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Hypoglycemic and Antihyperlipidaemic Effect of Ethanolic Extract of Aerial Parts of *Aerva lanata* Linn. in Normal and Alloxan induced Diabetic Rats

G. Appia Krishnan[1*], V. K. Rai[2], B. C. Nandy[2], K. C. Meena[2], S. Dey[2], P. K. Tyagi[2], L. K. Tyagi[3]

[1]*Pharmacy supervisor, Ministry of Health, Bisha Health Directorate, Kingdom of Saudi Arabia*
[2]*Shri R. N. S. College of Pharmacy, Gormi, Bhind, Madhya Pradesh, India*
[3]*Geetanjali College of Pharmaceutical Studies, Udaipur, Rajasthan, India*

ABSTRACT
The present study was designed to evaluate the preliminary phytochemical investigation of *Aerva lanata* Linn. (Family, *Amaranthaceae*), and anti-diabetic effect of Arial parts of plant *Aerva lanata* Linn. in normal and Alloxan induced diabetic rats. Ethanolic extract of Arial part of *Aerva lanata* Linn. were evaluated for four weeks treatment for hypoglycemic activity in diabetic albino rats; at doses of 50,100 and 200 mg kg $^{-1}$ body weight ,its effects on the blood glucose and other biochemical parameters like cholesterol, urea, creatinine, billirubin and SGPT in Alloxan induced diabetic rats were investigated. Diabetes was introduced by single tail-vein injection of Alloxan (60 mg kg $^{-1}$). Oral administrations of Aerva lanata Linn. extract to diabetic animals up to four weeks; dose dependently reduced the blood glucose level, which is comparable to standard dose of Metformin. Significantly decreased in body weight was observed with diabetic control, which was partially restored upon administration *Aerva lanata* Linn. extract (50, 100, 200 mg kg $^{-l}$). Alcoholic extract of Arial part of *A. lanata* Linn. also altered other biochemical parameters level. It was concluded from the results that the alcoholic extract of *A. lanata* Linn. possesses anti-diabetic effect in experimental animals.

Keywords: *Aerva lanata Linn.*, phytoconstituents, anti-diabetic.

INTRODUCTION

Hyperglycemia is a disease ailing humanity today and is a "gift" of the modern way of life. These afflictions usually occur is a common particularly in affluent classes. Clinically, the former results from deficiency of the hormone "insulin" in the body. It is also considered to the hereditary in many cases, if treated early these conditions are perfectly manageable, but if neglected, serious complications may arise. Hyperglycemia also weakens the body's natural defense mechanism and makes it more prone to infections by opportunistic germs. Modern medicine does not have any cure for these but can only temporarily relieve patients of their symptoms. Traditional herbal medicines, however, cure them from the aerial part. They actually enhance the production of Insulin in the body by restoring the proper functioning of the concerned organ (pancreas) [1] *Aerva lanata* Linn. (Amaranthaceae) known as 'chaya' in hindi and 'Bhadram' in Sanskrit and 'pulai' in Tamil. *Aerva lanata* Linn. is a ramous many branched hardy erect prostrate woody herb from 30 to 80 cm height, the main stem short but stout and woody at base from which arise 4 to 10 or more elongate hairy branches. The branches bears short petioles nearly orbicular leaves 8 to 20 mm long, numerous minute hairy white flowers. [2] *Aerva lanata* Linn (Amaranthaceae) is an herbaceous perennial weed growing wild in the hot region of India. *Aerva lanata* has been claimed to be useful as diuretic, anthelmintic, anti-diabetic, expectorant and hepato-protective in traditional system of medicine [3] Antimicrobial and cytotoxicity activity [4], diuretic [5], urolithiasis [6] and anti-inflammatory [7] activity. It has been reported that Canthin-6-one and β-carboline alkaloids were isolated from leaves of *Aerva lanata*. [8] The study was conducted to establish the traditional use of *Aerva lanata* Linn. as anti-

***Corresponding author: Mr. Appia Krishnan G.,**
Pharmacy Supervisor, Ministry of Health, Bisha Health Directorate, and Kingdom of Soudi Arabia
Tel: +91-9755183055; **Fax:** (+91) 7527-286503;
E-mail: appiakrishnan@yahoo.com

diabetic effect against Alloxan induced hyperglycemic effect in rats. *Aerva lanata* Linn. generally found as the perennial weed in all plains districts Tamil Nadu and upto 900 meters elevation in the hills the various parts of Tamil Nadu. *A. lanata* Linn. leaves are used as sap is used for eye-complaints, an infusion is given to cure diarrhea and Kidney stone, and root is used in snake bite treatment. A leaf decoction preparation used as gargle for treating sore throat and used in various complex treatment against guinea-worm. The Aerial parts of *A. lanata* used as demulcent traditionally. Till date no systematic study has been reported in this regard. In the present study, sincere effort has been attempted to establish the scientific validity of the hypoglycemic activity of the Aerial part of plant.

MATERIALS AND MATHODS

Collection and authentication of plant material

The aerial part of *A. lanata* Linn. were collected in the month of October, because during that period the phytoconstituent contents riches in a maximum levels and availability of the plant is plenty. It was collected form local area of Pulluthoort in Madurai district of Tamil Nadu, India, and authenticated by Prof. Ganeshar, the taxonomist at department of botany of Madura College, Madurai, Tamil Nadu, and the specimens voucher were deposited in the department herbarium for further reference. The aerial parts of plants shade dried, cut in to small pieces, air-dried and pulverized in to course powder by using a dry grinder and passed through the sieve before being stored in closed vessel for further use.

Drug and chemicals

The drug Alloxan monohydrate (Loba chemical, Mumbai) purchased from commercial sources. All other chemicals were analytical grade and used as such without further testing.

Extraction

The air dried powder of aerial part of the *A. lanata* Linn. was extracted with ethanol in soxhlet extraction for 48 hours. Alcoholic extract was concentrated under the vacuum in rotary flash evaporator and successively in hot air oven till solid to semisolid mass. Extracts were stored in an airtight container in refrigerator below 10°C.

Preliminary photochemical investigations

The extract of aerial parts of *Aerva lanata* were subjected to qualitative tests for the identification of various active constituents viz. glycosides, alkaloids, terpenoids, sterols, flavoniods, proteins and amino acids, volatile oils, free reducing sugar ,saponins using standard test procedures [9-10] (Table 2).

Determination of physical constants

The physical constants like moisture constant, total ash, acid insoluble ash, sulfated ash, alcohol soluble extractive, water soluble extractive were determined [11] (Table 1).

Pharmacological activity

Albino rats (Wister) of either sex weighing between 200±20 gm were used in this study. The animals were allowed to acclimatize to laboratory condition for 10 days after their arrival. The animals were keeping in separate plastic cages under standard laboratory conditions and maintained 12h/12h, light/dark cycle. The animals were fed with standard rat feed (Amrut rat feed) and allowed water *ad libitum.* [12]

Induction of diabetes

Diabetes was introduced to overnight fasted rats by single tail-vein injection of Alloxan solution (60 mg kg-1). Since Alloxan is capable of producing fatal hyperglycemia as a result of massive pancreatic insulin release, rats were treated with 20 % glucose solution (15-20 ml) intra-peritoneal after 6 h. The rats were then kept for the next 24 h on 5 % glucose solution bottles in their cages to prevent hypoglycemia. [13] Blood glucose was detected by applying glucose-oxidase and peroxidase methods and rats showing hyperglycemia with blood glucose $\geq$ 200 mg dl^{-1} after 48 h of Alloxan injection administration; which was selected to carried out the experiments.

Table1: Evaluation of physical constants aerial parts of *A. lanata* Linn.

S. No.	*Parameters*	Percentage
1.	Total Ash	9.91%
2.	Acid Insoluble ash	1.46%
3.	Sulphated ash	5.7%
4.	Alcohol soluble extractive	8.00%
5.	Water soluble extract	9.00%
6.	Moisture content	0.26%

Table 2: Qualitative chemical examination of ethanolic extract of aerial parts of *A.lanata* Linn.

S. No.	Chemical Test	Result
1.	Sterols	+
2.	Terpenoids	+
3.	Free reducing sugar	-
4.	Flavoniods	+
5.	Proteins and aminoacids	+
6.	Alkaloids	+
7.	Volatile oils	-
8.	Glycosides	+
8 .a	Cyanogenitic glycoside	+
8 .b	Anthracence derivative	+
8 .c	Cardiac glycoside	+
8 .d	Flavonoid glycoside	+
9.	Saponins	-

+, means presence of that component and -, means absence of respective component.

Experimental design for Hypoglycemic and antihyperlipidaemic studies

All the procedures were performed in accordance with the Institutional Animal Ethical. Committee (265/CPCSEA/Feb./09, Department of Pharmacology, Periyar College of Pharmaceutical Sciences for Girls, Trichy -620021) constituted as per the direction of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under Ministry of Animal Welfare Division, Government of India, New Delhi, India.

The rats were divided into six groups each containing six animals as follows:

- Group 1: Normal control, Received the vehicle (distilled water; orally).
- Group 2: Diabetic control, Received the Alloxan solution (60mg kg^{-1}; i.v).
- Group 3: Diabetic reference, Metformin was given at a dose of 25 mg kg^{-1}; .i.p.).
- Group 4: Diabetic, Ethanolic extract of *Aerva lanata* Linn. was given at a dose of 50 mg kg^{-1}; orally).
- Group 5: Diabetic, Ethanolic extract of *Aerva lanata* Linn. was given at a dose of 100 mg kg^{-1}; orally).
- Group 6: Diabetic, Ethanolic extract of *Aerva lanata* Linn. was given at a dose of 200 mg kg^{-1}; orally).

Doses of ethanolic extract were selected on the basis of LD_{50} and it was 550 mg per kg body weight of rat. All the treatment where given once daily by oral route, for four weeks on fixed time. During the study, standard food and water were freely supplied to the rats. At the end of four weeks, 1 ml of samples were collected from the tail vein and

Table 3: Determination of biochemical parameters after four weeks treatment with ethanolic extract of *Aerva lanata linn*

Parameters	Normal Control	Diabetic Control	Diabetic+ Metformin**	Diabetic+ ALE (50 mg kg $^{-1}$)	Diabetic+ ALE** (100 mg kg $^{-1}$)	Diabetic+ ALE ** (200 mg kg $^{-1}$)
Blood glucose (mg/dl)	88.87±3.01	345.59±16.08	130.89±8.92	171.57±5.39	142.06±4.41	130.78±5.06
Cholesterol (mg/dl)	83.79±3.71	147.03±9.04	102.14±3.84	112.73±5.84	107.34±4.46	102.37±4.08
Urea (mg/dl)	29.43±3.46	86.34±4.04	32.78±2.54	45.29±2.17	36.16±1.56	32.32±4.33
Creatinine (mg/dl)	0.76±0.06	1.75±0.07	0.98±0.09	1.11±0.08	1.03±0.02	0.99±0.01
Billirubin (mg/dl)	0.71±0.01	0.79±0.07	0.70±0.4	0.75±0.06	0.73±0.04	0.71±0.02
SGPT	51.53±2.65	85.72±7.03	63.30±2.45	74.27±5.068	69.61±6.31	64.53±5.07

All data's were calculated as Mean ± S.E.M. (n=6), **p< 0.05, diabetic control compared with normal control and drug treatment compared with diabetic control.

Table 4: Effects of ethanolic extract of *Aerva lanata linn* on body weight

Treatment	Body weight Mean ± S.E.M,(n=6)	
	Initial	After four weeks
Normal Control	220±10.25	224±9.6
Diabetic Control	215±16.5	176±10.5
Diabetic + Metformin	210±12.71	201±9.3*
Diabetic +ALE(50mg kg^{-1})	200±15.8	179±7.8
Diabetic +ALE(100 mg kg^{-1})	205±11.7	189±11.02*
Diabetic +ALE(200 mg kg^{-1})	202±17.6	192±8.45*

*p< 0.05, Compared with respective control.

kept in to centrifuge tube. Plasma was separated by centrifuging the samples at 5000 rpm for ten minutes and stored in refrigerator until analyzed. Blood glucose was detected by glucose-oxidase and peroxidase method [14] as described by using commercially available kit (Bayer Diagnostic, India).The biochemical parameters cholesterol, urea, creatinine, bilirubin and SGPT were analyzed by standard procedures using Transasia Chem-5 plus V2 auto analyzer using standard kit (Span Diagnostics).

Statistical Analysis

All the data are expressed as Mean ± S.E.M. and analyzed statistically using ANOVA followed by Dunnet test and compare with respective control group. A value of P<0.05 was considered significant.

RESULTS AND DISCUSSION

Dose dependent effects of ethanolic extract of *Aerva lanata Linn.* (ALE) on blood glucose and other biochemical parameters on four weeks treatment in Alloxan induced diabetic rats shown in Table 3. The blood glucose level was significantly (P < 0.01) elevated in diabetic rats as compare to normal rats. Metformin significantly reduced the blood glucose level. Oral administration of ALE dose dependently lowered the blood glucose as compare to diabetic control animals (non-diabetic) with the for weeks treatment of ALE.

Diabetic animals showed increase in the cholesterol level then the normal control; ALE and Metformin significantly (*P <0.05*) decreased it. Diabetic animals also showed significant elevation in urea and creatinine as compare to respective normal control. Metformin and ALE both reduce urea and creatinine significantly.

No significant change in billirubin level was noted after four weeks in diabetic animals as well as with Metformin and ALE treatment groups. The increased SGPT level was significantly (P< 0.05) reduced by treatment with ALE (100, 200 mg kg^{-1}) and Metformin.

Significant decreased in body weight was observed with diabetic control as shown in table 4, which was partially restored or improved upon administration of Metformin and ALE (100-200 mg kg^{-1}). From the phytochemical investigation (Table 2) it was seen that majority of the phytoconstituents were extracted in ethanolic extract of *Aerva lanata* Linn. Hence they are investigated for the anti-diabetic properties.

Alloxan a β-cell cytotoxin, induces chemical diabetes in a wide variety of animals species including rats by damaging the insulin-secreting β-cell of the pancreas. Alloxan causes time and concentration dependent degradation lesions of the pancreatic β-cell leading hyperglycemia. [15]

In the present study, a hypoglycemic effect of ethanolic extract of aerial part of *Aerva lanata* Linn. was evaluated in Alloxan induced diabetic rats. Four weeks treatment with ALE 50, 100 and 200 mg kg $^{-1}$ and Metformin lowers elevated blood glucose level, which were reported highly diabetic control animals. Maximum reduction in the blood glucose level noted with ALE 200 mg kg $^{-1}$. Thus ALE proved hypoglycemic activity in diabetic rats, which is comparable to Metformin.

The possible mechanism of hypoglycemic action by increasing either the pancreatic secretion of insulin from β-cell of islets of langerhans or its release from bound form have shown that the cholesterol, triglyceride and SGPT levels are increased in hyperglycemia. [16] Deficiency of insulin causes the increase in the level of enzyme in liver and serum of diabetic animals. They have also reported that the elevated level of enzymes in the liver and serum decreases significantly with the treatment of Phenformin and improve body weight. Metformin has also shown to be beneficial in improving the lipid profile mainly by correcting the abnormal glucose metabolism. [17] The result showed that all the doses of ALE and Metformin significantly decrease the serum cholesterol level. Treatment with Metformin and ALE (100, 200 mg kg $^{-1}$) shows an increase in body weight of diabetic rats, probably due to improvement in glycemic control.

In the present study ALE 100 and 200 mg kg $^{-1}$ and Metformin significantly reduces SGPT level as compare to diabetic control animals and no significant change in billirubin level was observed. This might suggest the protective action of extract and Metformin reversing any organ damage due to induction of experimental diabetes that is manifested by elevated level of SGPT.

Kidney maintains optimum chemical composition of body fluids by acidification of urine and removal of metabolic wastes such as urea, uric acid, creatinine and ions. During renal diseases, the concentration of these metabolites increases in blood. [18] In the present study it was observed that, ALE 50, 100 and 200 mg kg $^{-1}$ reduces elevates levels of urea and creatinine, which is comparable to the effect observed with may be possible in diabetic animals.

The present study indicates that oral administration of ethanolic extract of *Aerva lanata* Linn. dose dependently improve the blood glucose profile in four weeks treatment in diabetic rats. The extract lowers the body weight, cholesterol level and also showed improvements in Alloxan-induced metabolic alterations. Further pharmacological and biochemical investigations are essential to elucidate the mechanism of action.

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