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Evaluation Anti-hyperglycemic and antihyperlipidaemic activities of *Andrographis lineata* Nees on Streptozotocin induced diabetic Rats

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Abstract

The present study was performed to find out the antihyperglycemic and antihyperlipidaemic effects of methanol and aqueous extracts of *Andrographis lineata* (Acanthaceae) in normal and streptozotocin (STZ) induced diabetic rats. Diabetes was induced by injecting streptozotocin (STZ, 50 mg/kg) intraperitoneally in adult male albino Wistar rats. The methanol and aqueous extracts of *A. lineate* as well as the standard antidiabetic drug Glibenclamide were administered orally to different group's diabetic rats once a day for fifteen days in the dosages of 400 mg/kg b.wt of individual extracts and 500 µg/kg b.wt of standard drug glibenclamide, respectively. Blood glucose levels in all the rats (Both normal and diabetic) of different groups were determined on the 1st, 4th, 7th, 10th and 15th days after standard and sample drugs administration. The serum lipid profile like total cholesterol (TC), triglycerides (TG), phospholipids (PL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) were also determined in all the rats administration. Both extracts exhibited significant reduction in BGL as well as TC, LDL, VLDL and an increase in HDL in diabetic rats when compared to the standard drugs. The above results indicate that the plant is capable of ameliorating hyperglycemia in STZ induced diabetic rats. Hence this plant may be a potential source for the isolation of new orally active agent(s) for diabetic mellitus. The present investigation established pharmacological evidence to support the folklore claim of this plant being used as an antidiabetic.

Keywords: *Andrographis lineata*, Glibenclamide, Hyperglycemia, Streptozotocin.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder affecting approximately 10% of the global population. Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macro vascular complications of diabetes which are the major causes of morbidity and death (Bennet and Joslin's, 1998). Currently, the available therapy for diabetes includes insulin and various oral anti-diabetic agents such as sulfonylureas, metformin, etc. These drugs are used as monotherapy or in combination to achieve better glycemia control. Each of the above oral antidiabetic agents suffers of a number of serious adverse effects (Moller, 2001). Plants have played a major role in the introduction of new therapeutic agents. A medicinal plant, *Galega officinalis*, led to the discovery and synthesis of metformin (Aiman, 1970). Despite the considerable progress in the treatment of diabetes by oral hypoglycaemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. In recent times, there has been a renewed interest in the plant remedies (Dinesh puri and Mohapatra, 1997; Ratnakar and Murthy 1996).

Andrographis lineata Nees (Fam. Acanthaceae) is a small plant found in and around Salem district, Tamil Nadu, India. All parts of this plant are medicinally important in the traditional system of medicine in India and have been used extensively in snake bite and as anti-pyretic (Alagesaboopathi, 1999). It is also used as blood purifier and also in veterinary medicine. Three Flavonoids were isolated from the leaf extract (Hari kishore *et al.*, 2003). Leaves are used as hepatoprotective (Sangameswaran *et al.*, 2007) and they exhibit diuretic activity (Sangameswaran *et al*., 2007).

In present work, the plant selected is locally available in Salem district and has been used for a long time in local folklore medicine for the treatment of diabetes. Since not much study had been done to evaluate the pharmacological activity of this plant, the present study is focused on evaluating the anti-diabetic activity of the leaves of *A. lineata.*

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2. Materials and Methods

2.1. Plant material

Leaves of *A. lineata* were collected from foot hill of Yercaud, Salem, Tamil Nadu, India and were authenticated by Dr P. Jayaraman, Director of Plant anatomy Research Centre, Chennai, Tamil Nadu, India. Voucher specimens (AL/088) were deposited at our College Museum for future reference.

2.2. Preparation of the extract

The powdered material (500g) of leaves of *A. lineata* was extracted separately using methanol (1000ml) by Soxhlet technique and water by cold maceration (Evans, 1989). The extracts were dried under reduced pressure. The dried extracts were stored in desiccator and were subjected to further studies.

2.3. Preliminary phytochemical screening

The methanol and aqueous extracts were subjected to preliminary phytochemical screening to find out the presence of active constitution such as alkaloid, glycosides, flavonoids, tannins etc.

2.4. Animals

Male albino Wistar rats, 9-12 weeks old with average weight of 150-180 g were purchased from M/S Venkateshwara enterprises (P) Ltd, Bangalore and used for the study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each experiment, the animals were fasted for at least 18 h. The experimental protocols were approved by Institutional Animal Ethical Committee (No: P.Cog-12/07).

2.5. Acute toxicity studies

The animals were divided into five groups separately and were treated orally with aqueous and methanol extracts of *A. leneata* at 100mg, 200, 300 400 and 500 mg/kg, body weight doses. The animals were continuously observed for 1 hr., then frequently for 12 days. The animals were observed continuously for the initial 4 h and intermittently for the next six h and then again at 24 h and 48 h following drug administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate, and convulsion

2.6. Glucose tolerance test

Overnight fasted rats were divided into 4 groups. First group was kept as normal control which received 5% Tween 80 (0.5 ml, p.o), second group received standard drug Glibenclamide (500 µg/kg) third and fourth groups received methanol and aqueous extracts of *A. lineata* 400 mg/kg, respectively. The rats of all the groups were loaded with glucose (3 g/kg, p.o) after the administration of the sample: blood samples were collected at 0, 30, 60 and 120 min after the glucose loading.

2.7. Streptozotocin-induced diabetic rats

Streptozotocin (STZ), purchased from Sigma Aldrich chemical Co., Bangalore, was dissolved in ice-cold normal saline immediately before use. Diabetes was induced in rats by intraperitoneal (i.p) injection of streptozotocin at a dose of 50 mg/kg (Pulok K Mukarjee, 2002). Forty eight hours after streptozotocin administration, blood samples were drawn from tail and glucose levels were determined to confirm the on set of diabetes. The diabetic rats that exhibited blood glucose levels higher than 300 mg/dL were selected for the study. The rats were divided into 4 groups as follows: the first group served as normal control, received food and water; the second group served as (Group II to IV) diabetic control, received 0.5 ml of 5% Tween 80; the third group received glibenclamide (500 µg/kg); and the fourth and fifth groups received 400 mg/kg of methanol and aqueous extracts of *A. lineata*, respectively. The treatment was continued daily for 15 days. Blood drop was collected from the tail for glucose estimation, just before drug administration on 1st day and 1 h after sample administration on days 4, 7, 10 and 15 (Table 1).

Table 1. Effect of extracts of *A. lineata* and glibenclamide on oral glucose tolerance test

Treatment	Changes in blood Glucose levels (mg/dl)			
	0 min	30 min	60 min	120 min
Normal	68.42 ± 3.88	65.82 ± 3.20	66.52 ± 1.20	68.20 ± 0.20
Glibenclamide 500 µg/kg	73.50 ± 1.66	74.82 ± 0.82*	72.20 ± 3.98**	85.50 ± 0.82**
Methanol extract 400 mg/kg	68.46 ± 2.66	81.82 ± 1.42*	70.48 ± 1.32**	64.52 ± 1.20**
Aqueous extract 400 mg/kg	69.82 ± 2.30	77.63 ± 0.32	62.82 ± 0.2**	61.48 ± 0.82**

Values are mean ±SEM, n= 6. When compared with diabetic control *= p<0.05, **p<0.01
(One way ANNOVA Followed by Dunnette multiple comparison tests).

2.8. Anti-hyperlipidaemic activity

At the end of the experiment, the animals from each group were sacrificed by cervical dislocation for biochemical and histological studies. Blood was collected from the heart and allowed to clot and the serum was separated by centrifuged at 3500 rpm for 10 minutes. Serum was assayed either immediately or stored at -20^0 C. The tissue like pancreas was collected and used for histological studies.

Serum samples were analyzed spectrophotometrically for triglycerides, total cholesterol, high density lipoprotein (HDL-C), using their respective kits UV- visible spectrophotometer (Shimadzu-1601, Japan), VLDL-C and LDL-C were calculated as per Friedwald's equation (Richterich, 1981).

VLDL was calculated using the formula, $VLDL = \frac{\text{Triglycerides}}{5}$

LDL cholesterol was calculated as

$LDL = \text{Total Cholesterol} - HDL - \frac{\text{Triglycrides)}}{5}$.

2.9. Estimation of biochemical parameters

Serum lipid profiles like low density lipids (LDL), very low density lipids, high density lipids, triglycerides, and total cholesterol were determined standard procedures in an auto analyzer using Ecolin kits (E. Merck, Mumbai, India).

2.10. Statistical evaluation

All the data are presented as mean ±SEM. The differences between group were evaluated by one-way analysis of variance (ANOVA) followed by the Dunnette multiple comparisons test. P<0.01 was considered to be significant.

3. Results and Discussion

3.1. Preliminary chemical test

Our phytochemical studies indicated that methanol and aqueous extracts of leaves of *A. lineata* contain alkaloids, flavanoids, glycosides, saponins, terpenes and steroids.

3.2. Toxicity studies

In performing preliminary test for pharmacological activity in rats, aqueous and methanol extracts did not produce any significant changes in the behavioral or neurological responses up to 5000 mg/kg b. wt. Toxicity studies revealed the non-toxic nature of the aqueous and ethanol extracts of *A. lineata*. The result obtained from the LD_{50} study indicates that both methanol and aqueous extracts of leaves of *A. lineata* are safer to use in animals even at a dose of 500 mg/kg p.o.

3.3. Oral glucose tolerance test

Effect of methanol and aqueous extracts of *A. linetata* (each 400 mg/kg) and glibenclamide (500µg/kg) on glucose tolerance has been shown in Table 1. At 30 min after glucose administration, the blood glucose concentration increased rapidly from the fasting value and then attains nearly the same value at the end of the study.

3.4. Antihyperglycemic activity

The effects of extracts of *A. lineata* on blood glucose levels in normal and diabetic rats are reported in Table 2. Blood glucose level of the diabetic rats was significantly higher than those in normal rats. A significant decrease in blood glucose levels was observed in the rats treated with methanol extract of *A. lineata* from an initial level of 366 to 192 mg/dl. The present experiment was conducted to study the anti-diabetic effect of *A. lineata* in normal as well as streptozotocin induced diabetic rats.

Table 2. Anti-hyperglycemic activity of extracts of *A. lineata* on STZ induced diabetic rats

Treatment/ Dose	Changes in blood glucose level in mg/dl				
	1st Day	4th Day	7th Day	10th Day	15th Day
Normal control	96.50 ± 2.88	97.24 ± 2.24	96.00±2.62	96.08±2.42	96.60±2.81
Diabetic control	376.72 ± 0.25	380.62±1.66	386.00±1.24	390.16±1.42	396.22 ± 1.2
Glibenclamide 500 µg/kg	363.70±0.16	322.82±0.12	280.64± 0.42	242.00± 0.26	127.60±0.14**
Methanol extract 400 mg/kg	362.71±0.26	338.82±0.46	296.48±0.42	258.22±0.22	222.10±0.22**
Aqueous extract 400 mg/kg	366.5 ± 0.20	325.62±0.44	288.46± 0.48	246.00± 0.24	192.1 ± 0.10**

The values are mean ±SEM, n=6, When compared with diabetic control

** = p<0.001, (One way ANOVA followed by Dunnett's, multiple comparison test.

In group II (Diabetic control), the BGL significantly increased from 376.72 ± 0.25 to 396.22 ± 1.2 mg/kg. Methanol and aqueous extracts (400 mg/kg) treatment (Group IV and V) showed decreased blood glucose levels significantly from 362.71±0.26 to 222.10±0.22 and 366.5 ± 0.20 to 192.1 ± 0.10 mg/dl, where as in glibenclamide standard drug (500 µg/kg) treated diabetic rats (Group III), the BGL significantly decreased from 363.70±0.16 to 127.60±0.14 mg/dl, respectively.

3.5. Anti-hyperlipidaemic activity

The lipid profiles in the experimental rats are depicted in Table 3. In STZ induced diabetic rats, there was a significant (P<0.001) increase of total cholesterol, triglycerides, phospholipids, and low density lipoproteins (LDL) and very low density lipoprotein (VLDL) cholesterol and significant (p<0.001) decrease in high density lipoprotein (HDL) cholesterol in serum when compared with normal control. The extracts treated rats were significantly (p<0.001) decreased the total cholesterol, triglycerides, phospholipids and LDL and VLDL cholesterol and significantly (p<0.001) increased HDL cholesterol.

Table 3. Anti-hyperlipidaemic effects of extracts of *A. lineata* on STZ induced diabetic rats.

Treatment (mg/kg body wt)	Changes in mg/dl					
	TC	TG	HDL	LDL	VLDL	PL
Normal 10 ml/kg p.o	84.5 ± 7.9	87.3 ± 7.5	23.8 ± 2.1	23.8 ± 2.1	13.3 ± 1.7	144.3 ± 7.7
Diabetic control	192.3 ± 11.6	169.3 ± 5.1	12.3 ± 3.1	69.7 ± 8.3	28.3 ± 1.1	250.8 ± 10.6
Glibenclamide 500 µg/kg	131.7 ± 9.2**	112.7 ± 2.8**	17.2 ± 1.0*	38.2 ± 4.5**	15.8 ± 2.0**	174.8 ± 7.6**
Methanolic extract 400	152.7 ± 10.4*	125.0 ± 4.6**	16.2 ± 0.9*	44.2 ± 6.7*	18.7 ± 2.1**	190.0 ± 10.4**
Aqueous extract 400	146.2 ± 9.5**	118.5 ± 3.2**	18.9 ± 0.9*	42.2 ± 5.3*	17.0 ± 2.0**	184.2 ± 8.4**

The values are mean ± SEM n= 6, when compared with diabetic control, * = p<0.05, ** = p<0.01 (One way ANOVA followed by Dunnestt's, multiple comparison tests)

The present experimental result indicated that methanol and aqueous extracts exhibited a potent blood glucose lowering properties in STZ diabetic rats. A further exploration of the bioactive molecule responsible for the activity is under investigation in our laboratory.

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