ANTIHYPERGLYCAEMIC ACTIVITY OF *CICER ARIETINUM* SEEDS

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Summary

The objective of the present investigation was to evaluate antihyperglycaemic activity of petroleum ether extract of *Cicer arietinum* (PEECA) seeds at three different doses i.e. 100,200 and 400 mg/kg p.o. in alloxan (70 mg/kg i.v.)induced diabetic mice. The acute oral toxicity was performed which indicated no mortality upto 5000 mg/kg p.o. dose of PEECA. In both acute and subacute studies serum glucose level (SGL) was measured.The change in body weight was noted during subacute study. OGTT was performed in both diabetic and non-diabetic mice previously loaded with (2.5 g/kg p.o.) glucose.Glyburide (10 mg/kg) was used as a standard drug. The maximum reduction in SGL was observed in PEECA (400 mg/kg) group at 6h (137.17 mg/dl) in acute study and on 21st day (217.79 mg/dl) in subacute study respectively.In glyburide treated mice the maximum reduction in SGL was observed at 6h (194.97 mg/dl) and on 21st day (267.40mg/dl) respectively. PEECA (400 mg/kg) and glyburide (10 mg/kg) prevented loss of body weight in diabetic mice. OGTT showed increased glucose threshold in non-diabetic and diabetic mice. It is concluded that PEECA showed antihyperglycaemic activity comparable with glyburide.

Keywords: *Cicer arietinum*, Alloxan, Antihyperglycaemic, Body weight, Oral glucose tolerance test.

Introduction

Herbal medicines for the treatment of diabetes mellitus have gained importance throughout the world. The available literature shows that there are more than 400 plant species showing hypoglycaemic activity **(1)**.Though some of these plants have great reputation in the indigenous system of medicine for their antidiabetic activities, many remain to be scientifically established.The scientific basis of the beneficial effects of gram seeds is not clear. Therefore this study was designed to investigate the antidiabetic activity of petroleum ether extract of *Cicer arietinum* seeds to establish its potential therapeutic value.

Cicer arietinum Linn. (Papilionaceae) is commonly known as Bengal gram or chickpea in English.Chickpea is an erect or spreading , much branched annual herb cultivated in Sind, Bombay Presidency and as a pulse crop throughout India **(2)** It is 30-50 cm tall covered with glandular hairs. Leaves pinnately compound , 2.5-5 cm long usually with a terminal leaflet, stipules small , obliquely ovate, toothed , leaflets 9-17, flowers – white to various shades of pink or blue, fruits (Pods) 2-2.5 cm long, seeds 1-2 obovate, beaked variable in color (yellow, green, orange brown, pink or black) smooth, granular or tuberculate . The leaves of *Cicer arietinum* are astringent to bowels, used in the treatment of bronchitis and diabetes. Boiled leaves relieve sprains, dislocated limbs while leaf juice is used as stomachic and laxative . The seeds are stimulant tonic, aphrodisiac , anthelmintic used in the treatment of bronchitis, leprosy and skin diseases **(3)** It is reported that the seeds reduced postprandial plasma glucose and are useful in the treatment of diabetes **(4,5)** but the scientific evidence is not available.Therefore the objective of the study was to evaluate antihyperglycaemic activity of *Cicer arietinum* seeds.

Materials and Methods

Plant material

The dried seeds of *Cicer arietinum* Linn.were procured from the local market of Pune in Maharastra state.The seeds were authenticated by Dr. A.M. Mujumdar, Department of Botany, at Agharkar Research Institute,Pune and voucher specimen number was AHMA S-068.

Drugs and Chemicals:

The following drugs were obtained (from) Glyburide (Ranbaxy Pharma. Ltd. India), Alloxan monohydrate (Spectrochem,India),Glucose estimation kit (Accurex Biomedical Pvt. Ltd., India) , d –glucose (S.D.FineChem. Ltd, India) and petroleum ether (Merck,Mumbai,India).

Preparation of extract :

The air dried powder was subjected to hot continuous extraction with Petroleum ether in a Soxhlet extractor and filtered. The filtrate was evaporated at room temperature to concentrate extract . The yield of petroleum ether extract of *Cicer arietinum* (PEECA) seeds was 1.94 % v/w.Weighed quantity of PEECA was dissolved in distilled water using 1.5% Tween 80 to prepare drug solution of concentration of 100 mg/ml and used for pharmacological studies.

Detection of Phytochemical constituents
The PEECA was evaluated for presence of various phytochemical constituents as per the methods described by Khandelwal **(6)**.

Animals
Swiss albino mice (25-35 g) were purchased from the National Toxicology Centre, Pune, India. These were housed under standard condition of temperature 25 ± 1ºC 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. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Poona College of Pharmacy, Pune, India.

Toxicity studies
Non-diabetic adult Swiss albino mice of female sex were subjected to acute toxicity studies as per guidelines (AOT no. 425) suggested by the Organization for Economic Cooperation and Development **(7)**. The mice were observed continuously for 2 h for behavioral, neurological and autonomic profiles and for any lethality or death for the next 48 h.

Induction of diabetes
Alloxan monohydrate (70mg/kg i.v.) was administered to induce diabetes.After 2 days animals showing Serum Glucose Level (SGL) more than 200 mg/dl (diabetic) were selected for the study **(8).** All animals had free access to food pellets and water.

Determination of Serum Glucose Level (SGL)
Retro-orbital Puncture (ROP) technique was followed to collect the blood samples and glucose oxidase peroxidase (GOD/POD) method for detection of SGL (mg/dl) (9).

Determination of SGL
The diabetic mice were divided into five groups (n =6),
Group I-vehicle (1.5% Tween 80 in distilled water, 10 ml/kg)
Group II-glyburide (10 mg/kg)
Group III- PEECA (100 mg/kg)
Group IV- PEECA (200 mg/kg)
Group V- PEECA (400mg/kg).

All drugs were given orally.

Acute study :- SGL was determined at 0,2,4,6 and 24 hours after drug administration (10).

Subacute study :- All the animals were administered the respective drugs doses at prefixed time for 28 days. SGLs were estimated on 7^{th} ,14^{th} ,21^{st} and 28^{th} day. At the end of 28 days the drug administration was stopped and a rest period of 7 days was given. The SGLs were estimated on 35^{th} day.The data were represented as mean SGL ± standard error of mean (SEM).

Body weight
All the mice were weighed daily during study period of 35 days . The body weights were noted and presented as mean change in body weights.

Oral Glucose Tolerance Test (OGTT)
All the animals were fasted overnight before the experiment. Non-diabetic and diabetic mice were divided into three groups (n =6)
Group I – d-glucose (2.5 g/kg)
Group II – Glyburide (10 mg/kg)
Group III –PEECA (400 mg/kg)

d-glucose was administered in non-diabetic and diabetic mice at the 4th hour of pretreatment with PEECA and glyburide. Serum glucose levels were estimated before and 2 hours after glucose loading.

Statistical analysis
The results are expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by *post hoc* Tukey's test **(11)**.

Results

Phytochemical constituents :-The phytochemical tests indicated that the PEECA contains carbohydrates,reducing sugars, hexose sugars, amino acids, cardiac glycosides and sterols.

Toxicity studies :- No lethality or any toxic reactions were occurred upto the end of study period.The results indicated that PEECA was safe upto the dose of 5000 mg/kg body weight.

Acute study :- In acute study, PEECA (100,200 and 400 mg/kg) and glyburide (10 mg/kg) showed significant reduction in serum glucose levels at 4 and 6 h. The onset of reduction in serum glucose of PEECA (100, 200 and 400 mg/kg) treated mice was observed nonsignificant at 2 h (7.65,45.68 & 86.29 mg/dl respectively), significant at 4 h (34.40,70.52 & 103.55 mg/dl respectively) and peak at 6h (49.68,98.71& 137.17 mg/dl respectively) but effect was waned at 24 h. The onset of reduction in serum glucose levelof glyburide treated mice was at 2 h (90.09 mg/dl) at 4h (147.99 mg/dl) and the peak effect was at 6 h (194.97 mg/dl) **(Table 1)**.

Subacute study :- In the subacute study, repeated administration (once a day for 28 days) of the PEECA as well as glyburide caused significant ($P < 0.001$) reduction in the serum glucose level as compared with vehicle treated group. Maximum reduction in serum glucose level was observed (217.79 mg/dl) on 21st day in the diabetic mice treated with PEECA (400 mg/kg). Maximum reduction in serum glucose level was observed (79.18 mg/dl and 157.01 mg/dl respectively) on 28th day in the diabetic mice treated with PEECA(100 and 200 mg/kg respectively). Glyburide treated animals showed maximum reduction in serum glucose level (312.28 mg/dl) on 21stday **(Table 2)**.

Body weight:- Body weight of diabetic animals decreased during study period in vehicle and PEECA (100 & 200 mg/kg) treatment while it was increased in glyburide and PEECA (400mg/kg) treatment. This indicated that there is similarity in the effect of PEECA (400mg/kg) and glyburide (10 mg/kg) (Table-3).

Oral Glucose Tolerance Test:- In this study PEECA (400 mg/kg) indicated significant increase in glucose threshold in both non-diabetic (Table-4) and diabetic (Table 5) mice.
These results indicate that PEECA (400mg/kg) possessed antihyperglycaemic activity in alloxan induced diabetic mice.

Table 1:- Effect of PEECA on serum glucose level in alloxan-induced diabetic mice (acute study).

Groups (Treatment.mg/kg p.o.)	Mean fasting serum glucose level (mg/dl) ± SEM (% Change)				
	0h	2h	4h	6h	24h
Group I (Vehicle)	446.92 ± 11.61 _	454.37 ± 10.90 1.71	459.68 ± 12.12 2.88	465.90 ± 13.03 4.29	472.13 ± 10.43 5.86
Group II (Glyburide 10)	439.49 ± 12..24 _	349.40 ± 12.18*** -20.38	291.50 ± 14.16*** -33.69	244.52 ± 13.03*** -44.30	342.09 ± 16.35*** -22.13
Group III (PEECA 100)	454.13 ± 15.44 _	446.48 ± 14.52 -1.65	419.73 ± 14.81 -7.56	404.45 ± 15.37 -10.97	448.33 ± 15.88 -1.27
Group IV (PEECA 200)	461.31 ± 14.36 _	415.63± 13.63 -9.91	390.79± 12.32 -15.26	362.60± 13.75 -21.45	411.18 ± 12.90 -10.76
Group V (PEECA 400)	471.84 ± 12.76 _	385.55 ± 11.85 -18.31	368.29 ± 12.25 -21.54	334.67 ± 13.74*** -25.08	392.31 ± 15.23* -16.79

Values are mean ± SEM,n=6 in each group, data were analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software, *P<0.05, **P<0.01,***P<0.001 as compared with vehicle-treated group (1.5% Tween 80 in distilled water 10 ml/kg)

Table 2: Effect of PEECA on serum glucose level in alloxan-induced diabetic mice (subacute study).

Groups (Treatment.mg/kg p.o.)	Mean fasting serum glucose level (mg/dl) ± SEM (% Change)					
	Day 0	Day 7	Day 14	Day 21	Day 28	After day 7 rest period
Group I (Vehicle)	446.92± 11.61	480.65 ± 11.96	487.08 ± 11.38	492.97 ± 11.89	499.52 ± 13.58	523.39 ± 13.19
	_	7.80	9.17	10.43	11.87	17.25
Group II (Glyburide 10)	439.49± 12.24	292.96 ± 16.51***	246.14 ± 12.41***	172.09 ± 17.13***	192.35 ± 16.49***	205.71 ± 16.37***
	_	-33.34	-43.95	-60.79	-56.16	-53.14
Group III (PEECA 100)	454.13 ± 15.44	400.00 ± 11.32*	394.13 ± 13.21**	388.55 ± 13.49**	374.95 ± 12.95***	391.65 ± 12.07***
	_	-11.65	-12.64	-13.86	-16.88	-12.27
GroupIV (PEECA200)	461.31 ± 11.46	384.60 ± 15.03**	339.81 ± 15.09***	312.69 ± 14.42***	304.30 ± 13.37***	319.43 ± 13.52***
	_	-16.59	-26.33	-32.28	-34.08	-30.81
Group V (PEECA 400)	471.84 ± 12.76	341.40 ± 14.56***	305.04 ± 14.64***	254.05 ± 18.34***	262.77 ± 18.41***	286.56 ± 16.33***
	_	-27.71	-35.41	-46.36	-44.41	-43.11

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one way ANOVA followed by Tukey's test using Graphpad Instat software, ns- not significant, **P<0.01.All other values are significant (P<0.001) as compared with vehicle-treated group(1.5%Tween80 in distilled water,10ml/kg)

Table 3: Effect of PEECA on body weight in alloxan-induced diabetic mice.

Groups (Treatment mg/kg p.o.)	Mean Body Weight (g) ± SEM (% Change)					
	Day 0	Day 7	Day 14	Day 21	Day 28	After day 7 rest period
Group I (Vehicle)	30.83 ± 0.94	28.83 ± 0.94	27.00 ± 0.63	25.33 ± 0.66	24.33 ± 0.66	23.83 ± 0.70
	_	-6.49	-12.31	-17.77	-21.03	-22.63
Group II (Glyburide 10)	32.00 ± 0.85	33.33 ± 0.61*	35.00 ± 0.57***	34.66 ± 0.98***	35.50 ± 0.76***	35.83 ± 0.79***
	_	4.18	9.43	8.28	10.95	12.02
Group III (PEECA 100)	29.33 ± 1.14	28.33 ± 1.14	27.50 ± 1.05	28.66 ± 1.08	27.50 ± 0.76	26.83 ± 0.94
	_	-3.36	-6.05	-2.20	-5.93	-8.38
Group IV (PEECA 200)	30.33 ± 0.88	29.33 ± 0.80	28.83 ± 1.01	29.66 ± 0.88 *	28.33 ± 1.20	27.50 ± 1.33
	_	-3.25	-4.95	-2.05	-6.59	-10.42
Group V (PEECA 400)	32.16 ± 0.90	33.16 ± 1.07*	33.66 ± 0.98***	34.16 ± 0.70***	34.00 ± 1.03***	34.00 ± 0.63***
	_	3.05	4.66 ±	6.36	5.70	5.90

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software, *P<0.05, **P<0.01, ***P<0.001.All other values are not significant as compared with vehicle-treated group (1.5% Tween 80 in distilled water, 10 ml/kg).

Table 4: Effect of PEECA on oral glucose tolerance test (OGTT) in non-diabetic mice.

Groups (Treatment.mg/kg p.o.)	Mean fasting serum glucose level (mg/dl) ± SEM		
	0h	Before Glucose load	After Glucose load (6h)
Group I (Vehicle)	116.99 ± 8.25	141.28 ± 8.16	190.42 ± 11.22
Group II (Glyburide 10)	117.49 ± 9.53	131.00 ± 6.95	164.70 ± 9.00
Group III (PEECA 400)	114.01 ± 7.84	117.38 ± 9.10	141.21 ± 8.91*

d-glucose (2.5 g/kg) was administered in non-diabetic mice at the 4th h of pretreatment with PEECA and glyburide. Serum glucose levels were estimated before and 2 h after glucose loading. Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software, *P<0.001 as compared with vehicle-treated group (1.5% Tween 80 in distilled water, 10 ml/kg).

Table 5: Effect of PEECA on oral glucose tolerance test (OGTT) in diabetic mice.

Groups (Treatment.mg/kg p.o.)	Mean fasting serum glucose level (mg/dl) ± SEM		
	0h	Before Glucose load	After Glucose load (6h)
Group I (Vehicle)	410.18 ± 7.67	422.72 ± 9.48	528.27 ± 9.31
Group II (Glyburide 10)	413.51 ± 9.68	425.62± 10.55	362.66±11.72***
Group III (PEECA 400)	486.30 ± 7.10***	463.56 ± 6.76*	406.31±12.79***

d-glucose (2.5 g/kg) was administered in diabetic mice at the 4th h of pretreatment with PEECA and glyburide. Serum glucose levels were estimated before and 2 h after glucose loading. Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one- way ANOVA followed by Tukey's test using Graphpad Instat software, **P<0.05, ***P<0.001 as compared with vehicle-treated group (1.5% Tween 80 in distilled water, 10 ml/kg).

Discussion

It has been reported that seeds of *Cicer arietinum* contains chemical constituents like isoflavones (Biochanin A,B,&C) , flavonoids, formononetin, protensein, liquiritigenin, isoliquiritigenin,garbenzol, p-coumaric acid, pangamic acid, ciceritol, pseudouridine, pantothenic acid, riboflavin, vit B6, β sitosterol & volatile components has been reported in the seeds **(4).** The constituents like starch, glucose, fructose, polysaccharides, levulose, γ – galactan, betaine, choline, adenine, inositol, phytin, saponin and citric and oxalic acids are also reported**(12).**

OGTT study indicated that PEECA enhanced glucose utilization in non-diabetic & diabetic mice. Administration of PEECA 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. In this context, other medicinal plants, such as *Ficus racemosa* **(13)**, *Ficus religiosa* **(14)** and Psidium guajava **(15)** have been reported to possess similar effect.

Cicer arietinum seeds have been reported to contain isoflavones, flavonoids and its glucosides, cyanogenetic glycosides, protensein, garbenzol, ciceritol, β sitosterol **(4),** starch , sugars, adenine , choline, inositol, phytin, saponin and citric and oxalic acids.**(12)**.

In glucose loaded animals, the PEECA reduced the serum glucose levels. It is possible that the drug may be acting through potentiating the pancreatic secretion or increasing glucose uptake. Thus it is apparent that PEECA possesses antihyperglycaemic activity. Further study is required to isolate the active constituent responsible for antihyperglycaemic activity.

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