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In-vivo AND *In-vitro* ANTI-DIABETIC POTENTIAL OF THE STEM AND FLOWERS OF *Aerva lanata*

Shashikala Metri[1,✉], P. Samson Simharayallu[1], M. Ganga Raju[1], Hiral Dave[2] and Ceema Mathew[1]

[1]Gokaraju Rangaraju College of Pharmacy, Osmania University, Bachupally, Hyderabad-500090, Telangana, India

[2]Parul Institute of Pharmacy, Parul University, Limda, Vadodara-391760, Gujarat, India

✉Corresponding Author: shashimetri@rediffmail.com

ABSTRACT

Aerva lanata is known as Pashanabheda, a traditional plant used as an anti-calculus, hepatoprotective, anthelmintic, anti-diarrhoeal, and anti-diabetic. In the current investigation, the methanolic extract of *Aerva lanata* was assessed for its anti-diabetic effect in a streptozotocin-nicotinamide and dexamethasone-induced rodent model. The extract significantly lowered the blood sugar level in streptozotocin-nicotinamide-induced diabetic and glucose-loaded rats. The study showed a substantial decrease in serum total cholesterol, triglycerides, and low and very low-density lipoproteins while increasing the high-density lipoproteins significantly. Histopathology of the pancreas showed mild degenerative changes and revitalization of beta cells. Insulin and fasting blood sugar levels were significantly lowered in diabetic animals, induced by dexamethasone. The extract also effectively prevented dexamethasone-induced insulin resistance and demonstrated increased insulin sensitivity after 12 days of treatment. The extract also resulted in efficient inhibition of carbohydrase enzymes, *α*-glucosidase and *α*-amylase, *in vitro*. The above outcomes suggest that the flowers and stems of *Aerva lanata* have a potential anti-diabetic effect.

Keywords: *Aerva lanata*, Anti-Diabetic, Streptozotocin, Nicotinamide, Dexamethasone, Insulin Resistance, *α*-amylase, *α*-glucosidase

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disease that affects carbohydrates, proteins, and fat metabolism, resulting from a lack of insulin secretion as well as varying levels of insulin resistance.[1,2] Inhibition of the conversion of dietary complex carbohydrates into glucose and the diffusion of glucose across the intestinal membrane into the bloodstream are the two main goals of treating type II diabetes. Inhibiting the enzymes that hydrolyse carbohydrates can help to lower postprandial blood glucose levels.[3,4]

Many plants have demonstrated their ability to treat diabetes and repair islets of Langerhans and have fewer side effects than synthetic drugs.[5] Thus, they are being used more frequently as an alternative to traditional synthetic drugs in the modern healthcare system.[6] *Aerva lanata* (family; *Amaranthaceae)* is a prostrate or erect plant found in the tropical regions of the Philippines, Arabia, and Africa. It is a widespread weed in the southern states of India and is abundant in flavonoids, tannins, alkaloids, and steroids.[7]

The active constituents such as aervine, aervolanine, *β*-amyrin, betulin, campesterol, chrysin, stigmasterol, stigmasterol acetate, ergosterol, lupeol, 16-dioxymethyl ester, canthin-6-one, β-carboline, hentriacontane, and β-sitosterol were isolated from this plant.[8,9] Traditionally, *Aerva lanata* has been used as an anti-inflammatory, antirheumatic, antimalarial, anti-diabetic, and hepatoprotective.[10] Numerous pharmacological potentials including hepatoprotective, anti-inflammatory, antimicrobial, antihelminthic, and hypoglycemic properties, have been documented for the plant.[11,12]

The fractions rich in phenolic acids extracted from the plant showed significant anti-oxidant activity and inhibited α-glucosidase and α-amylase enzymes.[13] A review of the literature found that no studies have been done on the anti-diabetic activity of the stem and flowers of *Aerva lanata* on type II diabetes mellitus. Therefore, to ascertain *Aerva lanata*'s anti-diabetic effect, the study was conducted in both *in vitro* and *in vivo* models.

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EXPERIMENTAL

Preparation of Stem and Flower Extracts

The plant was collected from hedges in Hyderabad (India) in December 2021 and authenticated by botanist Mr. Suresh Babu at New Government Degree College, Hyderabad. The stem and flower were separated, cleaned under water, and dried in the shade at room temperature. The material was ground, subjected to soxhlet extraction with 80% methanol at 40^0 C, and concentrated under vacuum. The percentage yield was calculated and stored in the refrigerator until used for the experiment.

Preliminary Phytochemical Study

The methanolic extract was tested using standard procedures to identify different phytochemicals present in the extract.[14,15]

Test Animals

The study included Wistar Albino rats (180–280 g). The animals were kept in polymer cages with a temperature of 25±2°C and a relative humidity of 45–55%, with an alternating, light and dark cycle every 12 hours. The rats were acclimated to the lab environment before use. The animals were fed until the fasting phase with a regular animal diet and sufficient water. The treatment and care of the animals were in accordance with the internationally recognized ethical guidelines for the use of experimental animals.[16,17,18] The animal Ethics Committee of the institute approved every and each experimental procedure to carry out anti-diabetic activity.

Study on Acute Toxicity

Acute toxicity, as per OECD guidelines 425 was conducted on female Wistar albino rats. The methanolic extract of *Aerva lanata* (MEAL) was administered orally up to 2000 mg/kg,[19] Then the animals were supervised for any behavioral changes, toxicity, or mortality for 14 days. In the case of any mortality of 2 out of 3 animals, the dose is considered a toxic dose.

In vitro Anti-Diabetic Activity

Inhibitory Activity of *α*-amylase

The activity was performed using 3, 5 di-nitro-salicylic acid (Spectrum Chemical Mfg Corp., Mumbai). The stock solutions of the test and standard (100 µg/mL), were prepared in sodium phosphate buffer, and from these stock solutions, different concentrations (10 to 50 µg/mL) were prepared. Later, 500 µL of each test and standard concentration were treated with 500 µL α-amylase (GK Biochemical Corporation, Gujarat) and kept at 37 °C for 10 minutes. 500 µL, solution of starch (1%) was transferred to each concentration and again incubated for 10 minutes and 1 mL of di-nitro-salicylic acid was mixed subsequently in each test tube. The solutions were heated for 5 minutes and diluted with 10 mL of distilled water after cooling. The absorbance of each solution was taken in a UV-visible spectrophotometer (Shimadzu Corporation, Japan) at 540 nm. Acarbose (Bayer Pharmaceutical Pvt. Ltd., Thane) was used as a standard. The percentage of inhibitory activity was determined with the following formula,

Inhibitory activity (%) = (SA –CA) / SA * 100

Where SA = sample absorbance and CA = control absorbance.

α-Glucosidase Inhibition Activity

Test and standard stock solutions (100 µg/mL) were prepared in sodium phosphate buffer, and from these stock solutions, different concentrations of test and standard (10 to 50 µg/mL) solutions were prepared. Later, 500 µL solution from each concentration of test and standard solutions was pipetted out and mixed with 500 µL α-glucosidase and kept for 5 minutes at room temperature. A 500 uL solution of 37 mM maltose was added in each test tube and kept for incubation for 30 minutes and 1 mL glucose peroxidase reagent was mixed. The mixture was rested for 15 minutes at room temperature, and 1 mL Tris buffer was mixed. The absorbance was taken in a UV-visible spectrophotometer at 540 nm. The formula mentioned under the inhibitory activity of α-amylase was used to calculate the percentage inhibition of α-Glucosidase.[20,21]

***In vivo* Anti-diabetic Activity**

Animal Grouping

The rats were divided into six groups (n=6). Group I: normal control, (0.9% NaCl); Group II: STZ-NA/dexamethasone-induced diabetic control; group-III and IV: treated with 200 mg/kg and 400 mg/kg of MEAL respectively; and group-V: standard control, treated with metformin hydrochloride (5mg/kg). MEAL and metformin hydrochloride were suspended in normal saline and administered orally for 21 days and 12 days in the STZ-NA-induced and dexamethasone-induced diabetes model, respectively.

STZ-NA Diabetic Model

A single dose of Nicotinamide (150 mg/kg., Agro Cool India, Delhi, India), followed by STZ (55 mg/kg) (Sisco Research Laboratories Pvt. Ltd., Maharashtra), was given intraperitoneally to produce type II diabetes. The fresh STZ solution was prepared in citrate buffer (0.1 M, pH 4.5) and injected into overnight-fasted animals intraperitoneally. NA was administered before 15 minutes before STZ injection to prevent sudden hypoglycemic shock. A 5% glucose solution was given after 1 hr of STZ injection. The blood was withdrawn after 72 hrs from the tail and checked for elevated blood sugar levels. Animals having blood sugar levels higher than 200 mg/dL were included in the study.[22,23]

Effect on Serum Glucose and Serum Lipids

The blood was taken on the 0th, 7th, 14th, and 21st days of the treatments, from the retro-orbital sinus after anesthetizing animals with ether to estimate blood sugar levels using a semi-auto analyser (Tulip, India). On the 21st day of the treatments, overnight-deprived animals were anesthetized, and venous blood was collected to estimate lipid levels in serum. The blood sample was rested for 2 hrs and centrifuged to separate serum and used for the analysis of serum triglycerides, total cholesterol, low and very low-density, and high-density lipoproteins.[15,23]

Histopathological Study

The pancreatic tissue was dissected quickly after the blood collection and stored in a formalin buffer solution containing 10% formaldehyde. Later, the pancreatic tissue was examined using a light microscope (100X, 40X) by staining with hematoxylin and eosin stain, and photomicrographs were taken.[24]

Dexamethasone-Induced Diabetic Model

Injection of dexamethasone (4 mg/kg) (Zydus Alidac Health Care Ltd., Ahmedabad) was given intraperitoneally to induce diabetes. The standard metformin hydrochloride (5 mg/kg) and two doses of MEAL were given to the appropriate groups orally for 12 days. The blood was taken from the retro-orbital sinus on the last day of the treatment and the blood sugar level was estimated in a semi-auto analyser.[25] To analyse insulin sensitivity (IS) and resistance (IR) Homeostatic Model of Assessment (HOMA) was used with the following formula:

$$IR = (FI \times FG) / 405$$

$$IS = 1000 / (FI \times FG)$$

Whereas FI=Fasting insulin; FG= Fasting glucose

Glucose-Loaded Rat Model

Test animals, fasted for 18 hrs overnight, were selected. The standard metformin hydrochloride (5 mg/kg) (Cipla, Mumbai) and MEAL were given orally to the appropriate groups, and the initial blood sugar level was determined. Then glucose D, (2 g/kg) was given orally, and after 30 minutes of its administration, the blood sugar level was analyzed at 0, 30, 60, 90, and 120 minutes. The tail vein was punctured to withdraw blood, and the sugar level was estimated using a glucometer, (Accu Check, Roche Diagnostics, USA). [26]

Statistical Analysis

The statistical analysis was performed using ANOVA (one-way) and Dunnett's comparison method. The values were displayed as mean ± SEM, all statistical analysis was done with the GraphPad Prism software. We used Excel 2007 software to draw lines and bar charts.

RESULTS AND DISCUSSION

The preliminary phytochemical study of the extract found that it had a positive reaction with alkaloids, tannins, saponins, flavonoids, triterpenoids, and steroids, (Table-1), which confirmed their presence in the plant. The MEAL yield was determined to be 7.8% w/w.

Table-1: Preliminary Phytochemical Study of the MEAL

Active constituents	Phytochemical tests	Results
Alkaloids	Wagner's test	+
	Dragendorff's test	+
Flavonoids	Alcoholic sodium hydroxide	++
	Alcoholic lead acetate	++
Tannin and phenolics	Aqueous lead acetate	+
	Aqueous Ferric chloride	++
Steroids and triterpenoids	Salkowski test	++
	Liberman Buchard test	++
Saponins	Foam formation with water	+

(+): positive reaction indicates the presence of the constituents; (++): Fast reaction to the tests

Acute Toxicity Study

There were no indications of any signs or symptoms of mortality or toxicity in the animals. Even after 14 days of testing, none of the animals showed any over-toxicity within 24 hrs, and all were deemed safe.

In vitro Anti-diabetic Activity

The α-amylase of the pancreas breaks down starch into disaccharides and oligosaccharides, while the intestinal α-glucosidase converts disaccharides to glucose. Thus, inhibition of these two enzymes is one of the ways to treat hyperglycemia. MEAL inhibited the enzymes *in vitro* efficiently (Table-2) and exhibited a more significant inhibitory effect compared to a standard control, Acarbose, a competitive inhibitor of the enzymes.[20,21] The extract was found to have IC50 values of 28.95±0.31 µg/mL with % inhibition of 76.11±0.6 for α-amylase and 29.50±0.12 µg/mL with % inhibition of 72.34±0.13 for α-glucosidase enzymes at a concentration of 50 µg/mL. Acarbose showed IC50 values of 27.88±0.34 with α-amylase and 28.51±0.32 with α-glucosidase enzymes and % inhibition of 76.45 ± 0.27 and 72.5 ± 0.44 at 50 µg/mL, respectively.

Table-2: *In vitro* Inhibitory Activity of MEAL

Concentration (µg/mL)	% inhibition			
	α-Amylase inhibitory assay		α-Glucosidase inhibitory assay	
	MEAL	Acarbose	MEAL	Acarbose
10	17.66 ± 0.51	14.19 ± 0.60	17.1 ± 0.33	19.86 ± 0.53
20	31.26 ± 0.80	41.68 ± 0.88	38.75 ± 0.91	40.33 ± 0.17
30	62.33 ± 0.88	65.35 ± 0.32	57.74 ± 0.71	59.15 ± 0.79
40	70.91 ± 0.35	68.36 ± 0.43	67.58 ± 0.20	68.07 ± 0.51
50	76.11 ± 0.61	76.45 ± 0.27	72.34 ± 0.13	72.5 ± 0.44
IC_{50} values	28.95±0.31	27.88±0.34	29.50±0.12	28.51±0.32

n=3, mean ± SEM, IC50 = half maximal inhibitory concentration

Anti-diabetic Effect *in vivo*

STZ-NA Diabetic Model

The STZ-NA diabetic model is a preferable method for non-insulin-dependent type II (type II NIDDM) diabetes to study the anti-diabetic potential of natural compounds, and it is closer to human type II diabetes. In our study it was induced by injecting nicotinamide, followed by STZ injection in experimental animals. If STZ is given alone, prevents insulin secretion by binding to the pancreatic beta cells via the Glut-2 transporter. However, nicotinamide, when administered 15 minutes before STZ, reaches the pancreatic beta cells and promotes NAD^+ production, PARP-1 suppression, and free radical scavenging. Thus, the

combination of STZ and NA is effective in treating type II NIDDM in animals without significant changes in plasma insulin levels.[27,28] The MEAL resulted in a potential anti-diabetic activity against the STZ-NA diabetic model. Compared to the normal group the diabetic control group showed a significant rise in blood sugar level ($p<0.001$). The animals treated with MEAL and metformin hydrochloride showed a significant decrease ($p<0.001/p<0.05$) in blood glucose levels in comparison to the diabetic control respectively. MEAL significantly decreased the elevated blood sugar levels in diabetic animals dependent on the doses. Table-3 shows the results of the MEAL effect on the induced diabetes model. The possible mechanism of hypoglycemic action of the extract may be peripheral glucose uptake or secretion of insulin from the islets of Langerhans. The active constituents present in the extract like, steroids, alkaloids, phenolics, saponins, and flavonoids, might be responsible for this mechanism.[13]

Table-3: MEAL effect on STZ-NA-Induced Diabetes Model

Animal grouping (n = 6)	Levels of blood glucose (mg/dl)			
	Number of days			
	0	7	14	21
Group-I	93.500± 0.42	96.210± 0.57	96.66±0.49	95.302± 0.57
Group-II	288.166± 0.60*	291.166±0.47*	294.04±0.63*	297.5±0.42*
Group-III	266.33±0.49*#$	253.10±0.73*#$$$	198.12±0.57*#$	127.5±0.56*#$
Group-IV	280.66±0.66*#$$	265.51±0.42*#$	175.83±0.60*#$	112.83±0.60*#$
Group-V	277.16±0.60*#	250.83±0.60*#	163.66±0.66*#	104.5±0.42*#

The results are given in mean ± SEM. The treated groups of MEAL (Group III, IV) were compared statistically to normal control (Group-I) (*=$p<0.001$), diabetic control (Group-II) (# = $p< 0.001$), and standard control (Group-V) ($$$ = $p< 0.05$, $$ = $p<0.001$, $ = $p<0.001$) using Dunnett's multiple comparison method and ANOVA.

Lipids play a vital role in the pathophysiology of diabetes due to an increase in their mobilization from peripheral fat stores. Thus, there is often an elevation of serum lipid levels, and there is an increased risk of coronary heart disease. MEAL showed effective hypolipidemic action dependent on its doses. The action might result from enhanced sensitivity of insulin in peripheral fat cells and skeletal muscles[29,30]. Administration of MEAL and metformin hydrochloride for 21 days showed a significant reduction in the levels ($p<0.001$) of TC, LDL, TG, and VLDL and an increase in HDL levels significantly ($p<0.001$) in comparison to the diabetic group. The diabetic group showed a substantial ($p<0.001$) increase in the level of TC, VLDL TG, and LDL, and a significant ($p<0.001$) reduction in HDL level (Fig.-1) in comparison to the normal control. The results showed that 400 mg/kg was the more effective of the two tested doses.

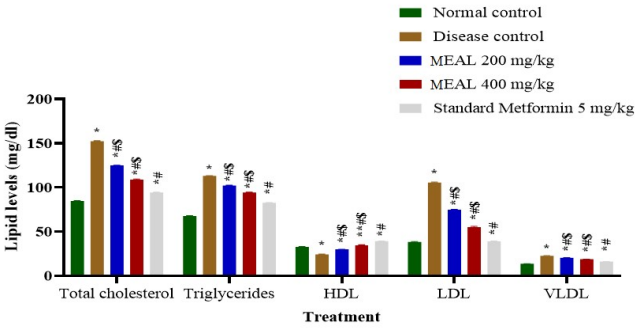

Fig.-1: Result of MEAL on Blood Lipid Levels in Diabetic Model Induced by STZ-NA

The results are given in mean (±) SEM, (n = 6). MEAL group was compared against control (* = $p<0.001$, disease control (# = $p< 0.001$), ** = $p<0.001$), and standard control ($ = $p<0.001$) statistically using Dunnett's multiple comparison method and ANOVA.

Histopathological Study

The normal morphology of beta cells was observed in normal control, whereas diabetic control showed severe degenerative changes with atrophy of islets of the pancreas. The MEAL-treated group showed

similar action to the standard control, treated with metformin hydrochloride, and protected the pancreatic tissue from damage (Fig.-2). The extract showed mild degenerative changes in beta cells, and there was increased insulin secretion evident from the regeneration of beta cells compared to the STZ-NA model. This action may also impart hypolipidemic action to the extract of *Aerva lanata.*

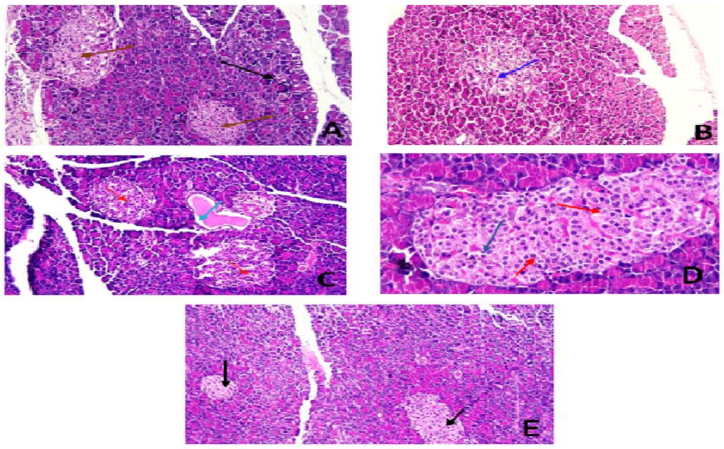

Fig.-2: Effect of MEAL on the Pancreatic Histology in STZ-NA Diabetes Model

A: No changes observed in normal morphology of beta islet cells in normal control, B: Disease control - severe degenerative changes with atrophy of beta islet cells, C: MEAL (200 mg/kg) - mild degenerative changes in beta islet cells, D: MEAL (400 mg/kg) - moderate hyperplasia and absence of dilation of beta islet cells, E: standard control (Metformin hydrochloride, 5 mg/kg) – an absence of dilation and moderate hyperplasia of beta islet cells.

Dexamethasone-Induced Diabetic Model

Dexamethasone causes insulin resistance by interfering with the ability of insulin to send signals inside the cells. Also, it downregulates substrate-1 insulin receptor, protein kinase B, and 3 kinase-phosphatidylinositol in fat cells skeletal muscles, and liver cells causing hyperglycemia.[25] We observed that the groups treated with MEAL significantly prevented resistance to insulin caused by dexamethasone and significantly ($p<0.001$) increased insulin sensitivity compared to the dexamethasone-induced diabetes group. MEAL also reduced FGIR (fasting glucose and insulin ratio) significantly compared to the diabetic rats. Table-4 presents a summary of outcomes.

Table-4: MEAL Effect on Dexamethasone-Induced Diabetic Rats

Groups (n=6)	Fasting sugar	Fasting insulin	Insulin resistance	Insulin sensitivity	FGIR (FG/FI)
Group I	87.91±0.48	64.46±0.47	13.63±0.16	0.167±0.002	1.37±0.007
Group II	286.21±0.60*	214.50±0.57*	151.5±0.41*	0.017±0.0006*	1.32±0.004*
Group III	129.33±0.49*$^{\#\$}$	166.83±0.65*$^{\#\$}$	53.27±0.37*$^{\#\$}$	0.045±0.0004*$^{\#\$}$	0.77±0.002*$^{\#\$}$
Group IV	104.33±0.71*$^{\#\$}$	124.16±0.60*$^{\#\$}$	31.96±0.21*$^{\#\$}$	0.075±0.0006*$^{\#\$}$	0.84±0.007*$^{\#\$}$
Group V	94.5±0.85*$^{\#}$	94.01±0.57*$^{\#}$	21.92±0.11*$^{\#}$	0.114±0.001*$^{\#}$	1.005±0.009*$^{\#}$

The results are presented in mean ± SEM. The treated groups of MEAL, (Group-III and IV) were compared statistically against standard control (Group-V) ($^{\$}$ = $p<0.001$), normal control (Group-I) (* = $p<0.001$), disease control (Group-II) ($^{\#}$ = $p<0.001$).

Activity on Glucose-Loaded Rat Model

The result of MEAL on glucose-loaded animals was similar to that of metformin and showed well-tolerated glucose against diabetic rats. Blood glucose levels were calculated at different intervals of time (minutes) and results are given in Table-5 and AUC in Fig.-3. The results showed there was an increased blood glucose level ($p<0.001$) in diabetic control against normal control. The animals administered with MEAL (200 mg/kg and 400 mg/kg) showed a substantial decrease ($p<0.001$/$p<0.001$) in blood glucose levels in

comparison to the diabetic control. It indicates that MEAL has strong anti-diabetic activity at both doses tested.

Table-5: Results of MEAL on Glucose-Loaded Rats

Animal grouping (n = 6)	blood glucose concentration (mg/dl) at time interval (minutes)				
	0	30	60	90	120
Group I	87.13±0.29	94.86±0.50	91.11±0.5	90.33±0.49	89.5±0.76
Group II	104.01±0.51*	122.33±0.71*	116.5±0.61*	105.16±0.60*	102.16±0.79*
Group III	92.16±0.79*#$	108.16±0.54*#$	98.23± .57*#$	93.16±0.65ns#$	81.83±0.70*#$
Group IV	83.66±0.55*#$$$	96.66±0.49ns#$$	87.66±0.55ns#$$$	83.33±0.76*#ns	72.16±0.60*#$$$
Group V	81.16±0.47*#	100.83±0.87*#	92.72±0.51ns#	80.15±0.78*#	67.66±0.65*#

The results are presented in mean ± SEM. MEAL-treated groups (Group-III and IV) were analyzed statistically using ANOVA and Dunnett's multiple comparison method against control (Group-I) (* = $p<0.001$), disease control (Group-II) (# = $p<0.001$); and standard control (Group-V) ($ = $p<0.001$, $$ = $p=0.001$, $$$ = $p<0.01$, ns = non-significant).

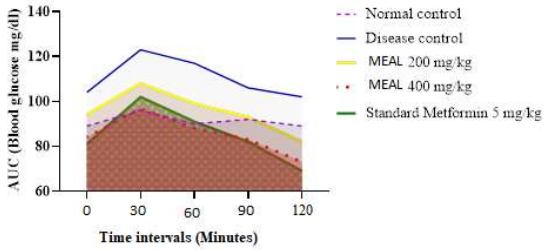

Fig.-3: Effect of MEAL on AUC of Glucose-Loaded Rat Model

CONCLUSION

The methanolic extract of *Aerva lanata* exhibited potential anti-diabetic activity against type II diabetic models such as STZ-NA and dexamethasone-induced. The MEAL protected the pancreatic tissue and increased insulin secretion in the STZ-NA induced model as well as significant improvement in measures like serum glucose and serum lipid profile. Furthermore, the extract also reduced HOMA-IR and brought about improvements in HOMA-IS and FGIR levels in the dexamethasone-induced model. Based on these results, it is evident that the plant can be effective for treating type II NIDDM.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing and editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

Shashikala Metri https://orcid.org/0000-0002-2072-6947
P. Samson Simharayallu https://orcid.org/0009-0000-3000-5142
M. Raju https://orcid.org/0000-0002-7355-8289
Hiral Dave https://orcid.org/0000-0002-3629-4536
Ceema Mathew https://orcid.org/0000-0001-5655-8263

REFERENCES

1. H. Rang, G. Henderson, R. Flower, J. Ritter, *Rang & Dale's Pharmacology*, Elsevier Health Sciences, London, pp. 380-92(2015), Available from: https://www.elsevier.com/books/rang-andampampdales-pharmacology/ritter/978-0-7020-5362-7
2. B. Jayaprasad, P.S. Sharavanan and R. Sivaraj, *Beni-Suef University Journal of Basic and Applied Sciences*, **5(1)**, 61 (2016), https://doi.org/10.1016/j.bjbas.2016.01.004
3. D.T. Eurich, F.A. McAlister, D.F. Blackburn, S.R. Majumdar, R.T. Tsuyuki, J. Varney, andJ.A. Johnson, *British Medical Journal* **335(7618)**, 497 (2007), https://doi.org/10.1136/bmj.39314.620174.80
4. N. Malviya, S. Jain and S. Malviya, *Acta Poloniae Pharmaceutica – Drug research*, **67(2),** 113 (2010).
5. A.C. Maritim, R.A. Sanders and J.B. Watkins, *Journal of Biochemical and Molecular Toxicology*, **17(1),** 24 (2003), https://doi.org/10.1002/JBT.10058
6. V.M. Munhoz, R. Longhini, J.R.P. Souza, J.A.C. Zequi, E.V.S.L. Mello, G.C. Lopes, J.C.P. Mello, *Revista Brasileria Farmacognosia*, **24(5)**, 576 (2014), https://doi.org/10.1016/j.bjp.2014.10.001
7. S. Netala, C.H. Rajeswari, D. Mounica, R. Manasa and D.S.N.B.K. Prasanth, *Pharmacognosy Journal,* **6(5)**, 29(2014), http://dx.doi.org/10.5530/pj.2014.5.6
8. S. Chandra, M.S. Sastry, *Fitoterapia*, **61**, 188 (1990).
9. A.A. Yuldashev, M.P. Yuldashev and V.N. Abdullabekova, *Chemistry of Natural Compounds*, **38**, 293(2002).
10. R. Rajesh, K Chitra and M.P. Padma, *Indian Journal of Natural Products and Resources*, **2(1)**, 5 (2011).
11. K.V Ramana and G. Vikram, *Medicinal Plant Research*, **5(4),** 1(2015), https://doi.org/10.5376/mpr.2015.06.004
12. G.A. Krishnan, V.K. Rai, B.C. Nandy, K.C. Meena, S. Dey, P.K. Tyagi and L.K. Tyagi, *International Journal of Pharmaceutical Sciences and Drug Research*, **1(3),** 191(2009), https://doi.org/10.3109/13880209.2012.761244
A. Pieczykolan, W. Pietrzak, U. Gawlik-Dziki and R. Nowak, *Molecules,* **26(12)**, 3486(2021), https://doi.org/10.3390/molecules26123486
13. C.K. Kokate, *Practical Pharmacognosy*, Vallabh Prakashan. Pitampura, Delhi, pp. 107-21(1999), Available from: https://thebookee.net.
14. Z.D. Kifle, J.S. Yesuf and S.A. Atnafie, *Journal of Experimental Pharmacology*, **9(12),** 151 (2020), https://doi.org/10.2147/JEP.S249964
15. Guide for the Care and Use of Laboratory Animals, The National Academic Press, Washington D, C, (2011), https://doi.org/10.17226/12910
16. V.H. Kumar, N.I.M. Nayak, S.V. Huilgol, S.M. Yendigeri and K. Narendar. *International Journal of Medical and Health Sciences*, **4(3),** 639 (2015), https://doi.org/10.5958/2319-5886.2015.00122.8
17. G.P.S. Kumar, P. Arulselvan, D.S. Kumar and S.P. Subramanian, *Journal of Health Sciences*, **52(2)** 283 (2006).
18. Test No. 425, acute oral toxicity- up-and-down procedure, OECD guidelines for the testing of chemicals, OECD Publishing, Paris (2008), https://doi.org/10.1787/9789264071049-en
19. M. Telagari and K. Hullatti, *Indian Journal of Pharmacology*, **47(4),** 425(2015), https://doi.org/10.4103/0253-7613.161270
20. E.J. Cooper, A.L. Hudson, C.A. Parker and N.G. Morgan, *European Journal of Pharmacology*, **482(1-3),** 189(2003), https://doi.org/10.1016/j.ejphar.2003.09.039
21. T. Szkudelski, *Experimental Biology and Medicine*, **237(5),** 481(2012), https://doi.org/10.1258/ebm.2012.011372
22. G.G. Rajiv and P. Sasikumar, *Asian Pacific Journal of Tropical Biomedicine*, **2(4)**, 281(2012), https://doi.org/10.1016/S2221-1691(12)60023-9
23. N.I.M. Nagendra, K.V. Hemanth, V.H. Shobha and K. Narendar, *Annals of Medical and Health Science Research*, **7,** 18(2017).
24. V. Mohan, V. Sornalakshmi, S.P. Tresina, P.M. Lincy and K Paulpriya, *International Journal of Toxicology and Pharmacology Research*, **8(1)**, 59(2016).

25. A. J. King. *British Journal of Pharmacology*, **166(3),** 877(2012), https://doi.org/10.1111/j.1476-5381.2012.01911.x.
26. P. Masiello, C. Broca, R. Gross, M. Roye, M. Manteghetti, D. Hillaire-Buys, M. Novelli, G. Ribes, *Diabetes*, **47(2)**, 224(1998), https://doi.org/10.2337/diab.47.2.224
27. R. Murali and R. Saravanan, *Biomedicine and Preventive Nutrition*, **2(4)**, 269(2012), https://doi.org/10.1016/j.bionut.2012.08.008
28. R.C. Latha and P. Daisy, *Chemico-Biological Interactions*, **189(1-2)**, 112(2011). https://doi.org/10.1016/j.cbi.2010.11.005

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