srivastava. Preliminary studies on the Hypoglycemic effect of peganum harmala I. seeds ethanol extract on normal and Streptozotocin induced diabetic rats. Indian Journal of Clinical Biochemistry, 2008; 23(4): 391-393.
4. Jatin jaiswal, hemant bhardwaj, shruti srivastava, hemendra gautam, surabhi sharma, ch. V rao. Anti-diabetic activity of Methanolic Extract of Calotropis Gigantea seeds on stz induced diabetic rats. International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6: 254-259.
5. B. Gandhare, S. Kavimani, and B. Rajkapoor, Acute and Subacute Toxicity Study of Methanolic Extract of ceiba pentandra (Linn.) Gaertn. on Rats. Journal of Scientific Research, 2013; 5(2): 315-324.
6. Anish Das1, Thomraj Khati Chhetry, Evaluation of Anti-Diabetic Activity of Mahonia nepalensis in STZ Induced Rat Model. International Journal of Pharmacognosy and Phytochemical Research, 2016; 8(7): 1104-1110.