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PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF ANTIDIABETIC ACTIVITY OFETHANOLIC LEAVES EXTRACT OF *LANTANA ACHYRANTHIFOLIA* PLANT

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ABSTRACT

Using graded oral doses (100 and 200 mg/kg body weight) for 15 days, Lantana Achyranthifolia ethanolic leaf extracts were given to diabetic Wistar rats that had been given "alloxan monohydrate" to induce diabetes. The purpose of the study was to assess the antidiabetic and antihyperlipidemic effects of the extracts. Significant antidiabetic action was demonstrated by the extract ($p<0.001$). Furthermore, after receiving extract treatments, the rats' reduced body weight considerably improved. When compared to the diabetic control group, the extract therapy also improved the serum insulin level in the diabetic rats. Utilizing in vivo methods, assess the antidiabetic potential of Lantana Achyranthifolia's ethanolic leaf extract. In the context of in vivo investigations, blood glucose levels were observed at various time points following the administration of different dosages of the extract to assess its hypoglycemic (100 mg/kg b.w.) and antihyperglycemic (200 mg/kg b.w.) effects in rats with diabetes and normoglycemia. When diabetic rats were given the extract orally every day for 15 days, their serum transaminase, triglyceride, and cholesterol levels were all significantly reduced; yet, their HDL-cholesterol levels were shown to be improved ($p<0.001$) when compared to the diabetic control group. In rats with diabetes and neither hyperglycemia nor hypoglycemia, the plant extract significantly reduced and prevented hyperglycemia.

Keyword: *Lantana Achyranthifolia*, Hypoglycemia, Plant Extract.

1. Introduction

Diabetes mellitus is a common endocrine disease that affects over 100 million individuals globally, accounting for about 6% of the global population. It is brought on by fluctuations in blood glucose levels due to inadequate insulin production from the pancreas. It has been demonstrated to negatively impact several bodily systems, including the heart, blood vessels, eyes, kidneys, and nerves. The two types of diabetes mellitus are insulin-dependent diabetes mellitus (Type-1) and non-insulin-dependent diabetes mellitus (Type-2). Type-2 diabetes is an autoimmune disease that is typified by a local inflammatory reaction in and around islets that is followed by the selective death of insulin secreting cells. This is in contrast to Type-2 diabetes, which is characterized by peripheral insulin resistance and decreased insulin secretion. Diabetes mellitus increases the chance of developing a number of problems, including peripheral vascular disease, stroke, neuropathy, renal failure, retinopathy, blindness, and amputations. Mostly, drugs are used to treat symptoms and preserve lives. The secondary goals are to prolong longevity by removing different risk factors and to prevent long-term diabetic problems.

Diabetes mellitus is a chronic disease that affects how proteins, fats, and carbs are metabolized. A weak or insufficient insulin secretory response leads to inefficient utilization of carbohydrates (glucose) and the ensuing hyperglycemias, which are the hallmarks of diabetes mellitus.4 Diabetes mellitus, also known as "sugar diabetes," is the most common endocrine disease. It usually results from either too little or no insulin, or in rare instances, from a malfunction in the insulin's functioning. The International Diabetes Federation estimates that there are currently 40.9 million diabetics in India; by 2025, that figure is projected to rise to 69.9 million. The pancreas secretes the hormones glucagon and insulin. The beta (ß) cells and alpha (α) cells are found in the islets of Langerhan's and release insulin and glucagon, respectively. Insulin transfers glucose into the muscles, liver, and adipose tissue, lowering blood glucose levels through glycogenesis. While erythrocytes and neural tissue do not require insulin for glucose use, alpha (α) cells are crucial for blood glucose regulation because they produce glucagon, which raises blood glucose levels by speeding up the process of glycogenolysis.

Diabetes mellitus is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycaemia and glucose intolerance, as a result of lack of insulin , defective insulin action, or both (Sicree *et al.,* 2006). Such complications arise due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids and proteins emanating from defective insulin secretion, insulin action, or both .

Diabetes mellitus is categorized according to its clinical manifestation and etiology. Thus, diabetes mellitus can be classified into four groups or types: type 1 diabetes, type 2 diabetes, gestational diabetes, and additional subtypes. Although type 1 diabetes is the most common form of the disease in younger age groups in the majority of wealthy nations, it is thought to represent a small portion of the overall diabetes burden in a population. Type 1 diabetes is becoming more common in both wealthy and developing nations. Moreover, there will soon be a movement toward type 1 diabetes in children at younger ages.

Type 2 diabetes accounts for 85–95% of all cases of diabetes in high-income countries, and this percentage is significantly greater in underdeveloped nations. It is closely linked to target cells' and tissues'

inappropriate insulin use. It is currently a major worldwide health hazard that is widespread. WHO (1994) states that a number of factors, including changing eating habits, decreased physical activity, aging populations, growing urbanization, fast cultural and social dynamics, and other hazardous lifestyle and behavioral patterns, have made this issue worse. Nowadays, practically every population in the world suffers from diabetes mellitus and other milder forms of glucose intolerance, notably impaired glucose tolerance. Epidemiological data indicates that diabetes will likely continue to rise worldwide in the absence of effective prevention and control initiatives.

2. MATERIALS AND METHOD

2.1 Collection of plant material

Leaves of Lantana Achyranthifolia were collected from vindhya herbal nursery of Bhopal.Drying of fresh plant parts were carried out in sun but under the shade. Dried leaves of Lantana Achyranthifolia were preserved in plastic bags and closed tightly and powdered as per the requirements.

2.2 Extraction of Plant Material

Leaves of *Lantana Achyranthifolia* was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. 89 gm of dried powdered leaves of Lantana Achyranthifolia has been extracted with solvent using ethanol in maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40^0C. The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

2.3 Phytochemical Screening

The *Abutilon g*

randifolium extract acquire was subjected to the precursory phytochemical analysis following standard methods by Khandelwal and Kokate. The extract was screened to identify the presence of various active principles of alkaloids, glycosides, phenols, flavonoids, Terpenoids, Saponins, Steroids.

2.4 Estimation of total Phenolic, flavonoid and alkaloid Content

2.4.1 Total Phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol.10 mg of dried extracted dissolve in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenols. 2 ml of extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

2.4.2 Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method.10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of extract dissolved in 10 ml methanol and filter. Three (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

2.5 *In -Vivo* Anti diabetic activity

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C). Separate group (n=6) of rats was used for each set of experiments. The ethanolic leaves extract of *Lantana Achyranthifolia* (100, 200 mg/kg/day) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under examination for mortality as well as any behavioral changes. After fasting, diabetes was induced by a single intraperitoneal injection of 120 mg/kg body weight of 'Alloxan monohydrate' in distilled water. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. These animals were tested for diabetes after 15 days and animals with blood glucose (fasting) were selected for experimentation. Animals were divided into five groups of 6 rats each.

Group I: Rats served as normal-control and received the vehicle (0.5 ml distilled water/day/rat)

Group II: Rats served as diabetic-control and received the vehicle (0.5 ml distilled water/day/rat)

Group III: Rats (diabetic) were administered of ethanolic leaves extract of *Lantana Achyranthifolia* (100 mg/kg p.o.) for 15 days

Group IV: Rats (diabetic) were administered of ethanolic leaves extract of *Lantana Achyranthifolia* (200 mg/kg p.o.) for 15 days

Group V: Rats (diabetic) were administered Glibenclamide (600µg/kg p.o.) for 15 days.

3. RESULTS AND DISCUSSION

3.1 Determination of Percentage Yield

The yield of extracts obtained from samples using Ethanol as solvents are depicted in the table 3.1.

Table 3.1: Percentage Yield of leaves of *Lantana Achyranthifolia*

S. No.	Solvents	% Yield
1.	Ethanolic	3.58

3.2 Phytochemical screening of extracts

The outcomes of the results are discussed separately in the table 3.2.

Table 3.2: Phytochemical screening of leaves extract of *Lantana Achyranthifolia*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Mayer's Test	**-ve**
	Wagner's Test	**-ve**
	Dragendroff's test	**-ve**
	Hager's test	**-ve**
2.	**Glycosides**	
	Modified Borntrager's Test	**+ve**
	Legal's test	**+ve**
3.	**Flavonoids**	
	Lead acetate	**+ve**
	Alkaline test	**+ve**
4.	**Phenolics**	
	Ferric Chloride Test	**+ve**
5.	**Proteinsand Amino acids**	
	Xanthoproteic test	**+ve**
	Ninhydrin Test	**-ve**
6.	**Carbohydrates**	
	Molisch's Test	**+ve**
	Fehling's test	**+ve**
7.	**Saponins**	
	Froth Test	**+ve**
	Foam test	**+ve**
8.	**Diterpins**	
	Copper acetate test	**+ve**

3.3 Results of estimation of total phenolic contents

Table 3.3: Total Phenolic and Total flavonoid content of ethanolic leaves extract of *Lantana Achyranthifolia*

S. No.	Extract	Total Phenol (mg/100mg)	Total flavonoid (mg/100mg)
1.	Ethanolic leaves extract of *Lantana Achyranthifolia*	2.412	2.071

3.4 Results of *in vivo*anti diabetic activity

Table 3.4:Effect of Ethanolic leaves extract of *Lantana Achyranthifolia* in treatment on blood glucose (mg/dl) in normal and diabetic rats

Group	Treatment	Blood glucose (mg/dl)		
		Days 0	Days 8	Days 15
I	Normal	89.72±2.5	90±3.2	91±2.5
II	Diabetic Control	290±2.5	308±2.4	341±3.5
III	Diabetic+ Ethanolic leaves extract of *Lantana Achyranthifolia (100mg/kg)*	249±3.5	202±3.4	184±3.1
IV	Diabetic+ Ethanolic leaves extract of *Lantana Achyranthifolia (200mg/kg)*	235±3.5	176±2.5	118±2.5
V	Diabetic + Glibenclamide (600µg/kg)	225±3.5	132±4.5	105±2.6

Table 3.5: Effect of ethanolic extract of *Lantana Achyranthifolia* treatment on biochemical parameters in normal and diabetic rats

Group	Treatment	TC (mg/dL)	TG (mg/dL)	Total protein(g/dl)
I	**Normal**	91.00 ± 3.00	85.50 ± 3.00	9.00 ± 1.50
II	**Diabetic Control**	178.0 ± 5.00	126.0 ± 6.00	4.90 ± 1.50
III	**Diabetic+ Ethanolic leaves extract of *Lantana Achyranthifolia* (100mg/kg)**	118 ± 5.55**	92.50 ± 6.00*	7.92 ± 2.50**
IV	**Diabetic+ Ethanolic leaves extract of *Lantana Achyranthifolia* (200mg/kg)**	103.5 ± 5.50**	88.57 ± 6.00*	8.4 ± 2.50**
V	**Diabetic + Glibenclamide (600µg/kg)**	101.1 ± 5.50**	84.50 ± 6.00*	8.25± 2.50**

Table 3.6: Effects of ethanolic extract of *Lantana Achyranthifolia* on body weight

Group	Treatment	Initial weight (g)	Final weight (g)
I	**Normal**	151.00 ± 8.00	185.10 ± 9.00
II	**Diabetic Control**	155.00 ± 8.00	141.00 ±9.00
III	**Diabetic+ Ethanolic leaves extract of *Lantana Achyranthifolia* (100mg/kg)**	154.00 ± 8.00	171.50 ± 8.00
IV	**Diabetic+ Ethanolic leaves extract of *Lantana Achyranthifolia* (200mg/kg)**	155.00 ± 8.00	188.20 ± 9.00
V	**Diabetic + Glibenclamide (600µg/kg)**	160.00 ± 8.00	195.20 ± 9.00

CONCLUSION

In light of the results, our study indicates that of ethanolic leaves extract of *Lantana Achyranthifolia* has good antidiabetic activity. ethanolic leaves extract of *Lantana Achyranthifolia* exhibited significant anti-hyperglycemic activities in alloxan-induced hyperglycemic rats without significant change in body weight; they can also improve the condition of Diabetic mellitus as indicated by parameters like body weight & lipid profile. The renewal of cells in diabetes has been studied in several animal models. The total cell mass reflects the balance between the renewal and loss of these cells. It is found that of ethanolic leaves extract of *Lantana Achyranthifolia* at high dose (200 mg/kg) is more effective than whole plant extract at low dose (100 mg/kg) after 15 days of treatment. Hence the above discussion revels that of ethanolic leaves extract of *Lantana Achyranthifolia* at high dose (200 mg/kg) is more effective and shows similar curative effect as standard that is, glibenclamide (600 µg/kg).

This could be due to the possibility that some-cells are still surviving to act upon by ethanolic leaves extract of *Lantana Achyranthifolia* to exert its insulin releasing effect. From the above discussion it conclude that ethanolic leaves extract of *Lantana Achyranthifolia* at high dose (200 mg/kg) exhibited significant antihyperglycemic activity than whole plant extract at low dose (100 mg/kg) in alloxan-induced diabetic rats. These extracts also showed improvement in parameters like body weight and lipid profile as well as regeneration of cells of pancreas and so might be of value in diabetes treatment. Further investigation is in necessary to determine the exact phytoconstituents responsible for antidiabetic effect.

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