### **ORIGINAL ARTICLE**

# Antihyperglycaemic activity of alcoholic extract of *Aerva lanata* (L.) A. L. Juss. Ex J. A. Schultes leaves in alloxan induced diabetic mice

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#### Summary

*Aerva lanata* is prostrate or decumbent to erect herb. The objective of the present investigation was to study the antihyperglycaemic activity of alcoholic extract of *A. lanata* leaves (AL-alc) on serum glucose levels, and on the oral glucose tolerance test (OGTT) in alloxan induced diabetic mice. AL-alc (100, 200 and 400 mg/kg) and glyburide (10 mg/kg) were administered orally in alloxan (70 mg/kg, i.v.) induced diabetic mice. In AL-alc (400 mg/kg), the onset was 4 h, the peak effect was 6 h but the effect waned at 24 h. In the subacute study, repeated administration (once a day for 28 days) of the glyburide and AL-alc (400 mg/kg) treatment prevented a decrease in the body weight of the diabetic mice. In the OGTT, AL-alc (400 mg/kg) increased the glucose threshold at 60 min after the administration of glucose. The AL-alc (400 mg/kg) showed significantly more antihyperglycaemic activity than AL-alc (100 and 200 mg/kg).

Key words: Aerva lanata - alloxan diabetes - serum glucose - body weight - OGTT

### **INTRODUCTION**

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. A worldwide survey has reported that diabetes mellitus

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effects nearly 10% of the population (Vetrichelvan and Jegadeesan 2002). Currently available synthetic oral hypoglycaemic agents have side effects after prolonged use (Edwin et al. 2006). Patients are therefore using herbal medicines which have fewer side effects, are more easily available and cheaper (Shah et al. 2006a).

Aerva lanata known as Polpala is a prostrate to decumbent, sometimes erect herb, found throughout tropical India as a common weed in fields and wasteland (Krishnamurthi 2003). The plant is useful for curing diabetes; it is anthelmintic, demulcent and is helpful in lithiasis, cough, sore throat and wounds (Pullaiah 2003). The plant has been reported to possess anti-inflammatory (Vertichelvan et al. 2000), diuretic (Udupihille and Jiffry 1986) and nephroprotective actions in rats (Shirwaikar et al. 2004). Alcoholic extract of shoots of *Aerva lanata* has shown significant antidiabetic activity in rats (Vetrichelvan and Jegadeesan 2002). A number of Indian medicinal plants have been used for their antidiabetic activity in the traditional system of medicine, but not all of them have been reported on scientifically (Rangari 2004). The objective of the present investigation was to study the effect of alcoholic extract of *A. lanata* on serum glucose levels and on the oral glucose tolerance test (OGTT) in alloxan induced diabetic mice.

### MATERIALS AND METHODS

### Collection and authentication of plant

The leaves of *Aerva lanata* were collected from the local area of Pune in Maharashtra state, India (Latitude: 180 31' and Longitude: 730 55') and authenticated at the Botanical Survey of India, Pune. A voucher specimen was deposited at that institute (Specimen No. YAC- 1).

### Drugs and chemicals

Glyburide (Ranbaxy Pharma. Ltd. India), alloxan monohydrate (Spectrochem, India), a glucose estimation kit (Accurex Biomedical Pvt. Ltd., India) D-glucose (S.D. Fine-Chem. Ltd, India) and petroleum ether 600 – 800 (Merck, Mumbai, India) were purchased from their respective representatives. Drugs were administered orally.

#### Experimental animals

Swiss albino mice (25-30 g) of either sex were purchased from the National Toxicology Centre, Pune, India and used for the study. They were maintained at a temperature of  $25 \pm 10$  °C and relative humidity of 45% to 55% under 12-h light :12-h dark cycle. The animals had free access to food pellets (Chakan Oil Mills, Pune, India), and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Poona College of Pharmacy, Pune, India.

#### Extraction and preparation of AL-alc

The leaves of *Aerva lanata* were dried in shade and powdered in a grinder. The air-dried powder was subjected to hot continuous extraction with alcohol in a Soxhlet extractor, and filtered. The filtrate was evaporated at room temperature and the extract concentrated in a water bath to a dry residue. The percentage yield of alcohol extract was 5.8 % w/w. The AL-alc was dissolved in distilled water to prepare a drug solution with a concentration of 100 mg/ml which was used for the pharmacological studies.

### Acute oral toxicity studies

Healthy adult albino mice of either sex were subjected to acute toxicity studies as per guidelines (AOT no. 425) suggested by the Organization for Economic Cooperation and Development (OECD 2001). The mice were observed continuously for 2 h for behavioural, neurological and autonomic profiles for any lethality or death for the next 48 h.

### Induction of experimental diabetes

Diabetes was induced in mice by a single intravenous injection of aqueous alloxan monohydrate (70 mg/kg i.v.) solution (Kameswararao et al. 1999). After 48 h, the animals with serum glucose levels above 200 mg/dl (diabetic) were selected for the study. All the animals had free access to water and pellet diet.

## Collection of blood and determination of serum glucose

Blood samples from the experimental mice were collected by the retro orbital plexus technique using heparinised capillary glass tubes. The collected blood samples were analyzed for glucose levels by the glucose oxidase peroxidase (GOD/POD) method as described earlier (Abdel-Barry et al.1997) and serum glucose levels were expressed in mg/dl.

### Effect of AL-alc on serum glucose in alloxan-induced diabetic mice

Diabetic swiss albino mice of either sex were fasted overnight and divided into five groups (n=6) viz; Group I – vehicle (distilled water, 10 ml/kg), Group II – glyburide (10 mg/kg), Group III – AL-alc (100 mg/kg), Group IV – AL-alc (200 mg/kg) and Group V – AL-alc (400 mg/kg). AL-alc and glyburide were given orally.

The acute study involved estimation of serum glucose levels at 0, 2, 4, 6 and 24 hour after AL-alc and glyburide administration. The animals had free access to feed and water after 6 h.

The subacute study involved repeated administration of AL-alc and glyburide for 28 days (once a day) at a prefixed time and serum glucose levels were estimated in samples taken after 2 h on day 7, 14, 21 and 28. At the end of 28 days, AL-alc and glyburide administration was stopped and a rest period of 7 days was given to the animals to study the effect of AL-alc and glyburide treatment on serum glucose levels after 7 days (Dunn and McLetchie 1943). The animals had free access to feed and water during this period. The data were represented as mean serum glucose levels and standard error of mean (SEM).

### *Effect of AL-alc on body weight in alloxan-induced diabetic mice*

During the study period of 35 days the mice were weighed daily and their body weights were recorded. From this data, mean changes in body weight and SEM were calculated and tabulated.

### *Effect of AL-alc on oral glucose tolerance test* (*OGTT*) *in normal and diabetic mice*

The diabetic animals were fasted overnight before commencing the experiment. Nondiabetic and diabetic mice were each divided into five groups (n=6) viz; Group I – vehicle (distilled water, 10 ml/kg), Group II – glyburide (10 mg/kg), Group III – AL-alc (100 mg/kg), Group IV – AL-alc (200 mg/kg) and Group V – AL-alc (400 mg/kg).

The mice of all the groups were loaded with D-glucose (2.5 g/kg p.o.) solution after half an hour of drug administration (Latha and Pari 2003, Badole et al. 2006a, b). Blood samples were taken using the retro orbital plexus technique before drug administration and at 30, 60, and 120 minutes after glucose loading. The serum glucose was estimated immediately thereafter.

### Statistical analysis

Data were expressed as mean  $\pm$  SEM and statistical analysis was carried out by one-way ANOVA with a post hoc Tukey's test performed using GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California USA, *www.graphpad.com*. The significance level was considered at  $2\alpha = 0.05$ .

### RESULTS

A single administration of AL-alc (100, 200 and 400 mg/kg) with glyburide (10 mg/kg) significantly reduced serum glucose levels at 2, 4, and 6 h after administration. The reductions in serum glucose from basal value (before) at 6 h after glyburide and AL-alc (100, 200 and 400 mg/kg) were administered were 216.76 and 45.83, and 98.44 and 164.42 mg/dl respectively. The onset of the antihyperglycaemic effect of glyburide was at 2 h and that of the AL-alc (400 mg/kg) was at 4 h; the peak effect was at 6 h but the effect waned at 24 h. AL-alc (400 mg/kg) resulted in lowered serum glucose from basal value (before) at 24 h was 100.74 mg/dl (Table 1).

In the subacute study, repeated administration (once a day for 28 days) of AL-alc and glyburide caused significant reductions in the serum glucose7 level as compared to the vehicle treated group. On the 35th day, the reductions in serum glucose level of glyburide and AL-alc (100, 200 and 400 mg/dl) were 274.80 and 101.19, and 139.29 and 226.11 mg/dl respectively (Table 2).

The body weight of vehicle treated diabetic mice decreased during the study period. Glyburide and AL-alc (400 mg/kg) prevented the decrease in body weight of diabetic mice (Table 3).

In the oral glucose tolerance test, the administration of the glucose load (2.5 g/kg) increased serum glucose levels significantly after 30 min in nondiabetic and alloxan treated diabetic mice. Glyburide (10 mg/kg) and AL-alc (100, 200 and 400 mg/kg) produced a significant increase in the glucose threshold within 60 min, which was then reversed at 120 min after glucose loading in nondiabetic (Table 4) as well as alloxan induced diabetic mice (Table 5).

### DISCUSSION

*Aerva lanata* is used in medicine for the treatment of diabetes. The antidiabetic properties of the whole plant of the *Aerva lanata* have been reported. In folklore practice hot water extract of *Aerva lanata* has been reported to be useful in diabetes mellitus (Vetrichelvan and Jegadeesan 2002).

Glyburide is a potent, second-generation, oral sulfonylurea antidiabetic agent used as an adjunct to diet in order to lower blood glucose levels in patients with diabetes mellitus. The hypoglycaemic action of glyburide is due to the stimulation of pancreatic islet cells, which results in an increase in insulin secretion. The effects of sulfonylurea are initiated by binding to and blocking on the ATP-sensitive K<sup>+</sup> channel, which has been cloned. The drugs thus resemble physiological secretagogues (e.g. glucose, leucine), which also lower the conductance of this channel. Reduced  $K^+$  conductance causes membrane depolarization and an influx of Ca<sup>2+</sup> through the voltage sensitive Ca2+ channel. Prolonged administration of glyburide also produces extra pancreatic effects that contribute to its hypoglycaemic activity (Shah et al. 2006b).

The results of both the acute and the subacute study hypothesized that the late onset of the action and prolonged duration of the action of AL-alc may result from improved pancreatic cytoarchitecture. These results confirmed the use of *Aerva lanata* in folklore practice as an antidiabetic (Krishnamurthi 2003). Subacute treatment for 35 days with the AL-alc in the treated doses brought about improvement in body weights, indicating its beneficial effect in preventing loss of body weight in diabetic mice (Xie et al. 2003). The ability of AL-alc to prevent body weight loss

Table 1. Effect of alcoholic extract of *Aerva lanata* on serum glucose level (mg/ dl) in alloxan-induced diabetic mice (acute study)

Dose (mg/kg. p.o.)	0 h	2 h	4 h	6 h	24 h
Vehicle	437.37±15.32ª	442.12±12.23	449.88±17.54	454.85±20.43	455.56±20.18
Glyburide (10)	441.57±6.80	351.31±19.84*	311.82±23.70*	224.81± 2.97*	376.01±26.38
AL-alc (100)	478.81±15.06	465.86±13.86	442.24±23.50	432.98±21.56	460.44±27.24
AL-alc (200)	473.65±18.14	431.16±22.58	419.44±18.18	375.21±22.76	444.51±20.99
AL-alc (400)	480.87±16.83	402.96 19.72	349.63±20.03*	316.45±31.56*	380.13±26.49

n = 6, data was analyzed by one-way ANOVA followed by post hoc Tukey's test using Graphpad Instat software \*statistically significant as compared with vehicle-treated group (distilled water, 10 ml/kg) <sup>a</sup> mean  $\pm$  s.e.m.

Table 2. Effect of alcoholic extract of *Aerva lanata* on serum glucose level (mg/dl) in alloxan-induced diabetic mice (subacute study)

Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21	Day 28	After day 7 rest period
Vehicle	437.37±15.32	469.47±18.26	473.9±14.25	486.31±18.53	498.54±10.14	512.12±19.10
Glyburide	441.57±6.80	340.92±19.19*	292.68±29.42*	234.71±32.21*	181.77±25.95*	166.77±25.99*
AL-alc	478.81±15.06	441.07±19.64	431.21±29.38	447.11±22.91	393.02±23.42	377.62±30.32*
AL-alc	473.65±18.14	418.97±18.79	390.07±24.68	356.41±20.74*	344.75±22.92*	334.36±31.51*
AL-alc (400)	480.87±16.83	396.19±23.70	366.58±21.16*	330.91±22.23*	315.84±31.53*	254.76±26.87*

Symbols as in Table 1

seems to be due to its ability to reduced hyperglycaemia.

AL-alc significantly enhanced glucose utilization in OGTT in both nondiabetic and diabetic mice. From the data obtained from OGTT, it is clear that administration of AL-alc effectively prevented an increase in serum glucose level without causing a hypoglycaemic state. The effect may be due to restoration of the delayed insulin response. In this context, other medicinal plants, such as *Pleurotus pulmonarius* (Badole et al. 2006a), *Cassia auriculata* (Latha and Pari 2003) have been reported to possess

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similar effects.

Flavonoids are potent antioxidants and are known to modulate the activities of various enzymes due to their interaction with various biomolecules (Catopano et al. 1997). Flavonoids, alkaloids, tannins and phenolics as bioactive antidiabetic principles are also reported (Kameswararao et al. 1997). Flavonoids regenerate the damaged  $\beta$ -cells in alloxan diabetic rats (Chakravarthy et al. 1980).

Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21	Day 28	After day 7 rest period
Vehicle	31±0.36	26±0.93	24±1 36	22±1 46	19±0.96	18±0.81
	20+0.00	20-0.91	20+1.22*	20+1.41*	20+1.20*	20+1.20*
(10)	29±0.68	28±0.81	30±1.23*	29±1.41*	30±1.29*	30±1.36*
AL-alc (100)	31±0.44	28±0.68	29±1.23	29±1.41*	28±1.06*	26±1.52*
AL-alc (200)	30±0.51	29±0.57	29±0.85	28±1.23	28±1.41*	27±1.18*
AL-alc (400)	29±0.63*	30±0.73*	31±1.18*	31±1.36*	31±1.23*	32±1.63*

Symbols as in Table 1

Treatment (mg/kg)	Before glucose	0 min	30 min	60 min	120 min
Vehicle	123.06±11.16	327.66±15.42	259.16±9.51	208.56±9.10	154.55±11.41
Glyburide (10)	118.88±12.03	300.92±16.40	181.74±9.59*	152.56±11.67*	169.54±10.78
AL-alc (100)	108.29±8.95	292.16±11.59	215.94±10.41	175.92±10.10	135.25±10.14
AL-alc (200)	111.26±7.82	310.00±11.01	201.82±12.17*	145.35±8.90*	164.42±11.75
AL-alc (400)	114.31±9.03	318.72±12.24	193.19±11.05*	131.82±9.84*	150.39±9.96

Table 4. Effect of alcoholic extract of Aerva lanata on oral glucose (mg/dl) tolerance test (OGTT) in nondiabetic mice

Symbols as in Table 1

Phytochemical studies on *A. lanata* revealed that it contains flavonoid glycosides, aervitrin, aervolanine, aervoside, amyrin betulin, campesterol, canthin-6-one, 10-hydroxy-canthin-6-one, carboline-1 propionic acid, chrysin,  $\beta$ -ecdysone, daucosterol, hentriacontane, narcissin,  $\beta$ -sitosterol, syringic acid, feruloyl tyramine and vanillic acid (Vetrichelvan and Jegadeesan 2002). Preliminary phytochemical analysis indicated that the leaf extract of *Aerva lanata* contain sterols, glycoside, flavonoids, carbohydrates and tannins.

Traditional medicinal plants with various active principles and properties have been used from ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes, coronary heart disease and cancer. Beneficial multiple activities like manipulating carbohydrate metabolism by various mechanisms, preventing and restoring the integrity and function of  $\beta$ -cells, releasing insulin activity, improving glucose uptake and utilization, and the antioxidant properties present in medicinal plants, offer an exciting opportunity to develop them into novel therapeutics (Tiwari and Rao 2002). The antihyperglycaemic activity of AL-alc may be due to the presence of several bioactive antidiabetic principles. It is thus apparent that AL-alc possesses an antihyperglycaemic activity.

Table 5. Effect of alcoholic extract of *A. lanata* on the oral glucose (mg/dl) tolerance test (OGTT) in diabetic mice

Dose (mg/kg)	Before glucose	0 min	30 min	60 min	120 min
Vehicle	399.82±17.44	505.70±14.78	434.74±18.61	389.68±8.16	494.57±10.96
Glyburide (10)	452.29±20.87	514.19±13.06	326.77±15.77*	314.61±7.28*	437.78±21.20
AL-alc (100)	476.60±17.73	531.60±18.79	472.13±11.08	389.33±12.95	492.45±13.49
AL-alc (200)	461.82±20.01	525.72±21.95	355.73±5.77*	314.52±11.34*	452.49±11.28
AL-alc (400)	478.48±16.40	538.96±17.08	340.80±12.43*	301.31±9.14*	469.88±12.02

Symbols as in Table 1

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### REFERENCES

- Abdel-Barry JA, Abdel-Hassan IA, Al-Hakiem MH: Hypoglycaemic and antihyperglycaemic effects of *Trigonella foenum-graecum* leaf in normal and alloxan induced diabetic rats. J. Ethnopharmacol. 58:149–155, 1997.
- Badole SL, Shah SN, Patel NN, Thakurdesai PA, Bodhankar SL: Hypoglycemic activity of aqueous extract of *Pleurotus pulmonarius* (Fr.) Quel-Champ in alloxan induced diabetic mice. Pharm.

Biol. 44:421-425, 2006a.

- Badole SL, Bodhankar SL, Thakurdesai PA: Study of interaction of aqueous extract of *Pleurotus pulmonarius* (Fr.) Quel-Champ with rosiglitazone in alloxan induced diabetic mice. Pharmacologyonline 3:64–72, 2006b.
- Catopano AL: Antioxidant effect of flavonoids. Angiol. 48:39–46, 1997.
- Chakravarthy BK, Gupta S, Gambir SS, Gode KD: Pancreatic β cell generation- A novel antidiabetic mechanism of *Pterocarpus marsupium* Rox. Ind. J. Pharmacol.12:123–127, 1980.
- Dunn JS, McLetchie NGB: Experimental alloxan diabetic in rats. Lancet 2:384 387, 1943.
- Edwin E, Sheeja E, Chaturvedi M, Sharma S, Gupta VB: A comparative study on antihyperglycemic activity of fruits and barks of *Ficus bengalensis* (L.). Adv. Pharmacol. Toxicol. 7:69–71, 2006.
- Kameswararao B, Giri R, Kesavulu MM, Apparao C: Herbal medicines: In the treatment of diabetes mellitus. Manphar Vaidya Patrika 1:33–35, 1997.
- Kameswararao BK, Kesavulu MM, Giri R, Apparao C: Antidiabetic and hypolipidemic effects of *Momordica cymbalaria* Hook. fruit powder in

alloxan-diabetic rats. J. Ethnopharmacol. 67:103–109, 1999.

- Krishnamurthi A: The Wealth of India, Vol. I. A Publication and Information Directorate. Council of Scientific and Industrial Research, New-Delhi 2003, pp. 92.
- Latha M, Pari L: Antihyperglycaemic effect of *Cassia auriculata* in experimental diabetes and its effect on key metabolic enzymes involved in carbohydrate metabolism. Clin. Exp. Pharmacol. Physiol. 30:38–43, 2003.
- Pullaiah T, Naidu CK: Antidiabetic Plants in India and Herbal Based Antidiabetic Research. Regency Publications, New-Delhi 2003, pp. 68–69.
- Rangari V D: Pharmacognosy and Phytochemistry. Part I, 1st ed. Career Publications, Nashik 2004, pp. 2–6.
- Shah SN, Bodhankar SL, Bhonde R, Mohan V: Hypoglycemic activity of the combination of active ingredients isolated from *Trigonella foenumgraecum* in alloxan induced diabetic mice. Pharmacologyonline 1:65–82, 2006a.
- Shah SN, Bodhankar SL, Badole SL, Kamble HV, Mohan VJ: Effect of trigonelline: an active compound from *Trigonella foenumgraecum* Linn. in alloxan induced diabetes in mice. J. Cell Tissue Res. 6:585–590, 2006b.

- Shirwaikar A, Issac D, Malini S: Effect of *Aerva lanata* on cisplatin and gentamicin models of acute renal failure. J. Ethnopharmacol. 90:81–86, 2004.
- Tiwari AK, Rao Madhusudana: Diabetes mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospects. Curr. Sci. 83:30–38, 2002.
- Udupihille M, Jiffry M T M: Diuretic effect of *Aerva lanata* with water, normal saline and coriander as controls. Indian J. Physiol. Pharmacol. 30:91–97, 1986.
- Vetrichelvan T, Jegadeesan M: Anti-diabetic activity of alcoholic extract of *Aerva lanata* (L.) Juss. Ex Schultes in rats. J. Ethnopharmacol. 80:103–107, 2002.
- Vertichelvan T, Jegadeesan M, Senthil Palaniappan S, Murali NP, Sasikumar K: Diuretic and anti-inflammatory activities of *Aerva lanata* in rats. Indian J. Pharm. Sci. 62:300–302, 2000.
- Xie TT, Wang A, Mehendale S, Wu J, Aung HH, Dey L, Qiu S, Yuan CS: Antidiabetic effect of *Gymnema yannaense* extract. Pharmacol. Res. 47:323–329, 2003.