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ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of Antdiabetic Activity of *Andrographics serpyllifolia* in Alloxan induced Diabetic rats

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ABSTRACT

The pharmacological principles work together in dynamic way to produce maximum therapeutic efficacy with minimum side effects with many of the marketed polyherbal formulations. Investigating the antidiabetic potential of the whole plant of Andrographis Serpyllifolia in comparison with a standard oral antidiabetic drug. The objective of the present investigation is therefore to evaluate the whole plant of Andrographis serpyllifolia for its antidiabetic activity in experimentally induced diabetic rats and to achieve this objective we came up the plan of work.

Keywords: *Andrographis Serpyllifolia,* Diabetes mellitus, *antidiabetic potential and therapeutic efficacy*

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INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia. The metabolic disturbance involves the disturbance in the metabolism of fats, proteins and carbohydrates, reflecting a state of insulin deprivation and possibly abnormally high amounts of glucagon and other counter regulating hormones such as glucagon hormone, sympathomimetic amines and corticosteroids. This occurs due to deficient insulin secretion and also to factors opposing the tissue effects of insulin or both. Diabetes mellitus is usually irreversible which it allows the patient to have reasonably normal life style, its complications results in a considerably reduced life expectancy [1]**.** Roughly 5-15% of all cases of diabetes are type 1 DM. it is the most common metabolic disorder of childhood usually starts in children aged 4 years or older, with the peak incidence of onset at age 11-13 years, coinciding with early adolescence and puberty. Also, a relatively high incidence exists in people with their large 30s and early 40s. onset of type 1 diabetes may be sudden [2]**.**

It is a relatively new class of diabetes found in tropical region in people who are grossly underweight and who have had a history of malnutrition in childhood. The etiology is far from clear, but one hypothesis is the increased consumption of foods containing cyanogenetic glycosides, (Ex: Wild cherry bark (Prunasin), Mustard (Sinigrin), Bitter almond (Amygdalin), Linseed (Linamarin) etc resulting in pancreatic damage.

S. Jain et al., [3] observed antidiabetic activity of *Paspalum scrobiculatum*in alloxan induced diabetic rats. Their results suggest the ethanolic extract treatment showed a significant increase in the liver glycogen and a significant decrease in glycated haemoglobin levels. The results demonstrate the *Paspalum scrobiculatum* possesses significant antidiabetic activity in diabetic rats.

K.L.Joy, R.Kuttan [4] investigated that antidiabetic activity of *Picrorrhizakurroa*extract. The chronic administration of an alcoholic extract of P.Kurroa significantly reduced the blood sugar as well as reduced the increased blood urea nitrogen & serum lipid peroxides in alloxan induced diabetic rats and inhibited the body weight reduction & leucopenia induced by alloxan administration. Their results indicated that *P. Kurroa*extracts were aboe to ameliorate bio-chemical damages indudced by alloxan in diabetic rats.

S. Abd Et Sattar El Batran *et al*., [5] investigated the *Momordica charantia*juice and alcoholic extract. Both showed a significant decrease in serum glucose levels in normal and diabetic rats. Two test compounds values did not show any significant difference in urea, creatinine, ALT, AST & AP in normal rat, while in diabetic rats the two test compounds caused a significant decrease in serum urea, creatinine, ALT, AST, AP, cholesterols and triglyceride levels. So, these results suggested that the extract possessed anti-diabetic, hepato-renal protective and hypolipidemic effect in alloxan induced diabetic rats. Thus, MC in alternative therapy in patients with diabetes mellitus.

R.R. Ortiz-Andrade *et al*., [6] investigated the anti-diabetic and hypoglycemic activities in rats due to administration of methanol extract from aerial parts of *Tournefortia hartwegiana,* which significantly lowered the blood glucose levels.

T.Vertichelvan, M.Jeegadeesan, [7] evaluated the effect of an alcoholic extract of *Aervalanta* and then found to reduce the increased blood sugar in alloxan-induced diabetic rats is a 2 week study. Their results suggest that the extract possesses anti-diabetic activity and were able to ameliorate biochemical damages in alloxan induced diabetic rats.

MATERIAL AND METHODS

Drugs and Chemicals:

Alloxan monohydrate was purchased from N.R. chemicals, Mumbai. Glibenclamide was a generous fift from HETERO Laboratories, Hyderabad, India. Alcohol, ether and Assay kits (GOD-POD, SGPT, SGOT, Cholesterol, Triglyceride, and HDL) were purchased from SS Pharma, Warangal. All other required chemicals and solvents were purchased and were of analytical grade.

Preparation of the Ethanolic Extract of *Andrographis serpyllifolia:*

The whole plant material was shade died at room temperature. The dried material was then crushed by mechanical grinding and stored in a dry place until use. The coarsely powered whole plant material was subjected to soxhlation using ethanol in 60:40 ratio for 72 hrs, at 60-80^{o}C. the concentrated extracts were obtained by evaporating the solvent, under reduced pressure in a rotary evaporator at 42-45^{o} C. The concentrated extracts were transferred to china dished and then dried at room temperature. The solid extracts were scraped before complete drying, and then dried to a constant weight. The percentage yield obtained was 19.06% w/w and kept in an air tight container until use. The dried *Andrographis serpyllifolia* ethanolic extract was suspended in 2% gum acacia and used for the present study.

Preliminary Phytochemical Screening of Ethanolic Extract

A. Detection of Glycosides:

About 50mg of extract was hydrolyzed with concentrated hydrochloric acid for two hours on a water bath, filtered and the hydrolysate was subjected to the following tests.

Borntrager's test: To 2ml of filtered hydrolysate 3ml of chloroform was added and was shaken, chloroform layer was separated and 10% ammonia solution was added to it. Pin colour indicates presence of glycoside.

Legal's test: About 50mg of the extract was dissolved in pyridine. Sodium nitroprusside solution was added and made alkaline by using 10% sodium hydroxide solution. Presence of glycoside is indicated by pink colour.

Keller – Killiani Test: To a small quantity of the crude ethanolic extract acetic acid and a drop of ferric chloride solution and to it concentrated Sulphuric acid was added along the sides of test tube. A brown ring was observed at the junction of two layers indicating the presence of de-oxy sugars.

B. Detection of Flavonoids: Magnesium and HCL Reduction Test: The crude ethanolic extract was dissolved in a few ml of alcohol and few pieces of Magnesium ribbons and concentrated Hydro Chloric acid was added drop by drop. Pink or crimson red colour developed indicating the presence of flavonoids.The extract and fractions showed positive response for the above test.

C. Test for Phenolic compounds:Ferric Chloride Test: To the ethanolic extract Ferric chloride solution was added. The appearance of blue color indicates the presence of Phenolic compounds.

D. Test for Steroids/Terpenoids:Liebermann-Burchard Test: The extract was dissolved in acetic anhydride by heating the mixture to boiling, cooled and then 1ml of cold concentrated sulfuric acid was added along the sides of the test tube. Color change at the junction was observed. Steroids/Triterpenoids and their glycosides give red, pink or blue color.

Phytochemical Investigations: The various qualitative chemical tests performed on the test extract showed that the extract contained glycosides, flavonoids, and phenolic compounds, steroids, terpenoids, alkaloids and saponins.

Acute Toxicity Test: The acute toxicity test was performed on the mice and no abnormality or mortality was seen with 2000 mg/kg dose of test extract given orally. Hence the test dose was fixed as 100 and 200 mg/kg.

RESULTS AND DISCUSSIONS

Pharmacological Investigations

Hypoglycemic effect of ethanolic extract of A*ndrographis serpyllifolia*in normal rates is tubalated as follows (Table1).

Table 1 Hypoglycemic effect of ethanolic extract of *Andrographis serpyllifolia* in normal rats.

Group n=6	Treatment	FASTING BLOOD GLUCOSE LEVEL (mg/dl)			
		0hr	2hr	4hr	6hr
I	Vehicle Control (2% gum acacia)	97.40±2.19	98.95±3.10	94.44±3.17	97.97±3.62
II	Standard (10 mg/kg)	95.83±2.08	83.96±2.08	63.02±1.49**	54.42±1.30**
III	Test low dose (100 mg/kg)	94.44±1.44	86.36±1.29	74.74±1.49	83.34±1.82*
IV	Test high dose (200 mg/kg)	96.96±1.35	83.33±1.29	64.14±1.82**	82.32±1.44*

Data represents Mean±S.E.M. (n=6). *p<0.05 **p<0.01 ***p<0.001 significant compared to control.

The table 1 and fig 1 show that when Glibenclamide and two doses of the ethanolic extract of A.S were administered to the animals, and glucose levels were tested in serum at different time points, a hypoglycemic effect was observed in all the groups. However, the effect was less when compared with group treated with Glibenclamide. A significant hypoglycemic effect in all groups was observed at 4th hour. The percentage reduction in glucose levels were 34.23% in group II, 20.85% in group III and 34.56% in group IV respectively.

GLUCOSE TOLERANCE TEST

Effect of ethanolic extract of *Andrographis serpyllifolia*on the glucose tolerance test is presented in Table 2.

Table 2 Effect of ethanolic extract of *Andrographis serpyllifolia* on the glucose tolerance test

Group n=6	Treatment	FASTING BLOOD GLUCOSE LEVEL (mg/dl)				
		0 min	60 min	90 min	120 min	180 min
I	Vehicle Control (2% gum acacia)	97.48±5.34	126.3±3.19	110.6±1.70	103.5±1.44	93.93±1.10
II	Standard (10 mg/kg)	83.34±1.82	99.49±1.82**	87.87±3.22**	76.26±1.82**	67.17±1.82**
III	Test low dose (100 mg/kg)	89.50±1.76	109.9±2.97*	90.74±2.82*	85.18±1.35*	75.92±1.58*
IV	Test high dose (200 mg/kg)	88.88±1.35	108.0±2.42**	87.03±1.58**	78.39±2.55**	74.69±1.76**

Data represents Mean± S.E.M. (n=6). *p<0.05 **p<0.01 ***p<0.001 Significant compared to control

Table 2 show that when Glibenclamide and two doses of the ethanolic extract of A.S were administered to the animals, and glucose levels were tested in serum at different time points, a hypoglycemic effect was observed in all the groups. However, the effect was less when compared with group treat with Glibenclamide. A significant effect in all groups was observed at 180 min. the percentage reduction in glucose levels were 19.40% in group II, 15.17% in group III and 15.96% in group IV respectively.

ANTIDIABETIC ACTIVITY

Effect of *Andrographis serpyllifolia*ethanolic extract on fasting blood glucose level in diabetic rats is tabulated as follows (Table 3).

Table 3 Effect of *Andrographis serpyllifolia*ethanolic extract on fasting blood glucose level in various experimental groups.

Group n=6	Treatment	FASTING BLOOD GLUCOSE LEVEL (mg/dl)			
		0th day	5th day	10th day	15th day
I	Negative control (2% gum acacia)	90.08±1.86	88.02±2.19	90.32±1.86	85.71±2.08
II	Diabetic Control	286.1±2.90	291.7±2.23	279.6±2.15	272.4±3.35
III	Standard (10 mg/kg)	285.6±2.81	201.0±2.08***	179.0±2.16***	139.5±2.69***
IV	Test low dose (100 mg/kg)	288.9±4.27	246.9±1.80***	198.4±2.46***	161.9±3.01***
V	Test high dose (200 mg/kg)	283.3±4.12	233.3±1.92***	174.7±3.27***	147.6±2.72***

Table No.3Data represents Mean± S.E.M. (n=6). *p<0.05 **p<0.01 ***p<0.001 Significant compared to Diabetic control.

Table 3 and Fig. 3 show that the ethanolic extract 100 mg/kg, 200 mg/kg and standard drug 10 mg/kg exhibited a significant (p<0.001) antihyperglycemic effect after 15 days of the treatment. The higher ethanolic extract does lowed the blood glucose level maximally 47.89% at the end of 15 days treatment and standard drug lowed the blood glucose level to 51.15%. the antihyperglycemic effect of the ethanolic

extracts at both the doses is significant but less than that of standard drug. The results were considered significant at P<0.001.

Test for Serum Biomarkers: The effect of ethanolic extract of *Andrographis serpyllifolia* on serum biomarkers SGOT and SGPT in diabetic rats was studied and the results are presented as follows. (Table4).

Table 4 Effect of ethanolic extract of *Andrographics serpyllifolia* on serum biomarkers in various experimental groups

Group n=6	Treatment	SGOT(IU/L)	SGPT(IU/L)
I	Negative Control (2% gumacacia)	57.94 0.54	62.66 2.62
II	Diabetic Control	138.9 6.72	135.6 2.19
III	Standerd (10 mg/kg)	63.45 1.87	77.75 1.90
IV	Test low dose (100mg/kg)	89.21 1.18	88.94 1.26
V	Test high does (200mg/kg)	70.35 0.82	81.02 1.07

Data represents Mean S.E.M. (n=6). *p<0.05 **pV0.01 ***P<0.001 significant compared to Diabetic control.

Table 4 that after 15 days of ethanolic extract administration, there was a significant diminution in serum GOT levels. This was significant when compared to that of standard treatment. The percentage reduction in serum GOT levels of ethanolic extract in doses of 100 gm/mg and 200 mg/kg were 35.77% and 49.35 respectively, when compared to diabetic control, the effect being significant (p<0.001) and also effect was comparable to that of the standard drug.

Table 4 show that after 15 days of ethanolic extract treatment, there was significant diminution in serum GPT levels. The percentage reduction in Serum GPT levels were 42.66% in group III, 34.41% in group III, 34.41% in group IV and 40.25% in group V respectively. The results were significant at P<0.001.

Serum Lipid Profiles: The effect of ethanolic extract treatment and Glibenclamide on serum lipid profile in diabetic rats is shown in table 5.

Table 5: The effect of ethanolic extract treatment and Glibenclamide on serum lipid profile in diabetic rats

Group n=6	Treatment	Cholesterol(mg/dl)	Triglycerides(mg/dl)	HDL(mg/dl)	LDL(mg/dl)
I	Negative Control (2% gumacacia)	142.4±2.19	103.3±1.07	48.90±0.99	109.0±1.62
II	Diabetic Control	256.3±2.04	215.6±1.02	27.79±0.64	216.2±0.76
III	Standard (10 mg/kg)	157.5±3.09***	117.3±1.35***	52.42±0.70***	102.2±0.87***
IV	Test low dose (100mg/kg)	191.0±1.51***	184.3±2.04***	37.45±0.70***	174.0±1.33***
V	Test high does (200mg/kg)	174.9±1.79***	149.0±1.48***	43.17±1.09***	139.6±1.20***

Data represents Mean±S.E.M (n=6). *p<0.05**p<0.001 significant compared t Diabetic control.

Table 5 and Fig 6 show that ethanolic extract 200 mg/kg significantly lowered the serum cholesterol level after 15 days of treatment. The percentage reduction in serum cholesterol level in 100 mg/kg and 200 mg/kg ethanolic extract treated groups were 25.47%, 31.75% respectively, which was comparable to the reduction observed in standard group, i.e., 38.54% and was statistically significant (p<0.001). also, the effect on the serum triglyceride levels was also similar i.e., 14.51%, 30.89% and 45.59% respectively in groups IV, V and III. Here also the reductions were significant at P<0.001. table 6.5 and Fig 6.8 indicate that the ethanolic extract at a higher dose significantly (p<0.001) elevated the serum HDL cholesterol level after 15 days of treatment and the effect is comparable to that of standard drug. Likewise, the percentage reduction in serum LDL cholesterol level in ethanolic extract treated groups was comparable with the effect seen in standard drug treated group.

Body Weight Changes: The effect of the ethanolic extracts of *Andrographis serpyllifolia* and the standard drug was studied on body weight of the diabetic rats in the 15-day study period, by noting the initial body weight of all the animals before treatment and then weighing the animals at the end of the study period. The average change in the body weight is given in table 6.

Table 6 Effect of ethanolic extract of *Andrographis serpylligolia*on body weight of the experimental animals.

Group (n=6)	Average change in body weight (gms)
Diabetic control	32.1±1.815
Standard (10 mg/kg)	14.5±0.7638
Test low dose (100 mg/kg)	19±0.8944
Test high dose (200 mg/kg)	16.5±1.688

Table 6 show that diabetic control group animals were lost considerable body weight during the 15 days study. Alloxan monohydrate caused the reduction in body weight, whereas the various drug treatments have affected the body weight reduction favorably compared to the diabetic control. The weight loss was less in drug treated groups.

DISCUSSION

In our study we could induce diabetes by alloxan monohydrate. Alloxan monohydrate, a beta-cytotoxin, induces "chemical diabetes" (Alloxan Diabetes) in a wide variety of animal species.

The present study shows that *Andrographis Serpyllifolia* ethanolic extract has reduced the glucose level in normal, glucose loaded animals and in animals made diabetic with Alloxan monohydrate. The continuous treatment with ethanolic extract of *Andrographis serpylligolia* for a period of 15 days produced a significant decrease in the bold glucose levels of diabetic rats. These results confirmed the use of *Andrographis serpyllifolia* ethanolic extract in folklore practice as an antidiabetic.

Alloxan monohydrate has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of alloxan induced free-radical damage. It was reported earlier that ethanolic extract of *Andrographis serpyllifolia*can act as a free radical scavenger in vitro [8]. Alloxan monohydrate damages a large number of β-cells, resulting in decrease in endogenous insulin release, which paves the way for established that sulphonylureas produce hypoglycaemia by increasing the secretion of insulin from pancreas and these compounds are active in mild alloxan-induced diabetes, but they are inactive in intense alloxan diabetes [4]. Since our results showed that glibenclamide reduced the blood glucose levels in hyperglycemic rats, the state of diabetes is not severe. The acute antihyperglycaemic and insulin-tropic effects of the *Andrographis serpyllifolia* ethanolic extract (200 mg/kg) were similar to those of glibenclamide. The possible mechanism by which the plant extract mediates its antidiabetic action might be by potentiation of pancreatic secretion of isnsulin from existing residual β-cell of islets or due to enhanced transport of blood glucose to periphery. The progressive reduction in the blood glucose levels of alloxan-diabetic rats on treatment with ethanolic extract of *Andrographis serpyllifolia* might be due to a cumulative action of the extract during the period of treatment and also associated with an increase in the blood insulin levels, as is the possibility with the standard drug.

Oral glucose tolerance test (OGTT) measures the body's ability to use glucose, the body's main source of energy. It can be used to diagnose prediabetes and diabetes. In our study, it was found that the ethanolic extract has also caused hypoglycemic effect, which may be due to the presence of hypoglycemic flavonoids, phenolic compounds or glycosides, alkaloids, saponins, terpenoids. A further investigation is needed to conclude anything for sure about the hypoglycemic effect [1].

`Elevation of serum biomarker enzymes such as SGOT, SGPT was observed in diabetic rats indicative of hepatic damage the diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated transaminase activity [8]. This study substantiated the hepatic damage by Alloxan monohydrate. The elevated trasaminase activities were significantly reduced by ethanolic extract of *Andrographis serpyllifolia* from this, one can assume the ethanolic extract of *Andrographis serpyllifolia* to be heatoprotective also.

It is well known that in uncontrolled diabetes mellitus serum lipid profile changes significantly. There will be increase in total cholesterol, triglycerides and LDL cholesterol along with decrease in HDL cholesterol [3]. In the present study the total cholesterol triglycerides, LDL cholesterol was increased in diabetic control groups and it was reduced in 15 days treatment with ethanolic extract of *Andrographis serpyllifolia*as well as the HDL cholesterol level was significantly increased. This proves to be a further beneficial effect of the extract along with the antidiabetic effect.

Diabetes mellitus causes failure to use glucose for energy which leads to increased utilization and decreased stores of protein causing reduction of body weight essentially by depletion of the body proteins . The results of the study indicated that upon treatment with standard drug and extract the loss

in body weight improved when compared to the diabetic control group. This could be due to an improvement in utilization of glucose so that protein breakdown for energy purposes decreased thus leading to an improvement in body weight.

CONCLUSION

The ethanolic whole plant extracts of *Andrographis serpyllifolia*at high dose (200 mg/kg) exhibited significant antihyperglycemic activity than at low dose (100 mg/kg) in alloxan induced diabetes mellitus in rats. These extracts also showed improvement in parameters like body weight and lipid profile as well as serum enzymes and thus may be of value in diabetes treatment. Further investigation is necessary to determine the exact phytoconsituent (s) responsible for antidiabetic effect.

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