Research Article

AN INVESTIGATION ON ANTI-DIABETIC ACTIVITY IN AQUEOUS EXTRACT OF WHOLE PLANT OF *RUSSELIA EQUISETIFORMIS* IN EXPERIMENTAL RATS

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ABSTACT:

Diabetes mellitus (DM) is a combination of heterogeneous disorders characterized by hyperglycemia with an increasing global prevalence and incidence. The prevalence of diabetes mellitus is reported to be rising globally in parallel with an increasing prevalence of obesity. DM is a slowly developing but progressive condition and it is irreversible once established. The objective of present study is to evaluate the anti-diabetic activity of aqueous extract of whole plant of *Russelia equisetiformis (*AERE*).* Anti-diabetic activity of AERE was investigated against Alloxan induced diabetic rats using Glibenclamide as a standard. AERE was administered orally at doses of 200 mg/kg and 400 mg/kg for 30 consecutive days in diabetic rats. Fasting blood glucose level, serum lipid profiles as well as changes in body weight were evaluated. The significantly ($p< 0.05$) reduction in blood glucose levels were observed in the std & AERE treated diabetic animals at 30 day and body weight improves by the extracts during treatment period. These results indicate that the plant *Russelia equisetiformis* possess significant anti-diabetic activity.

Keywords: Anti-diabetic activity, Alloxan, Glibenclamide, Russelia equisetiformis, Body weight.

INTRODUCTION

Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism in the endocrine system due to absolute or relative deficiency of insulin secretion with/without varying degree of insulin resistance characterized by increased fasting and post prandial blood sugar levels. This leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves. Type 1- which affect the younger population; Type 2- which affect affects the population above 40; and gestational diabetes-which affect the women during third trimester of their pregnancy[1]. Chronic hyperglycemia often leads to micro vascular complications that includes nephropathy, retinopathy, neuropathy and macro vascular Complications that includes coronary artery disease, leading to myocardial infarction (heart attack) or angina, stroke (mainly ischemic type), peripheral vascular disease, which contributes to intermittent claudication (exertion-related foot pain) as well as diabetic foot and these complications leads to significant morbidity and mortality in patients with diabetes[2,3].

According to the recent estimation by International Diabetes Federations, India has 50.8 million people with diabetes by 2010 that is predicted to increase 87 million by 2030, will still top the list. Diabetes caused 1.5 million deaths in 2012. Hyperglycemia caused an additional 2.2 million deaths, by increasing the risks of cardiovascular and other diseases. 43% of these 3.7 million deaths occur before the age of 70 years[4].

Traditionally, numerous herbs have been recommended for treatment of diabetes. Presently, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents (therapeutic agent) for the treatment of diabetes mellitus [5].

Russelia equisetiformis (RE) recently introduced into the new monophyletic family Plantaginaceae, is native to Mexico. It is an evergreen, perennial, weeping shrub, commonly named as firecracker plant, coral plant. RE is traditionally used in Nigeria to cure malaria, cancer, inflammatory disorders, diabetes, leukemia, and in hair preparations to promote hair growth. The fresh entire plant decoction is taken orally to cure kidney stones in Colombia, and the whole plant is utilized as complementary therapy for DM2 patients in Mexico. Additionally anti-oxidant, anti- inflammatory, antinociceptive and

analgesic properties were reported for different extracts of RE, as well as antibacterial, antimicrobial, cytotoxic, CNS depressant, hepatic function, membrane stabilizing activity[6].

MATERIALS AND METHODS

Collection of plant material

The fresh plants of *Russelia equisetiformis* were collected in the month of July-August from the local areas of Mangalore district, Karnataka state, India. The taxonomic were authenticated by Dr Shobha, Botanist, Mangalore, Karnataka. The dried whole plants were pulverized into coarse powder at plant mill and 100g of the powered samples were extracted with water by using Soxhlet apparatus. The extracts obtained were dried and used for anti-diabetes studies.

Preparation of Aqueous Extract

The powdered material was subjected to batch extraction in Soxhlet apparatus by using water as solvent. The powdered material (150g) of whole plants of *Russelia equisetiformis* was packed in Soxhlet extractor and extracted using water as solvent for 12 cycles. The temperature was maintained on an electric heating mantle with thermostat control. Appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was concentrated by using rotary flash evaporator. The concentrated extract was then air dried at room temperature, weighed and percentage yield was calculated. The colour and consistency of the extract were noted.

Preliminary phytochemical screening[7]

About 50 mg of the solvent-free extract was stirred with little quantity of dil HCl & then filtered. The filtrate was subjected to preliminary phytochemical screening for detection of major chemical constituents this revealed the presence of alkaloids, Carbohydrates, Steroids, Proteins, Tannins, Flavonoids, Glycosides, Saponins and terpene.

Experimental animals

Healthy Wistar albino rats (150–200g) of either sex were used for the experiment were procured from the animal house of Srinivas College of Pharmacy, Mangalore. They were maintained under standard conditions. The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. All the experimental protocols were reviewed and approved by the institutional animal ethical committee (Approval no SCP/IAEC/F150/P111/2017) prior to the initiation of the experiment and the care of the laboratory animals were taken as per the CPCSEA regulations. The animals were acclimatized for at least one week before use.

Alloxan induced anti-diabetic activity[8]

Hyperglycemia was induced by single i.p injection of 100 mg/kg of Alloxan monohydrate in normal saline in animals except group I. After 2 days of Alloxan injection, the animals having blood sugar level >200 mg/dl was selected for the study and divided into four groups for the anti-diabetic study. The treatment was given orally once daily for entire 30 days except diabetic control group. The animals had free access to feed and water ad libitum.

Experimental Design

The Wistar albino rats (150-200g) of either sex were randomly divided into five groups of six each. The different groups were assigned as follows.

Group I : Normal control (Vehicle)
Group II : Diabetic control (Alloxan100 mg/kg)
Group III : Diabetic animals (Alloxan100 mg/kg + Glibenclamide 5mg/Kg)
Group IV : Diabetic animals (Alloxan100 mg/kg + AERE 200mg/kg)
Group V : Diabetic animals (Alloxan100 mg/kg + AERE 400mg/kg)

Evaluation

Starting from the first day of treatment, blood was collected every week from retro orbital puncture and glucose level was estimated. On 30th day, Body weight noted and post treatment blood was collected; serum was separated and used for estimation of various biochemical parameters like fasting glucose, Total cholesterol, triglycerides, HDL- cholesterol, LDL-Cholesterol, SGPT and SGOT.

STATISTICAL ANALYSIS

The values are expressed as mean ± SEM. The results were analyzed for statistical significance using one-way ANOVA, followed by Dunnett's test. P value less than 0.05 was considered significant.

METHODS FOR ESTIMATION OF BIOMARKERS

The animals were sacrificed at the end of experimental period of 30 days by decapitation. Blood was collected, sera separated by centrifugation at 3000 rpm for 10 minutes. serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) levels in the normal, diabetic control and drug treated rats was measured spectrophotometrically as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit using Semi Autoanalyser.

RESULTS

Table1: Preliminary phytochemical screening of aqueous extract of whole plant of *Russelia equisetiformis.*

Sl. No.	Test	Result
1.	Alkaloids	+ve
2.	Carbohydrates	+ve
3.	Flavonoids	+ve
4.	Glycosides	+ve
5.	Saponins	+ve
6.	Steroids	+ve
7.	Tannins	+ve
8.	Proteins	+ve
9.	Volatile oil	-ve

Table 2: Effect of aqueous extract of whole plant of Russelia equisetiformis on blood glucose level in Alloxan induced diabetic rats

Groups	Blood glucose level(mg/dl)				
	Initial	Day 7	Day 14	Day 21	Day 30
Normal control	71.69 ± 1.441	76.84± 2.204	72.16± 1.504	77.20± 1.465	72.31± 1.526
Diabetic control	303.3± 3.705	326.33± 6.305	337.2± 7.231	321.0± 8.002	312.6± 12.40
Glibenclamide (5mg/kg)	310.8± 3.809	283.1± 8.200***	227.7± 13.88***	171.3± 5.76***	132.6± 3.11**
AERE (200mg/kg)	317.6± 3.905	299.3± 6.729*	262.9± 4.954**	234.7± 10.65*	183.4± 5.632*
AERE (400mg/kg)	312.77± 3.567	289.2± 4.011*	233.3± 9.323**	188.6± 3.633**	148.5± 2.188**

Values are expressed as mean ± S.E.M, n=6 in all group except in diabetic control One way ANOVA followed by Dunnett's t- test. *p<0.05, **p<0.01, ***p<0.001, when compared with diabetic control rats.

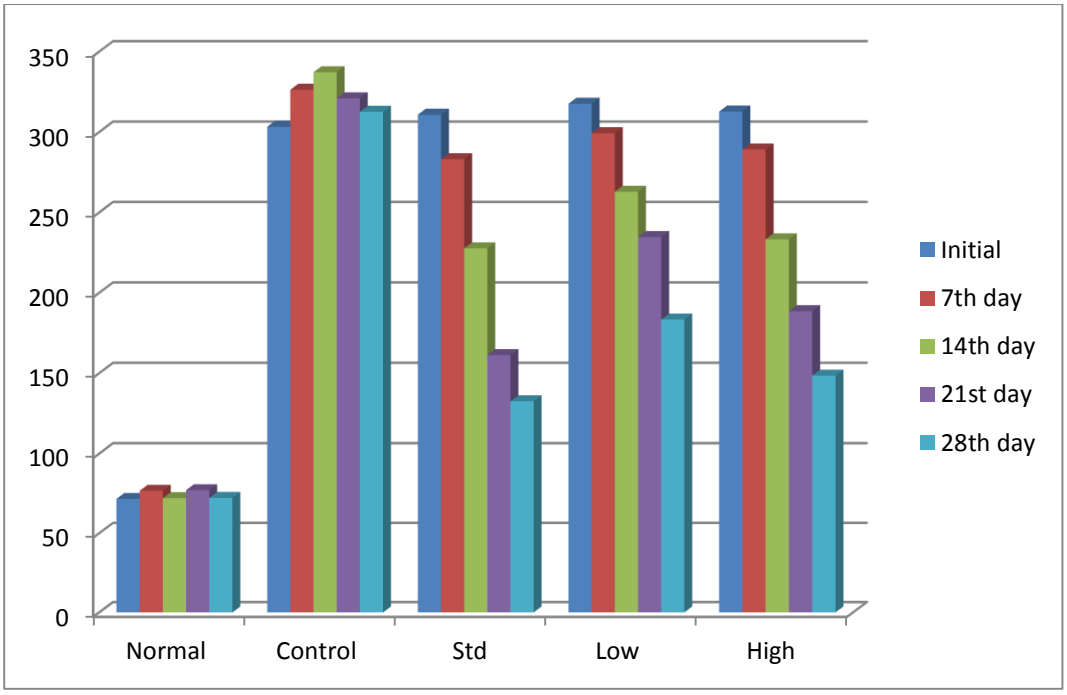

Fig. 1: Effect of AERE on blood glucose level in Alloxan induced diabetic rats

Table 3: Effect of AERE on body weight in Alloxan induced diabetic rats

GROUPS	BODY WEIGHT (Grams)			
	Day 0	Day 7	Day 14	Day 21
Normal control	180.8± 0.32	187.1± 0.91	192.3± 1.98	204.2± 0.75
Diabetic control	186.6± 0.67	158.6± 0.65	145.1± 3.65	135.8± 0.45
Glibenclamide (5 mg/kg)	182.8± 0.87**	183.8± 1.33**	191.7± 0.54***	196.4± 2.66***
AERE (200 mg/kg)	190.0± 0.81**	192.7± 2.33*	195.9± 0.75**	199.8± 1.08*
AERE (400 mg/kg)	192.8± 0.77**	195.6± 1.33**	197.8± 0.60*	203.4± 2.55**

Values are expressed as mean ± S.EM, n=6 in all except in diabetic control, one way ANOVA followed by Dunnett's test. *p<0.05, **p<0.01 and ***p<0.001 when compared to diabetic control.

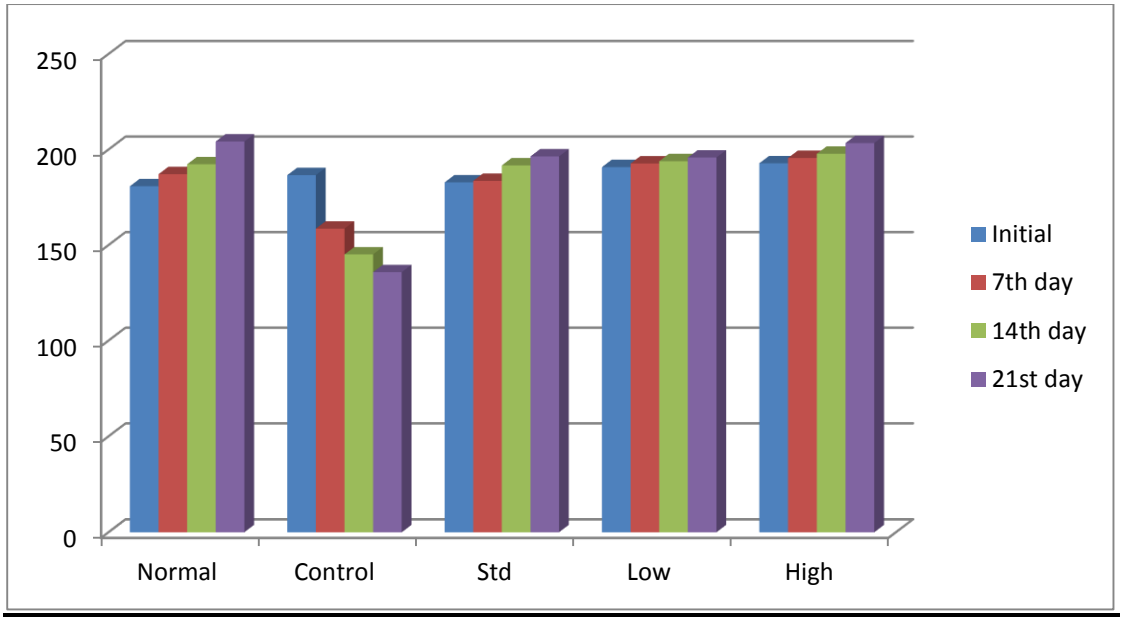

Fig. 2: Effect AERE on animal body weight in Alloxan induced diabetic rats

Table 4: SGPT and SGOT levels in diabetic rats

Groups	Alloxan	
	SGPT	SGOT
Normal control	53.43± 0.34	60.33± 0.76
Diabetic control	106.02± 0.77	111.87± 0.65
Glibenclamide (5 mg/kg)	61.22± 0.48***	67.72± 0.66**
AERE (200 mg/kg)	93.38± 0.62*	89.44± 0.54*
AERE (400 mg/kg)	80.04± 0.53**	76.34± 0.73**

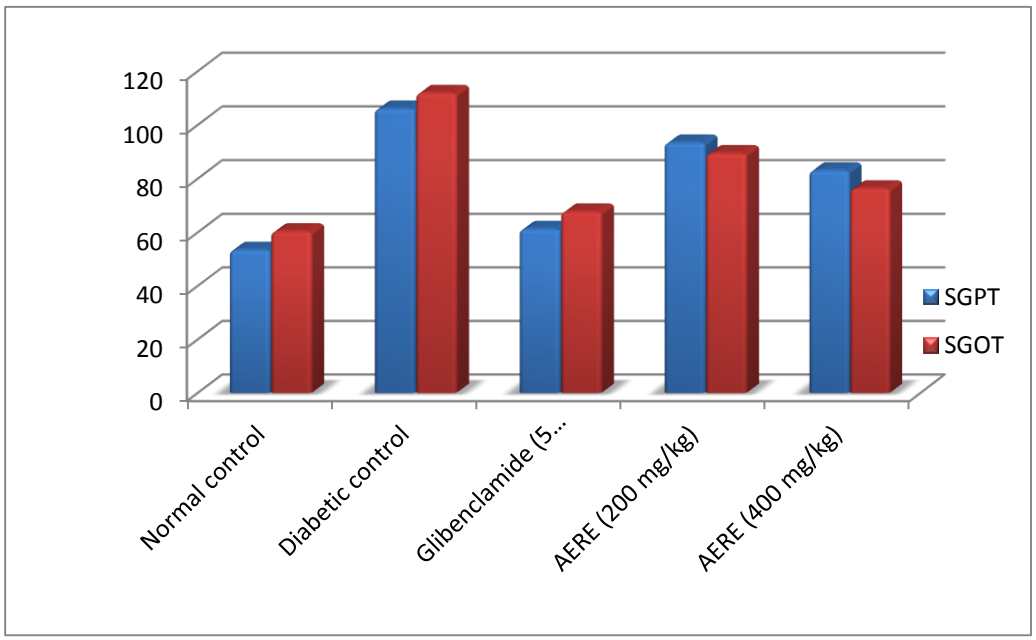

Fig. 3: Effect AERE on SGPT and SGOT level in Alloxan induced diabetic rats

RESULT AND DISCUSSION

Glibenclamide is often used as a standard antidiabetic drug in Alloxan induced diabetes to compare the efficacy of AERE*;* it is second generation sulphonylurea derivative and found to be effective in diabetic rats that retain functioning of islet β-cells. Hence the principle mechanism of action is to stimulate the production and secretion of insulin by the β-cells of pancreas. This drug may lower down the output of glucose from the liver by insulin independent mechanism.

Fasting blood glucose level (FBG) was within the range of 70-80 mg/dl in all the groups at day 0. After treatment with Alloxan in normal saline (100 mg/kg, i.p) a marked rise in fasting blood glucose level observed in diabetic control compare to normal controls rat. Aqueous extract of RE at a dose 200 and 400 mg/kg exhibited a dose dependent significant anti-hyperglycemic activity on 7th, 14th, 21st and 30th day post treatment. But antihyperglycemic effect of AERE was found less effective than the standard drug Glibenclamide.**(Table2,Fig 3)**.

Animals, which received Alloxan, showed a significant reduction in body weight, and increase in water and food intake as compared to normal control, which is significantly reversed by AERE after 21 days of treatment **(Table 3, Fig 2).**

Assessment of SGPT and SGOT levels during diabetes the measurement of enzymatic activities of phosphatases such as acid phosphatase (ACP) and alkaline phosphatase (ALP) is of clinical and toxicological importance. In this study, serum ALP increased in Alloxan induced diabetic rats[9]. Elevated level of this enzyme in diabetes may be due to extensive damage to liver by Alloxan. Treatment with AERE in diabetic rats produces a decline in SGPT and SGOT level **(Table 4, Fig 3).**

The phytochemical screening of RE has revealed the presence of triterpenes, phenylethanoid glycosides, russectinol and russeliaoside**.** Leaves yielded alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids. Flavonoids, sterols/triterpenoids, alkaloids, saponins and phenolics have bioactive antidiabetic principles[10]. Flavonoids can regenerate the damaged -cells in the alloxan induced diabetic rats. Thus, the presence of flavonoids, tannins and other polyphenolic compounds in the AERE might responsible for its antidiabetic activity.

CONCLUSION

From this experimental study it can be concluded that the aqueous extract of whole plant of *Russelia equisetiformis* showed significant anti-diabetic activity in Alloxan induced diabetic rats in dose-dependent manner. Antidiabetic activity of RE at 400 mg/kg was found to be more effective than 200 mg/kg. These extracts also showed improvement in parameters like body weight and SGPT and SGOT levels.

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