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Research Article

Pharmacological Screening of *Andrographis serpyllifolia* for Antidiabetic Activity

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ABSTRACT

Diabetes mellitus is chronic disease characterized by high blood glucose levels due to absolute or relative deficiency of circulating insulin levels. Though different types of oral hypoglycemic agents are available along with the insulin for the treatment of diabetes mellitus. The management of diabetes without any side effects is challenge to the medical system. Plants have played major role in the introduction of new therapeutic agents. In view of that we came up with the following plan of work.An ethanolic whole plant extract of *Andrographis serpyllifolia* (Acanthaceae) was found to lower the serum glucose level in normal rats. Maximum reduction in serum glucose level was observed after 4th hr at a dose levels of 100, 200 mg/kg body weight of the extract. In normal rats the serum glucose level reduction at 4th h was 20.85% by 100 mg/kg body weight and 35.56% by 200 mg/kg body weight. In alloxan induced diabetic rats chronic administration of the extract significantly reduced the serum glucose levels from 5th day to till the end of experiment. The extract was also found to reduced the elevated serum biomarker enzymes as well as improvement in parameters like lipid profile and body weight thus may be of value in diabetes treatment. These results indicate that *Andrographis serpyllifolia* extracts are able to ameliorate biochemical damages induced by alloxan in diabetic rats.

Keywords: Antidiabetic activity, *Andrographis serpyllifolia, alloxan,* Liver function, Glucose.

INTRODUCTION

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. Lack of the insulin affect the metabolism of carbohydrate, protein and fat. though different types of oral hypoglycemic agents are available along with insulin for the treatment of the diabetes mellitus. Management of diabetes without any side effects is a challenge to the medical system. Plants have played a major role in the introduction of new therapeutic agents and the beneficial multiple activities like, manipulating carbohydrate metabolism by various mechanisms, preventing, restoring integrity and function of β cells, insulin releasing activity, improving glucose uptake and utilization and antioxidant properties in medicinal plants, offer exciting opportunity to develop them into novel therapeutics.

The multifactorial pathogenesis of diabetes demands multimodal therapeutic approach. The pharmacological principles work together in dynamic way to produce maximum therapeutic efficacy with minimum side effects with many of the marketed polyherbal formulations. The multiple activities of plant based medicinal preparations meant for diabetes offers enormous scope for treatment of diabetes (Ashok K. Tiwari et al., 2002).

This plant is rare in open foot hills, and moist localities in forests. This plant is available at various places of thirumala hills near , nagapetla reserve forest near rangam peta, dhanambanda and near waterfalls in talakona, vedapatasala and sanakasonanda theertham in tirumala. A number of plants with known and unknown medicinal values are available here, which have to be explored to for their use in the effective treatment of dibetes mellitus. A literature search of herbal antidiabetic plants has showen that Andrographis serpyllifolia has not been explored for antidiabetic activity (Dr.K. Madhavashetty, K. Tulasi Rao) , although the ayurvedic ancient literature claims whole plant of *Andrographis serpyllifolia* to have antidiabetic

activity. The scientific reports on the plant revealed that it is rich in flavonoids, glycosides, unsaturated fatty acids and Phenolic compounds, alkaloids, Saponins, terpenoids, tannins, tannins, andrographolides which may possess antidiabetic activity(Sudhashan Deepa V et al., 2010).

Due to the presence of different classes of compounds of pharmacological importance and since the whole plant has not been investigated for the pharmacological activities, the present work was undertaken to evaluate its traditional medicinal claim in the treatment of diabetes scientifically.

In view of above, we have thought of investigating the antidiabetic potential of the whole plant of Andrographis Serpyllifolia in comparison with a standard oral antidiabetic drug.

2. MATERIALS AND METHODS

2.1 Collection of plant material

The whole plant of *Andrographis serpyllifolia* was collected from different regions of Chittoor District, Andhra Pradesh, India. The plant specimen was authenticated by Dr.K. Madhava chetty, Assistant Professor, Department of botany, Sri Venkateshwara University, Tirupati.

2.2 Drugs and Chemicals

Alloxan monohydrate was purchased from N.R. Chemicals, Mumbai. Glibenclamide was a generous gift 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.

2.3 Preparation of Plant extraction

The whole plant material was shade dried 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°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 dishes 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.

2.4 Experimental Animals

Wistar albino rats weighing 150-200 g were purchased from Sainath enterprises, Hyderabad, India. The animals were housed in standard polypropylene cages, and maintained under standard conditions (12:12 hour light and dark cycle; at an ambient temperature of 25 $\pm$ 5^{o}C; 35-60% of relative humidity). The animals were fed with standard rat pellet diet and water *ad libitum.* The maintenance and the handling of animals were done according to Committee for the Purpose of Control and Supervision on Experimentation on Animals (CPCSEA) guidelines. All the pharmacological investigations were carried out only after obtaining Institutional Animal Ethical Committee (IAEC) approval.

2.5 Acute Toxicity Study (OECD Guidelines-423)

Acute Toxicity study for the ethanolic extract of *Andrographis serpyllifolia* was carried out on mice according to OECD guidelines. Three mice were fasted overnight and maintained with water *ad libitum.The*. Each animal received single dose of ethanolic extract of *Andrographis serpyllifolia* (2000 mg/kg, p.o). After administration of the test compounds, animals were observed individually and continuously for 30 min, 2 hr and 24 hr to detect changes in the autonomic or behavioral responses and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep and coma and then monitored for any mortality for the following 15 days. No, mortality or any other autonomic or behavioral responses such as tremors, convulsion, salivation, diarrhea, lethargy, sleep and/or coma were observed during first 15 days.

2.6 Assessment of Hypoglycemic Activity in Normal Healthy Rats

For hypoglycemic study, rats were divided into four (4) groups (n=6) and were administered (2%w/v, 2 ml/kg, p.o.) gum acacia suspension ethanolic extract (100 mg/kg, p.o.), ethanolic extract (200 mg/kg, p.o.) and Glibenclamide (10mg/kg, p.o.) respectively. Blood samples were withdrawn from the retro orbital sinus under ether anesthesia at 0, 2, 4, 6 hr of extract administration. The fasting blood glucose levels were analyzed for blood glucose content using GOD-POD method.

2.7 Assessment of Oral Glucose Tolerance Test (OGTT)

The Oral Glucose tolerance test was performed in overnight fasted animals. Rats were divided into groups (n=6), and were administered (2% w/v, 2 ml/kg, p.o.) Gum acacia solution, ethanolic extract (100mg/kg, p.o), ethanolic extract (200mg/kg, p.o.) and Glibenclamide (10mg/kg, p.o.) respectively.

Glucose solution (2g/kg body weight; per oral) was fed 30 min after the administration of extract doses and the standard drug. Blood was withdrawn from the retro-orbital sinus under ether anesthesia (to minimize the distress) at 0, 60, 90, 120 & 180 min after the extract and standard drug administration.

The fasting blood glucose levels were analyzed for blood glucose content by using GOD-POD method.

2.8 Induction of Diabetes

After overnight fasting rats weighing 150-200gm are injected intraperitoneally with 150mg/kg Alloxan monohydrate dissolved in normal saline. (Mohammed et al., 2010). Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 0.5ml of 20%w/v glucose solution after 3 hours of Alloxan administration. The rats were then kept for the next 24 hours on 5% w/v glucose solution bottles in their cages to prevent hypoglycemia (Dhandapani et al., 2002).The diabetes was assessed in the treated rats by determining the blood glucose concentration 72 hours after injection of alloxan. The rats with blood glucose level above 250mg/dl were selected for the study.

After the induction of diabetes mellitus in the male Wistar albino rats, the animals were divided into experimental and control group. All animals were fasted for 16-18 hr. before treatment. Body weight of each animal was recorded before beginning the treatment. Fasting blood glucose estimation was done on day 0,5,10, 15 of study. Blood was collected from retro-orbital sinus samples were analyzed for blood glucose content using GOD-POD method. Standard and Test substances were given to diabetic rats for 15 days.

On day 15, the body weight of each animal was recorded again and blood was collected from retro-orbital sinus by using heparinised capillary tubes under ether inhalation(to minimize the distress) from overnight fasted rats and serum was separated and analyzed for serum cholesterol, serum triglycerides, HDL cholesterol and LDL cholesterol and liver function tests(SGOT and SGPT).

2.9 Statistical Analysis

All results are expressed as mean ±S.E.M (standard error of mean). Statistical evaluation was done using one way analysis of variance (ANOVA), followed by Dunnett's method. Results were considered significant when P values were less than 0.05($P<0.05$) or $p<0.001$.Statistical calculations and the graphs were prepared using Graph pad prism version 5.0

3. RESULTS

3.1 Phytochemical Investigations

The various qualitative chemical tests performed on the test extract showed that the extract contained Glycosides, Flavonoids, Phenolic compounds, Steroids, Terpenoids, Alkaloids and Saponins.

3.2 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(OECD guidelines 423).

3.3 Pharmacological Investigations

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

Group n=6		Treatment		Fasting Blood glucose level(mg/dl)	
		0 hr	2 hr	4 hr	6 hr
I	Vehicle Control (2%gum acacia)	97.40±2.19	98.95±3.10	94.44±3.17	97.97±3.62
II	Standard (10mg/kg)	95.83±2.08	83.96±1.81	63.02±1.49**	54.42±1.30**
III	Test low dose(100mg/kg)	94.44±1.44	86.36±1.29	74.74±1.49	84.34±1.82*
IV	Test high dose (200mg/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

3.2 Table 2: Effect on 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)	97.98±5.34	126.3±3.19	110.6±1.70	103.5±1.44	93.93±1.10
II	Standard(10mg/g)	83.34±1.82	99.49±1.82**	87.87±3.22**	76.26±1.82**	67.17±1.82**
III	Test low dose (100mg/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 (200mg/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.

3.3.3 Table 3: Effect 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(100mg/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***

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

3.3.4 Table 4: Effect on serum biomarkers

Group n=6	Treatment	SGOT (IU/L)	SGPT (IU/L)
I	Negative Control (2% gum acacia)	57.94±0.54	62.66±2.62
II	Diabetic Control	138.9±6.72***	135.6±2.19***
III	Standard (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 dose(200 mg/kg)	70.35±0.82***	81.02±1.07***

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

3.3.6 Table 6: Effect on body weight changes

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

4.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 serpyllifolia* for a period of 15 days produced a significant decrease in the blood 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 (Halliwell & Gutteridge, 1985). 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* (Sudarshana Deepa V et al., 2010). Alloxan monohydrate damages a large number of β-cells, resulting in decrease in endogenous insulin release, which paves the way for decreased utilization of glucose by the tissue (Saravanan and pari, 2005). It is well 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 (Nammi et al.,2003). 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 (200mg/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 insulin 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 that body’s ability to use glucose, the body’s main source of energy. It can be used to diagnose

prediabetes and diabetes (Md. Asaduzzaman et al. 2010). 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. (Sudarshana Deepa V et al. 2010).

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 (Gosh, Suryawansi, 2001). This study substantiated the hepatic damage by Alloxan monohydrate. The elevated transaminase activities were significantly reduced by ethanolic extract of *Andrographis serpyllifolia.* From this, one can assume the ethanolic extract of Andrographis serpyllifolia to be hepatoprotective also.

It is well known that in uncontrolled diabetes mellitus serum lipid profile changes significantly. There will be an increase in total cholesterol, triglycerides and LDL cholesterol along with decrease in HDL cholesterol (Arvind et al., 2002). 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 (Guyton and hall, 2000). 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.

5. CONCLUSION

The ethanolic whole plant extracts of *Andrographis serpyllifolia* at high dose (200mg/kg) exhibited significant antihyperglycemic activity than at low dose (100mg/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 phytoconstituent (s) responsible for antidiabetic effect.

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