Available online on 15.07.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Evaluation of Antidiabetic Activity of Ethanolic Extract of *Ajuga Parviflora* in Diabetic Rats

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ABSTRACT

The aim of the study was to evaluate the antidiabetic activity of ethanolic extract of whole plant of *Ajuga parviflora* in diabetic induced Sprague Dawley rats. Acute toxicity study was done on screened rats for 14 days. The rats were divided into four groups like normal, diabetic, treated and standard (diabetes treated with Glibenclamide). Streptozocin 160mg /kg body weight was administered intra peritoneally to rat for the induction of diabetes. After the confirmation of diabetes, the rats were subjected to further *in-vivo* studies. The formulation obtained by ethanolic extract of whole plant of *Ajuga parviflora (60mg/kg)* were administered orally to diabetic rats for 28 days. The body weight and fasted blood glucose level measured in diabetic and non-diabetic rats at the end of experiments. The formulation shows significant reduction in blood glucose level in Streptozocin induced rats that is comparable to *Glibenclamide.*. Methanolic extract of medicinal herb *Ajuga parviflora* Benth. was evaluated for phytochemical screening the plant extract showed the presence of aromatic compounds, carbohydrates and others.

Keywords: *Ajuga parviflora, Sprague Dawley rat, Streptozocin , Phytochemical screnning.*

Article Info: Received 09 May 2019; Review Completed 15 June 2019; Accepted 21 June 2019; Available online 15 July 2019

Cite this article as:

Kumari R, Amit, Kumar P, Ahmed R, Evaluation of Antidiabetic Activity of Ethanolic Extract of *Ajuga Parviflora* in Diabetic Rats, Journal of Drug Delivery and Therapeutics. 2019; 9(4):112-115 **http://dx.doi.org/10.22270/jddt.v9i4.2984**

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1. INTRODUCTION

Diabetes mellitus (**DM**), commonly referred to as **diabetes**

Diabetes mellitus is the third leading cause of death in many developed countries. It affects 2 to 3% of the general population . The complications of diabetes affect eyes,kidney and nervous system.

1. Types of Diabetes

Diabetes mellitus is a clinical condition characterized by increased blood glucose level due to insufficient or inefficient insulin . . An important feature of diabetes is that the body cells are starved of glucose despite its very high concentration around that is scarcity in plenty. For a comprehensive understanding of diabetes, the relevant hormones, namely insulin and glucagon , homeostasis of blood glucose , besides the biochemical aspects of diabetes .

Regulation of Insulin

40 to 50 units of insulin is secreted daily by human pancreas. The normal insulin concentration in plasma is 20-30µU/ml.

Factors stimulating insulin secretion:

Glucose

Amino acids

Epinephrine

2. MATERIALS AND METHODS

2.1. Materials

2.1.1 Plant Materials and Authentication

AJUGA PARVIFLORA, (500 kg) were collected from Himachal Pradesh (India) in October.

The specimen was identified and authenticated by NBRI Lucknow .

2.1.2 Animals for experiment

Sprague Dawley rat either sex, weighing 155-255 g purchased from AIIMS animal house, Delhi. All test animals are given excess to water for drink, before experiment animals was withdrawn.

2.2 Methods

2.2.1. Preparation of plant extract of *Ajuga parviflora*

Whole plant of the *Ajuga parviflora* were thoroughly checked and freed from any adulteration; The flowers were collected from Himachal Pradesh were Authentified and then

extracted by soxlet apparatus at 27°c properly with 99% ethanol for 7 days to get a high quality ethanolic extract. The extract were concentrated in a rotary flask evaporator and after drying in a desecator made ready for study.

Fig. 1 ***Ajuga parviflora***

2.2 Animal Grouping, Feeding and Extract Administration

2.2.1Experimental design:

Animals were fasted for 24 hours before the experiment with free access to water.

2.2.2Preparation ofdrugs

All drugs and vehicle (distilled water) were given in the volume of 12 ml/kg and doses were calculated according to the body weight of animals. The doses of ethanolic extract of *Ajuga Parviflora* selected in the manner of mg/kg body weight of rat respectively. Streptozocin Standard drug administered at a dose along with distilled water solution.

The ethanolic extract of *Ajuga Parviflora* was suspended in distilled water and used for oral administration. Each time fresh preparations of the extracts were prepared.

2.3 Assessment of Antidiabetic Activity

MODEL: Streptozocin induced diabetes model

2.3.1 Preparation of Feed

Normal food was given to the rats for 30 days prior to induce diabetes.

Table.1 Classification of groups according to their dose

GROUP	N	TREATMENT	DOSE
Group 1	6	Normal control	Saline
Group 2	6	Stz. + A. parviflora whole plants extract	(160 mg/kg.) + (60 mg/kg)
Group 3	6	Stz treated control	(160 mg/kg.)
Group 4	6	Stz + Standard drug, Glibenclamide	(160 mg/kg.) + (5 mg/kg)

N= Number of animals in each group STZ= Streptozocin

2.3.2 Evaluation of Diabetic Activity

The animal was starved for whole night and diabetes was made diabetic by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (162 mg/kg b.w.) in distilled water. The animals were allowed to drink 5% glucose solution to overcome the drug induced hypoglycemia . by the fourth day of Streptozocin -injection, the rats were starved for 4h and blood was taken by retro orbital puncture under light chloroform anesthesia. Rats having middle range of increased blood glucose level (250–400 mg/dl) were taken for the experiment In the experiment, a total of 24 rats (18 diabetic surviving rats and six normal rats) were used. The rats were divided into four groups of six rats.

2.3.3 Parameters observed

Body Weight of Spargue Dawely Rats

Fasting Blood Glucose level

Blood Glucose level

Plasma Insulin level

Different Parameters of blood

Different liver contents

Kidney parameter

2.3.4 Acute toxicity study

Healthy male Spargue Dawley rats were randomly divided into 4 groups with 6 animals in each group. The animals were kept fasting overnight providing only water, after which the hydro-methanolic extract of *Ajuga parviflora* benth. extract was administered orally with increasing doses (10, 50, 100, 500, 1000 and 2000 mg/kg) by intra gastric tube to determine the safe doses by up and down staircase method . The animals were observed continuously for 1 h, then frequently for 4 h and later at the end of 24 h for general behavioral, neurological and autonomic profile. Further, one group was administered high dose of *Ajuga parviflora* benth. extract orally once daily for 15 days and observed for any lethality and death .

2.4 ACTIVITY ANALYSIS

Streptozotocin was purchased from Wokhart Pvt. Ltd.. Glibenclamide (standard drug) was purchased from Wokhardt Pvt. Ltd. All other commercial reagents used were of analytical grade.

2.4.1 Induction of experimental diabetes

The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (50 mg/kg b.w.) in 0.1 M sodium citrate buffer (pH 4.6) The animals were allowed to drink 10% glucose solution to overcome the drug induced hypoglycemia. On the third day of STZ-injection, the rats were fasted for 6 h and blood was withdrawn by retro orbital puncture under light chloroform anesthesia. Rats having diabetes with blood glucose of (244–455 mg/dl) were taken for the analysis.

Table 2: Body Weight of Spargue Dawely Rats

Groups	Mean Body Weight In Gram(g)				
	Initial('0' day)	final('15th ' day)	change	% of increase	% of decrease
Normal control	224	258	34	15.17	
Diabetic control	226	180	46		20.35
Diabetic test	228	247	19	8.33	
Diabetic standard	222	252	30	13.51	

Table 3: Blood Glucose level: fasting blood glucose level mg/dl

Group	initial	1hrs	2hrs
Group-1	97.25	133.59	148.34
Group-2	246.36	280.47	311.56
Group-3	251.20	274.17	285.61
Group-4	254.47	273.17	276.37

Table 4: Glucose Level in Blood

Blood sugar (mg/dL)		
	Initial	**On 22nd day**
Group I	71.2	73.61
GroupII	297.6	314.83
GroupIII	282.61	240.1
GroupIV	283.21	249.27

Table 5: Insulin concentration in plasma

Group	**Different Parameters of blood**			
Parameter	Grp. I	Grp. II	Grp.III	Grp.IV
Hemoglobin (mg/dL)	13.46	8.27	11.90	09.56
Serum creatinine (mg/dL)	0.94	2.23	0.98	1.74
Serum urea (mg/dL)	32.87	85.10	37.12	78.54
Total protein (g/dL)	7.57	5.22	7.18	6.13

Parameter/Groups	Group I	GroupII	GroupIII	Group IV
Alkaline phosphate (IU/L)	116	291	128.4	242.7
SGOT (IU/L)	19.14	45.27	20.29	42.63
SGPT (IU/L)	25.21	57.63	28.07	51.48
Total cholesterol (mg/dL)	128.9	259.8	154.4	242.8
Triglycerides (mg/dL)	81.1	179.7	90.3	163.1
HDL (mg/dL)	51.30	17.61	48.34	22.72

Table 6: Kidney parameter

Groups	Kidney content		
	SOD (units/mg protein)	**CAT (mmol/min/mg protein)**	**GPx(mmol/min/mg protein**
Group-I	14.74	72.32	12.98
Group-II	8.53	51.67	7.23
Group-III	13.23	71.23	10.99
Group-IV	9.82	58.32	8.92

Table 7: Different liver contents

Group	Liver content		
	SOD (units/mg protein)	**CAT (mmol/min/mg protein)**	**GPx (mmol/min/mg protein**
Group-I	8.34	86.43	10.31
Group-II	3.74	30.25	5.42
Group-III	6.24	78.35	10.00
Group-IV	4.75	78.27	7.12

3 DISCUSSIONS

In India, hundreds of plants are used traditionally for the execution of diabetes mellitus. Unfortunately only a few of such Indian medicinal plants have received scientific scrutiny. Now a days study was therefore designed to study the reduction of glucose level of *Ajuga parviflora* benth. Extract for streptozotocin induced diabetic rats. Continuously treated animals from *ajuga parviflora* benth. It was extracted till 21 days and produced a significant decrease in blood glucose level in diabetic rats in is comparision to that of standard and diabetic control group.

An increase in blood glucose seen in the oral glucose tolerance test (OGTT) was significantly greater in the diabetic rats than in the non-diabetic rats. Oral administration of *Ajuga parviflora* extract 160 mg/ kg significantly improved the impaired glucose tolerance in the diabetic rats with change in plasma insulin level. From the results it is assumed that extract of *Ajuga parviflora* benth. could be responsible for stimulation of insulin and the observed restoration of metabolic activity. The decreased level of total hemoglobin in diabetic rats is mainly due to the increased formation of HbA1c. During diabetes mellitus, the excess glucose present in the blood reacts with hemoglobin to form HbA1c. The amount of HbA1c increase is directly proportional to the fasting blood glucose level .

Administration of *Ajuga parviflora* benth. extract to diabetic rats reduced the glycosylation of hemoglobin by virtue of its normoglycaemic activity and thus increase extract against strepto- zotocin induced diabetic rats. The levels of hemoglobin in diabetic rats. The concentrations of lipids, such as cholesterol, triglycerides (TG) and HDl, were significantly higher in diabetic rats than in the control group.

The elevation of biomarker enzymes such as SGOT, SGPT, and ALP was observed in diabetic control rats and indicates the hepatocellular damage From this point of view *Ajuga parviflora* benth. extracts may act as hepatoprotective agent The diabetic hyperglycemia induces elevation of the serum level of urea and creatinine which was considered as significant markers of renal dysfunction . An increase in serum level of urea and creatinine levels in STZ-diabetic rats may indicate diminished ability of the kidney to filter these waste products from the blood and excrete them in the urine.

4. CONCLUSION

The whole plant extract has antidiabetic activity. It decreases the systemic glucose level in diabetic rats by the treatement of 25 days. There is an increase in serum cholesterol, triglycerides, LDL, VLDL and the level of HDL and creatinine decreases in the diabetic rats treated with the plant dose and standard drug and all the results were statistically significant. These results show that the ethanolic whole plant extract of *A. parviflora* was used for cure of diabetes of Type II. which may be responsible for its hypoglycemic property. Further pharmacological and biochemical investigations are underway to find out the active constituents responsible for antidiabetic activity and to elucidate its mechanism of action.

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