EVALUATION OF ANTIHYPERGLYCEMIC AND ANTIHYPERLIPIDEMIC ACTIVITY OF *ALTERNANTHERA PUNGENS KUNTH* ON ALLOXAN INDUCED DIABETIC RATS

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Abstract:

To control blood sugar levels, traditional healers have used this plant as remedies. The aim of this study is to investigate hypolipidemic and hypoglycemic effect of the whole plant of *Alternanthera Pungens Kunth* against Alloxan induces hyperglycemia in rats.

An injection of Alloxan monohydrate 150 mg/kg (i.p.) was used to induce hyperglycemia. After 72 hrs, the rats were selected for the investigation whose blood glucose level (BGL) was above 150 mg/dl. The two different doses of aqueous, and ethanolic extracts (200 mg/kg and 400 mg/kg b.w.) were observed for antidiabetic effect. BGL was monitored and compared with metformin (250 mg/kg).

Both extracts showed significant ($P<0.05$) antihyperglycemic activity after doses of both the extracts have been given orally in a dose-dependent manner in alloxan-induced diabetic rats. After 21 days of treatment with ethanolic extract at dose of 400 mg/kg, the BGL decrease from 268.34±4.82 to 104.68±4.28 mg/dl, the reduction in BGL with aqueous extract at dose 400 mg/kg from 269.46±7.09 to 114.38 mg.

The ethanolic and aqueous extract resulted in reduction of the blood glucose level, it is primarily because of the flavonoids constituents, present in it. So, this current study supports the traditional use of Alternanthera Pungens Kunth in the treatment of the diabetes and contributes as evidence for their use in traditional medicine.

Key Words: Antihyperglycemia, Blood Glucose Level (BGL), Diabetes mellitus, Metformin, Alternanthera Pungens Kunth etc.

1. INTRODUCTION

Diabetes Mellitus is a metabolic disorder, may be due to various variables such as interaction of hereditary and environmental factors, damaged β-cells of the pancreas and risk of vascular disease [1]. Group of metabolic abnormal conditions, characterized by hyperglycemia, glycosuria, altered metabolism of proteins, lipids and carbohydrate. Oral hypoglycaemic agents for type II and insulin for type I Diabetes mellitus is available but the demand of natural product is growing now days [3]. The use of traditional herbal plant has a long history for the treatment of diabetes mellitus. Therefore, the researcher continued looking for newer plant with more effective and have high safety margins [4]. In recent years, the scenario become changed, there is considerable attention diverted towards identification of plant with antidiabetic activity [5].

Alternanthera Pungens Kunth (family-Amarathaceae) a spiny flat branched weed which can be found near roadside, at waste land and at dry open regions. the leaves of the same pair unequal, diagonally elliptic to orbicular, with fruits, flowers throughout the year, commonly spread as a wild plant in the tropics and sub-tropics region of the whole world. It was noticed that the some of the species of Alternanthera were used as antidiabetic in the remote area by local public [6]. Hence, the aim of the current study is to investigate antidiabetic effects of *Alternanthera Pungens Kunth* in alloxan induced diabetic rats to ascertain the folkloric claims.

2. MATERIALS AND METHODS

The plant was collected from roadside of waste land of Jabalpur, Madhya Pradesh, India during mid August- September 2018. This plant species forms dense mats of stem and leaves in rainy season. It was collected freshly and authenticated. Plant was dried under the shade and with mechanical grinder it has been pulverized into coarse powder. The powder was then passed through sieve (no 30) and powder material collected in polythene bags at room temperature (25^{o}C) for further extraction process.

2.1 Chemicals

Alloxan monohydrate and metformin HCL were purchased from Sigma chemical co. Mumbai, India. The diagnostic kit for measurement of blood glucose. All the other chemicals and reagents were of analytical grade and were purchased locally.

2.2 Extraction process

The dried crude powder was firstly defatted with petroleum ether (60- 80^{o}C) with continuous hot soxhletion method. The defatted powder thus obtained was further extracted with same method with ethanol (95% v/v). Fresh crude powder was then used for aqueous extraction by cold maceration method. Under low pressure, the solvent was removed through distillation. The resulted semisolid material was vacuum dried through rotary evaporator. To find out the presence of various phytoconstituents, Qualitative analysis of ethanolic and aqueous extracts was carried out [7].

2.3 Pharmacological activity

2.3.1Experimental animals

Wister albino rats (150-200g) of either sex and of near same age group were used for this experiment. The rats were fed with standard pellet diet (Hindustan Lever Ltd. Bangalore) and they were housed to standard condition of temperature and water *ad libitum*. The experimental procedure was approved prior of the animal experiment by Institutional Animal Ethics Committee.

2.3.2 Acute toxicity study

The acute toxicity study of plant extract was carried out in albino mice according to OECD guideline 420 (fixed dose method). The extract was administered to animal orally in increasing dose level of 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg body weight. All animals were observed continuously for behaviour changes up to 14 days [8].

2.4 Experimental procedure

2.4.1 Alloxan induced diabetes

Intraperitoneal (IP) injection of Alloxan monohydrate (150 mg/kg i.p) in normal saline solution was administered in 12 hr fasting rats to induce hyperglycemia [9]. After 1 hr of alloxan injection the rats were fed with pellet diet and water *ad libitum*. Under mild anesthesia blood samples was collected by tail tipping method and blood glucose level was measured with glucometer. After 72 hr, the rats which having above 150 mg/dl blood glucose level were selected for the further experiments.

2.4.2 Experimental design

The selected animals (BGL above 150 mg/dl) were divided into seven groups of six animals in each group.

Group I : Control, received only normal saline.
Group II : Standard, received metformin HCL 250 mg/kg in alloxan treated rats.
Group III : Diabetic control, received Alloxan monohydrate (150 mg/kg i,p)
Group IV & V : Received aqueous extract of plant at dose 200 and 400 mg/kg resp. in alloxan treated rats.
Group VI & VII: Received ethanolic extract of plant at dose 200 and 400 mg/kg resp. in alloxan treated rats.

2.4.3 Biochemical estimation of blood sample

Blood samples were collected from each group by amputation of the tail tip of rats. Glucometer (Accu check Active, Germany) was used to measure the blood glucose level. At the end of experiments, rats were anesthetized and sacrificed by decapitation method. Blood samples of free running blood were collected for measurement of lipid profile. For the estimation of total cholesterol (TC), span diagnostic kit was used which followed cholesterol oxidase/peroxidase [10]. Span diagnostic kit was used for estimation of triglycerides (TG), which followed end point colorimetric enzymatic test using glycerol-3-phosphate oxidase [11] High density lipoprotein (HDL), low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were determined by standard method [12,13]. AST, ALT and ALP were estimated by using standard kits [14,15]. The kidney function, serum urea concentration and serum creatinine concentration were determined by method of Bartels et al, Fawcett et al [16, 17].

3. RESULTS

3.1 Phytochemical studies

The ethanolic and aqueous extracts were subjected for phytochemical screening which confirms the presence of different phyto-constituent in it, mentioned in table 3.1.

Table 3.1: Phytochemical constituents of plant extracts of *Alternanthera Pungens Kunth*

Sr. No.	**Phytochemical constituents**	***Alternanthera Pungens Kunth***		
		Petroleum ether	**Ethanol**	**Aqueous**
1.	Glycoside	+	+	+
2.	Alkaloid	–	+	+
3.	Carbohydrate	+	+	+
4.	Flavonoids	–	+	+
5.	Saponins	–	+	+
6.	Tannins	–	+	+
7.	Phytosterols	–	+	–
8.	Phenolic compound	–	+	+
9.	Fixed oil & fat	+	+	–
10.	Protein and amino acid	–	+	+
11.	Gun and mucilage	–	–	+

Where, + ive= Present, – ive= absent

3.2 Acute toxicity study

This acute toxicity study revealed the non-toxic nature of ethanolic and aqueous extracts of plant. There was no lethality or toxic reactions were found during and at the end of study period. On the bases of these results the dose of 200 and 400 mg/kg were selected to determine hypoglycaemic activity in alloxan induce diabetic rats.

3.3 In-vivo studies

The ethanolic and aqueous extracts of plant at dose 200 and 400 mg/kg show a significant decrease in blood glucose level in alloxan induce diabetic rats (Table 3.2) the administration of ethanolic extract significantly (P<0.05) reduced the blood glucose levels of diabetic rats at 3rd and 7th days after given alloxan.

The TC, TG, LDL and VLDL level has been decreased, while HDL level increased by aqueous and ethanolic extract, at different days resulted in a statistically significant of $p<0.05$ to $p<0.01$, analysed by using ANOVA method followed by Dunneyy's t-test. The extent of decrease in lipid parameters except HDL of the 21days treated rats with metformin then followed by aqueous and ethanolic treated groups (Table 3.3).

The ethanolic and aqueous extract at the dose of 200 mg/kg and 400 mg/kg lowered significantly lipid profile when compared to diabetic control group. Liver enzyme AST, ALT and ALP in blood serum were evaluated for increasing level in liver because these enzyme levels were increased due to liver cells dysfunction and leakage in liver cells membrane. Results showed a significant increase in this enzyme level of serum in alloxan induced diabetes (Table 3.4).

Kidney function of different groups was shown in Table 3.5. Diabetic rats treated with aqueous and ethanolic extract in both doses 200 and 400 mg/kg produce significant decreases in serum urea and creatinine concentration that comparable to standard group. After treatment with aqueous extract with 200 and 400 mg/kg doses highly significant decrease were shown in serum urea from 84.40 ±5.46 mg/dl to 66.52±4.60 mg/dl and 53.42±3.28 mg/dl respectively. The creatinine concentration decreases from 3.8±0.28 to 2.6±0.74 and 1.8 ±0.67 mg/dl respectively. Treatment with ethanolic extract (200 and 400 mg/kg dose) decrease significantly serum urea from 84.40±5.46 mg/dl to 47.42±3.62 mg/dl and 46.83±3.92 respectively. The creatinine concentration decreased from 3.8±0.28 mg/dl to 1.8±0.29 mg/dl and 1.2±0.24 mg/dl respectively.

Table 3.2: Ethanolic and aqueous extract of whole plant of *Alternanthera Pungens Kunth* effect on blood glucose level of normal and experimental animals for 21 days treatment

Group	Treatment and Dose (mg/kg)	Blood glucose level (mg/dL)				
		Initial	3rd day	7th day	15th day	21st day
I.	Normal control	73.5±2.53	75.53±5.32	76.18±5.53	76.73±.3.63	74.32±4.9
II.	Standard treated metformin (250 mg/kg)	267.3±5.83*	252.4±5.87*	158.44±6.56*	123.33±5.59*	89.23±5.45*
III.	Diabetic control alloxan only (150 mg/kg)	269.67±5.45**	281.36±9.43**	285.93±9.52**	291.27±8.26**	293.46±9.46**
IV.	Aqueous ext. (200 mg/kg)	267.56±5.31*	266.58±6.62*	178.36±7.05*	132.22±7.24*	122.36±8.23*
V.	Aqueous ext. (400 mg/kg)	269.46±7.09*	262.32±7.93*	172.46±4.37*	130.85±7.3*	114.38±09.24*
VI.	Ethanolic ext. (200 mg/kg)	270.36±7.51*	258.35±6.34*	170.32±4.53*	130.32±4.62*	112.16±4.72*
VII.	Ethanolic ext. (400 mg/kg)	268.34±4.82*	255.67±5.12*	164.82±5.08*	128.36±5.34*	104.68±4.28*

Values are expressed in mean ± S.D, each group contain 6 animals (n=6). One-way ANOVA followed by Dunnett's test (P< 0.05) is used.*P < 0.01 and **P< 0.05 vs. Normal control

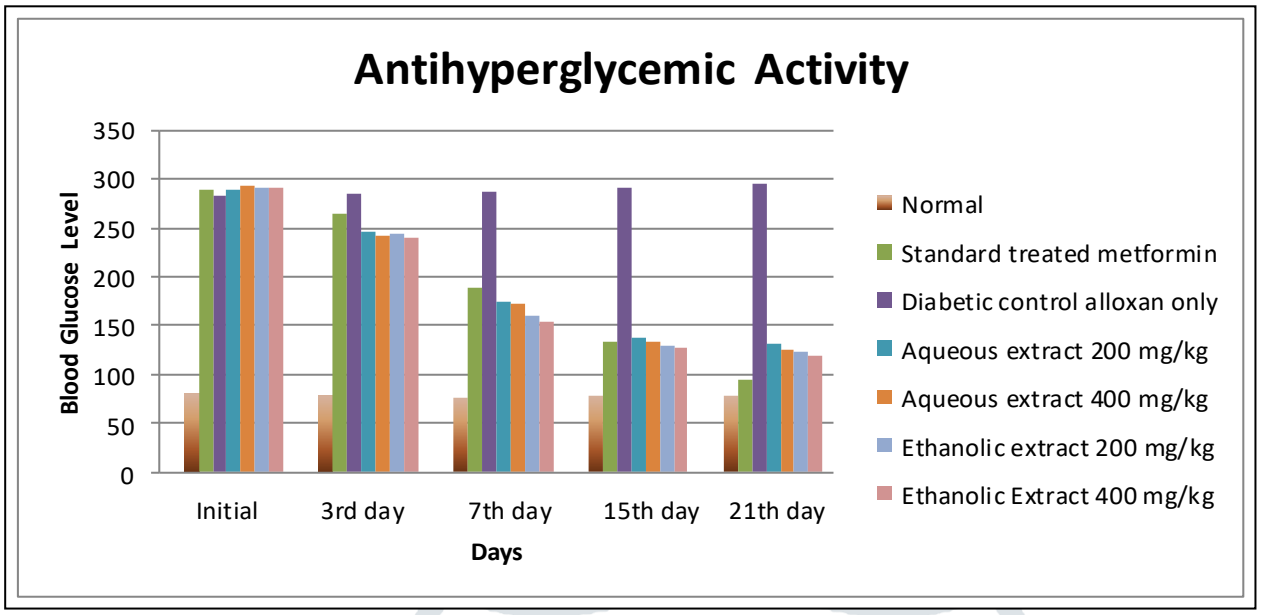

Figure 3.1: Effect of Ethanolic and aqueous extract of *Alternanthera Pungens Kunth* on blood glucose level of normal and experimental animals for 21 days treatment

Table 3.3: Ethanolic and aqueous extract of *Alternanthera Pungens Kunth* effect on lipid profile of different treatment groups of rats

Treatment and Dose (mg/kg)	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)
Normal Control	46.32±2.46	80.34± 3.17	9.24±3.34	11.22±3.34	24.35±0.42
Diabetic control alloxan only (150 mg/kg)	82.26±2.52	168.64± 2.32	38.62± 1.43	32.62± 1.43	10.62±1.27
Standard treated metformin alloxan (250 mg/kg)	58.26 ±6.37**	87.28± 2.24**	16.66±4.28**	18.66±4.28**	21.28±0.18**
Aqueous ext. (200 mg/kg)	61.18±3.28*	94.38 ± 2.69**	24.52±3.18**	21.52±3.18**	18.23± 1.57**
Aqueous ext. (400 mg/kg)	59.22±1.82**	90.64 ± 2.28**	23.42± 33.46**	20.42± 3.46**	19.26±0.61**
Ethanolic ext. (200 mg/kg)	62.28±1.56**	90.46 ± 1.18**	23.86 ± 3.28*	20.86 ± 3.28*	19.26±1.28*
Ethanolic ext. (400 mg/kg)	60.46±3.73**	89.24 ± 1.22**	22.26 ±3.14**	19.26 ±3.14**	20.28±0.36**

Values are expressed in Mean± SD, each group contain 6 animals (n=6) One-way ANOVA followed by Dunnett's t-test, *P<0.05 compared with diabetic control values.

Table 3.4: Effect of ethanolic and aqueous extract of *Alternanthera Pungens Kunth* on liver enzymes in blood serum collected from treatment groups of rats

Treatment and Dose (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal control	38.41±2.45	39.26± 3.62	44.26±2.58
Diabetic control alloxan only (150 mg/kg)	132.32±9.28	122.26± 8.64	136.62±10.42
Standard treated metformin alloxan (250 mg/kg)	48.62 ±3.56**	48.62± 3.26**	58.43±4.36 **
Aqueous ext. (200 mg/kg)	64.24± 6.38*	68.36 ±6.52*	76.51± 7.53*
Aqueous ext. (400 mg/kg)	48.82±6.24**	50.26 ± 4.53**	54.31±5.44**
Ethanolic ext. (200 mg/kg)	60.24±6.24*	61.22 ±4.22**	61.24±4.88**
Ethanolic ext. (400 mg/kg)	47.44±6.18**	44.72 ±4.72**	48.34±5.36**

Values are expressed in Mean± SD, each group contain 6 animals (n=6). One-way ANOVA followed by Dunnett's t-test *P<0.05, **P<0.01 compared with diabetic control values.

Table 3.5: Effect of alcoholic and aqueous extract of *Alternanthera Pungens Kunth* on kidney function profile of different treatment groups of rats

Treatment and Dose (mg/kg)	Urea (mg/dL)	Creatinine (mg/dL)
Normal control	44.22±2.18	0.9± 0.06
Diabetic control alloxan only (150 mg/kg)	84.40±5.46	3.8± 0.28
Standard treated metformin alloxan (250 mg/kg)	46.62 ±2.81**	1.4± 0.42**
Aqueous ext. (200 mg/kg)	66.52± 4.60*	2.6 ±0.74*
Aqueous ext. (400 mg/kg)	53.42±3.28**	1.8 ± 0.67**
Ethanolic ext. (200 mg/kg)	47.42±3.62*	1.8±0.29**
Ethanolic ext. (400 mg/kg)	46.83±3.92**	1.2 ±0.24**

Values are expressed in Mean±SD each group contain 6 animals (n=6)) One-way ANOVA followed by Dunnett's t-test, *P<0.05, **P< 0/01 compared with diabetic control values.

4. DISCUSSION

The phytochemical screening of plant extract reveals the presence of different compound like alkaloid, glycoside, flavonoid, saponin, carbohydrate, fixed oil & fat, tannins etc. A beta cytotoxin agent "Alloxan" destroy β-cell of islet of langerhans of pancreas of animal which cause reduction in the release of insulin and lead to increase in blood glucose level [18]. On treatment with the plant extract, there were signicicant reduction in blood glucose level in alloxan induce diabetic rat. The ethanolic and aqueous extracts of plant were administered at a dose of 200 and 400 mg/kg in alloxan induce diabetic rats. After 21 days of treatment with ethanolic extract at dose of 400 mg/kg, the blood glucose level decrease from 268.34±4.82 to 104.68±4.28 mg/dl. The reduction in blood glucose level by aqueous extract at dose 400 mg/kg from 269.46±7.09 to 114.38 mg/dl, both the extract decreases blood glucose level but on comparison with standard drug Metformin which decrease BGL from 267.3±5.83 to 89.23±5.45 mg/dl. This result reveals that both extracts have a significant role in reduction of BGL of alloxan induced diabetic rats.

However, blood glucose level of diabetic rats who were treated with ethanolic extract were similar or slightly lower than those of the standard treated group rats, suggesting that hypoglycaemic component in the plant are greater solubility in ethanol than aqueous. The reason behind it may be due to the stronger extraction capacity of ethanol to extract out the greater number of active components which have blood glucose lowering activity. Therefore, the mechanism of the both extracts is possibly an insulin- independent mechanism. Alloxan induces hyperglycaemia by pancreatic cell damage mediated through generation of oxygen free radicals. The primary target of these is the DNA of pancreatic cells causing DNA fragmentation [19].

Insulin deficiency as well as increase in blood glucose level increases cholesterol and triglyceride level due to fat storage in the liver [20]. Treatment with ethanolic and aqueous extract may improve insulin level and that will reduce the fat storage in liver. Diabetic condition also causes secretion and release of liver enzyme in blood circulation due to destruction of cells by change in membrane structure [21]. The ethanolic and aqueous extracts at dose 200 and 400 mg/kg significantly reduces the enzyme level in blood.

The presence of flavonoids and phenolics in both the extracts might have enzyme inhibition activity. This result supports the possible mechanism followed by flavonoid compound to control blood glucose level. This result could be correlated with significant enzyme inhibitory activity of ethanolic and aqueous extract of plant. This may interfere or delay the absorption of dietary carbohydrate and reduces blood glucose level.

5. CONCLUSION

The present study provides evidence for the use of herbal plant as medicine. *Alternanthera Pungens Kunth* has significant activity to reduce blood glucose level especially postprandial glucose level and also reduces liver enzyme and lipid component level. It was further required to investigate, which component were responsible for the reduction of BGL. This study may be beneficial for the development of the new antidiabetic agents from indigenous plant sources. Hence, to explore the possible mechanism of action, a further investigation was needed.

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