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Antihyperglycaemic Activity of Ethanol Extract of *Aerva javanica* Leaves in Alloxan- Induced Diabetic Mice

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ABSTRACT

The antihyperglycaemic activity of ethanol extract of *Aerva javanica* (EEAJ) leaves was studied by administering three doses of EEAJ (i.e. 100,200 and 400 mg/kg, p.o.) and glyburide (10 mg/kg) to alloxan (70 mg/kg, i.v.) induced diabetic mice. The serum glucose levels and body weights of mice were determined. In acute study the maximum reduction in serum glucose level was observed at 2h, peak at 6h but antihyperglycaemic effect was vanished at 24h. The sub acute study was also carried out which showed maximum reduction in serum glucose level at the dose of 400 mg/kg on 35th day. EEAJ (400 mg/kg) showed increased glucose threshold in non-diabetic and diabetic mice at 60 min after administration of glucose. EEAJ (400 mg/kg) prevented the loss of body weight. The EEAJ (400 mg/kg) showed significantly more hyperglycaemic activity than EEAJ (100 and 200 mg/kg).

Keywords: *Aerva javanica* , Alloxan-induced, Antihyperglycaemic, Body weight, OGTT.

INTRODUCTION

Diabetes mellitus is a common metabolic disorder with multiple etiologies and is associated with variety of irreversible complications. It is well known that the incidence of diabetes mellitus is high all over the world, especially in Asia. Different types of oral hypoglycaemic agents such as biguanides and sulphonylurea are available along with insulin for the treatment of diabetes mellitus, but have side effects associated with their uses. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low costs. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown. Even the World Health Organization (WHO) approves the use of plant drugs for different diseases, including diabetes mellitus. Therefore, studies with plant extracts are useful to know their efficacy and mechanism of action and safety.

The plant *Aerva javanica* (Amaranthaceae), is a perennial herb and widely distributed in various parts of the world. It is a native to Africa and also found to occur in some of the Asian countries (1). In traditional herb is used as diuretic, diabetic demulcent and the decoction of the plant are used to remove swelling (2). Powder of the plant is applied externally to ulcers in domestic animals. The seeds are used to relieve headache and also used rheumatism. The presence of steroids (3), triterpenoids (4), carbohydrates (5), flavonoids(6) has been reported earlier. To the best of our knowledge no report is available on the antihyperglycaemic activity of *A.javanica* leaves. The present study was undertaken to verify the claim and evaluate the antihyperglycaemic activity of ethanol extract*Aerva javanica* leaves.

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MATERIALS AND METHODS

Plant collection and authentication

The leaves of *Aerva javanica* were collected in October from siddipet, karimnagar district, Andhrapredesh, India and identified by Dr. Raju S.Vastavya, Taxonomist, Department of Botany, Kakatiya university, warangal and authenticated by comparing with the voucher specimen. The leaves were shade dried and then milled in to powder by a mechanical grinder. The powder was passed through sieve and used for further studies.

Drugs and Chemicals

Glyburide (Ranbaxy Pharma. Ltd. India), alloxan monohydrate (Spectrochem,India), glucose estimation kit (Accurex Biomedical Pvt. Ltd., India) and D -glucose(S.D.FineChem. Ltd, India) and ethanol (Merck,Mumbai,India) were purchased from respective vendors.

Preparation of the extracts

The dried powder of *Aerva javanica* leaves was subjected to hot continuous extraction with ethanol in a soxhlet extractor and filtered. The filtrate was evaporated at room temperature and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in desiccators. The extract was dissolved in distilled water to prepare the drug solution of concentration of 100 mg/ml and used for pharmacological studies.

Animals

The male albino mice weighing 22-30 gm were obtained from Mahaveera enterprises, Hyderabad. All the animals were stored in standard cages and maintained at 27ºc ± 2ºc under 12hrs dark/light cycle. The animals were fed with standard rat feed and water was given *ad libitum.* Ethical clearance for handling of animals and the procedures used in the study was obtained from the institutional animal ethical committee prior to the beginning of the study.

Induction of experimental diabetes

Diabetes was induced in mice by a single intravenous injection of aqueous alloxan monohydrate (70 mg /kg i.v.). After

Table 1: Effect of ethanol extract of *Aerva javanica* in alloxan-induced diabetic mice (acute study).

GroupsTreatment (mg/kg)	Mean Fasting Serum Glucose Level(mg/Groups g/dl) ± SEM				
	0hr	2hr	4hr	6hr	24hr
Group I (Vehicle)	438.50±9.66	445.74±12.58	448.60±13.26	453.40±16.58	458.55±12.67
Group II (Glyburide 10)	447.83±12.63	361.79±11.89**	298.24±11.43**	218.93±18.90**	386.09±20.45**
Group III (EEAJ 100)	476.33±14.03	461.57±10.70	443.71±11.71	425.30±12.33*	460.25±13.15
Group IV (EEAJ 200)	472.15±11.46	435.20±15.47	421.61±15.50**	358.85±17.38**	441.10±16.48
Group V (EEAJ 400)	478.61±13.24	402.47±14.28**	342.38±14.32**	298.74±16.93**	378.34±16.05

*Values are mean± S.E.M., n = 6 in each group, data were analyzed by one way ANOVA followed by Dunnet's test. *P<0.05, **P<0.01 as compared with vehicle treated group (distilled water, 10 ml/kg).*

Table 2: Effect of ethanol extract of *Aerva javanica* on serum glucose level in alloxan-induced diabetic mice (subacute study)

Groups Treatment(mg/kg)	Mean Fasting Serum Glucose Level(mg/Groups g/dl) ± SEM					
	Day 0	Day 7	Day 14	Day 21	Day 28	After 7 rest period
Group I (Vehicle)	438.50±9.66	471.38±14.67	474.93±14.81	486.01±15.59	497.67±16.91	511.67±15.72
Group II(Glyburide 10)	445.83±12.63	320.23±17.56**	299.42±16.70**	236.55±20.02**	183.11±19.59**	156.05±19.21**
Group III (EEAJ 100)	477.10±11.46	459.33±13.32	448.97±13.56*	427.77±14.23**	399.30±13.64**	364.62±15.12**
Group IV (EEAJ 200)	472.33±14.03	445.88±17.73*	410.75±17.70**	381.13±16.24**	372.34±14.16**	308.91±18.41**
Group V (EEAJ 400)	468.33±13.24	393.37±17.02**	332.82±16.18**	301.13±17.04**	289.13±19.71**	226.47±14.76**

*Values are mean± S.E.M., n = 6 in each group, data were analyzed by one way ANOVA followed by Dunnet's test. *P<0.05, **P<0.01 as compared with vehicle treated group (distilled water, 10 ml/kg).*

Table 3: Effect of ethanol extract of *Aerva javanica* on body weight in alloxan-induced Diabetic mice

Groups Treatment (mg/kg)	Mean Fasting Serum Glucose Level(mg/Groups g/dl) ± SEM					
	Day 0	Day 7	Day 14	Day 21	Day 28	After 7 rest period
Group I (Vehicle)	31.23±1.14	27.33±1.06	25.50±0.89	22.01±1.03	20.50±1.08	19.33±1.11
Group II(Glyburide 10)	34.16±0.94	33.50±0.71**	34.70±1.31**	34.83±0.92**	36.83±1.14**	37.16±1.18**
Group III (EEAJ 100)	29.83±0.55	28.50±0.91	27.33±0.84	27.66±0.89*	27.00±0.82**	26.83±0.77**
Group IV (EEAJ 200)	30.02±0.84	28.87±0.61*	29.46±0.86**	29.86±1.11**	30.33±0.86**	32.16±0.88**
Group V (EEAJ 400)	31.16±1.46	32.16±0.77**	33.23±0.78**	33.93±0.69**	34.66±0.68**	36.01±0.63**

*Values are mean± S.E.M., n = 6 in each group, data were analyzed by one way ANOVA followed by Dunnet's test. *P<0.05, **P<0.01 as compared with vehicle treated group (distilled water, 10 ml/kg).*

Table 4: Effect of ethanol extract of *Aerva javanica* on oral glucose tolerance test (OGTT) in nondiabetic mice

Groups Treatment (mg/kg)	Mean Fasting Serum Glucose Level(mg/dl) ± SEM				
	Before glucose	0min	30min	60min	120min
Group I (Vehicle)	128.08±12.16	331.66±15.42	269.18±9.41	217.66±9.12	158.45±13.41
Group II(Glyburide 10)	121.76±11.04	310.83±14.20	186.84±8.63**	162.36±10.54**	169.44±12.74
Group III (EEAJ 100)	110.19±7.92	298.14±12.46	258.72±11.24	192.76±11.21*	144.21±13.08
Group IV (EEAJ 200)	114.45±6.78	310.20±12.12	251.62±11.16*	159.23±7.50**	157.82±10.64
Group V (EEAJ 400)	116.22±8.10	319.38±12.44	193.11±10.08**	131.42±7.62**	134.27±7.76

*Values are mean± S.E.M., n = 6 in each group, data were analyzed by one way ANOVA followed by Dunnet's test. *P<0.05, **P<0.01 as compared with vehicle treated group (distilled water, 10ml/kg).*

Table 5: Effect f ethanol extract of *Aerva javanica* on the oral glucose tolerance test (OGTT) in diabetic mice

Groups Treatment(mg/kg)	Mean Fasting Serum Glucose Level(mg/dl) ± SEM				
	Before glucose	0min	30min	60min	120min
Group I (Vehicle)	398.71±16.41	502.60±13.48	438.84±17.41	391.48±8.14	496.67±11.31
Group II(Glyburide 10)	449.26±18.84	518.18±12.04	327.64±13.81**	316.81±9.37**	438.88±20.10
Group III (EEAJ 100)	473.50±16.53	531.42±16.46	422.12±10.12	374.23±11.90*	489.35±12.49
Group IV (EEAJ 200)	468.72±18.06	520.68±17.92	381.65±14.68**	324.62±12.22**	442.39±14.58
Group V (EEAJ 400)	476.28±18.40	530.84±16.18	330.40±10.32**	278.41±7.81**	449.78±11.02

48 h, the animals showing serum glucose levels above 200 mg /dl (diabetic) were selected for the study (7). All the animals were allowed free access to water and pellet diet.

Collection of blood and determination of serum glucose

Blood samples were collected by retro-orbital puncture (ROP) technique. The collected blood samples were analyzed for glucose levels by the glucose oxidase peroxidase (GOD/POD) method (8) and serum glucose levels were expressed in mg/dl.

Effect of EEAJ on serum glucose in alloxan-induced diabetic mice

The diabetic mice were divided into five groups (n =6), viz.: group I-vehicle (distilled water, 10 ml/kg); group II-glyburide (10 mg/kg); group III EEAJ (100 mg/kg); group IV- EEAJ (200 mg/kg); group V- EEAJ (400mg/kg). All drugs were given orally.

The acute study involved estimation of serum glucose at 0, 2, 4, 6 and 24 h after EEAJ and glyburide administration (9).

In subacute study involved repeated administration of drug for 28 days at prefixed times and serum glucose levels were estimated on the 7th, 14th, 21st and 28th day. At the end of 28 days the drug administration was stopped and a rest period of 7 days was given to the animals to study effect of drug treatment on blood glucose after 7 days i.e. on 35th day. The data were represented as mean serum glucose level and standard error of mean (SEM).

Effect of EEAJ on body weight in alloxan-induced diabetic mice

The mice were weighed daily during the study period of 35 days and their body weights were noted and presented as mean change

in body weights.

Effect of EEAJ on oral glucose tolerance test (OGTT) in non-diabetic and diabetic mice

Non diabetic and diabetic mice were divided into five groups (n=6),viz.groupI-vehicle(distilled water,10ml/kg), group II- glyburide (10mg/kg);group III- EEAJ(100 mg/kg), Group IV- EEAJ(200 mg/kg) and Group V-EEAJ(400mg/kg). The animals were fasted overnight before commencing the experiment. 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. Blood samples were collected by the ROP method just prior to drug administration and at 30, 60 and 120 min after glucose loading. Serum glucose level was measured immediately.

Statistical analysis

The results are expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by Dunnet's test (10).

RESULTS

Effect of EEAJ on serum glucose in alloxan-induced diabetic mice

In acute study, administration EEAJ (100,200 and 400 mg/kg) as well as glyburide (10 mg/kg) in diabetic albino mice, showed significant reduction of serum glucose levels at 2, 4, and 6 h interval. The onset of reduction of serum glucose of EEAJ (100, 200 and 400 mg/kg) treated mice was observed at 4h and peak effect at 6 h from basal value (51.03, 113.3 and 179.87mg/dl respectively) but effect was waned at 24 h after administration. The onset of antihyperglycaemic effect of glyburide(10mg/kg,p.o) was at 2 h (86.04 mg/dl) the peak effect was at 6 h (228.90 mg/dl) (Table 1).

In the subacute study, repeated administration (once a day for 28 days) of the EEAJ as well as glyburide caused significantly reduced in the serum glucose level as compared with vehicle treated group. Significant decreases in serum glucose of the diabetic mice were seen at doses of 100,200 and 400mg/kg in dose-dependent manner as compared with vehicle treated group. Maximum reduction in serum glucose level from basal value was observed (241.86 mg/dl) on 35th day in the diabetic mice treated with EEAJ 400mg/kg.Glyburide treated animals showed maximum reduction in serum glucose level from basal value (289.78 mg/dl) on 35th day (Table 2).

Effect of EEAJ on body weight in alloxan-induced diabetic mice

Administration of vehicle (distilled water, 10ml/kg.p.o.) in alloxan induced diabetic mice resulted in gradual decrease in body weight during the study period. Body weight of EEAJ (100,200 and 400 mg/kg) treated diabetic mice prevented further loss of body weight in diabetic mice. On the other hand, mice gained body weight which indicated beneficial effect of EEAJ (Table 3).

Effect of EEAJ on oral glucose tolerance test (OGTT) in non-diabetic and diabetic mice

In oral glucose tolerance test, the administration of the glucose load increased serum glucose levels significantly after 30 min in nondiabetic and alloxan treated diabetic mice. Glyburide (10mg/kg) and EEAJ (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

Preliminary phytochemical analysis indicated the leaf extract of *Aerva javanica* contain sterols, flavonoid glycosides, carbohydrates, phenolics and tannins. EEAJ (100, 200 and 400 mg/kg) showed significant decrease in serum glucose level at 2, 4 and 6 h. Continuous treatment with EEAJ (100, 200 and 400 mg/kg) for a period of 35 days showed a significant decrease in the serum glucose level in diabetic mice. Maximum reduction of serum glucose level in acute and subacute study occurred at the dose of 400 mg/kg. Subacute treatment for 35 days with the EEAJ in the treated doses brought about improvement in body weights indicating its beneficial effect in preventing loss of body weight in diabetic mice. The ability of EEAJ to prevent body weight loss seems to be due to its ability to reduce hyperglycaemia.

OGTT study indicated that EEAJ enhanced glucose utilization in non- diabetic & diabetic mice. Administration of EEAJ effectively prevented the increase in serum glucose level without causing a hypoglycaemic state. The effect may be due to restoration of the delayed insulin response. The antihyperglycaemic activity of EEAJ may be due to the presence of several bioactive antidiabetic principles. Present efforts are directed to isolate the active constituents from ethanol extract of *Aerva javanica* leaves and elucidation of mechanism of action.

CONCLUSION

Thus it may be concluded that the ethanol extracts of leaves of *Aerva javanica* ., is endowed with significant antihyperglycaemic activity justifying its use in traditional system of medicine.

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