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Antidiabetic activity of *Cicer arietinum* seeds in alloxan induced animal model

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Abstract
The objective of the present investigation was to evaluate antihyperglycaemic activity of *Cicer arietinum* seeds at three different doses i.e. 100,200 and 500 mg/kg p.o. in alloxan (150 mg/kg i.p.) induced diabetic mice. The acute oral toxicity was performed which indicated no mortality upto 2000 mg/kg p.o. dose of *Cicer arietinum* seeds. 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 diabetic rat previously loaded with (2.5 g/kg p.o.) glucose. glibenclamide (5 mg/kg) was used as a standard drug. The maximum reduction in SGL was observed in *Cicer arietinum* seeds (500 mg/kg) group at 6h (145.54±9.62) in acute study and on 21st day (248.59±9.84) in subacute study respectively. In glibenclamide treated mice the maximum reduction in SGL was observed at 6h (194.97 mg/dl) and on 21st day (267.40mg/dl) respectively. *Cicer arietinum* seeds (500 mg/kg) and glibenclamide (5 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 glibenclamide showed antihyperglycaemic activity comparable with glibenclamide.

Keywords: *Cicer arietinum*, alloxan, glibenclamide, tween 80 (5%), ethanol, 5% glucose

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. 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 ethanolic 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. 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 It is reported that the seeds reduced postprandial plasma glucose and are useful in the treatment of diabetes 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
The following drugs were obtained from Alloxan monohydrate (Ozone International Mumbai), Glibenclamide (Ranbaxy Pharma. Ltd. India), ethanol, Normal saline solution 5% glucose. Tween 80 (5%),

Extraction of the Plants
The air dried powder was subjected to hot continuous extraction with ethanol in a Soxhlet extractor and filtered. The filtrate was evaporated at room temperature to concentrate extract. The yield of ethanolic extract of *Cicer arietinum* seeds 8.32 w/w. Weighed quantity 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. The individual extracts of *Cicer arietinum* L which was prepared above were mixed in equal proportions using 5% Tween 80 solution for pharmacological experiments.

Authentication of plant
Cicer arietinum L seeds were collected from local market of chikhli town and authenticate from shri Shivaji Senior College botany department chikhli dist-buldana Maharashtra India,

Animals
Male albino rats were purchased from Crystal biological solution Pune Maharashtra. 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 and water. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) approved 751/PO/Re/S/03/CPCSEA 03.03.2003 of Anuradha College of Pharmacy Chikhli. Dist-Buldana The experimental design was approved by institutional Animal Ethical committee and the study was performed according to the committee for the purpose of control and supervision of experiments on Animals (CPCSEA) guidelines for the use and care of animals.

Toxicity studies
Non-diabetic adult Male albino rats of male sex were subjected to acute toxicity studies as per guidelines (AOT no. 425) suggested by the Organization for Economic Cooperation and Development. The rats 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
The rats were injected alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body weight, intraperitoneally (IP). Since alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution (15–20 ml) intraperitoneally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycaemia.

Chronic treatment model
Rats were divided into six groups of five rats (n = 5) each. Groups 1 & 2 served as control and diabetic untreated control respectively. Group 3, 4 & 5 was treated with the ethanolic extract of *Cicer arietinum* L at of and 100 mg/kg, 200 mg/kg and 500 mg/kg per oral/day. Group 6 served as standard and was treated with 5 mg/kg/day glibenclamide for 21 days. Blood glucose levels and body weight were measured on day 1, 7, 14 and 21 of the study.
The diabetic rats were divided into six groups (n =5),

- **Group I:** Normal control rats received 5% Tween 80 in distilled water p.o.at 5 ml/kg b.w.
- **Group II:** Diabetic control rats received 5% Tween 80 in distilled water p.o.
- **Group III:** Diabetic rats received *Cicer arietinum* seeds 100mg/kg b.w.,p.o.
- **Group IV:** Diabetic rats received *Cicer arietinum* seeds 200mg/kg b.w., p.o.
- **Group V:** Diabetic rats received *Cicer arietinum* seeds 500mg/kg b.w., p.o.
- **Group VI:** Diabetic rats received glibenclamide at the dose of 5mg/kg b.w. p.o.

The administrations of extracts were continued for 21 days, once daily. Blood samples were collected through the lateral tail vein on days 1, 7, 14 and 21 after drug administration and the blood glucose levels were estimated using Accu-check glucometer.
All drugs were given orally by oral feeding needle.

Acute study
Acute toxicity study of *Cicer arietinum* seeds was carried out in rats. It was observed that there was no gross evidence of any abnormalities up to 4 hrs and no mortality was observed in animals up to the end of 48 hours at the maximum tested dose level of 2000 mg/kg b.w. in rats. This was considered as Maximum Tolerated Dose (MTD) and thus, 1/10th of MTD i.e., 100 mg/kg b.w. was taken as test dose and the test dose i.e., 500 mg/kg b.w. was also selected for the experimental studies.

Subacute study
All the animals were administered the respective drugs doses at prefixed time for 28 days. GLs were estimated on 1, 7, 14 and 21 days. At the end of 21 days the drug administration was stopped and a rest period of 7 days was given. The GLs were estimated on 21th day. The data were represented as mean GL ± standard error of mean (SEM).

Body weight
All the rats were weighed daily during study period of 21 days. The body weights were noted and presented as mean change in body weights.

Oral Glucose Tolerance Test (OGTT)
Animals were fasted for 24 hours before experiment but were allowed free access to water. Fasted rats were divided into three groups of 6 animals each (WHO, 1999)

- **Group I:** Control animals received 5% Tween 80 in distilled water at 5ml/kg b.w.p.o.
- **Group II:** 100 mg/kg b.w. of *Cicer arietinum* seeds p.o.
- **Group III:** 200 mg/kg b.w. of *Cicer arietinum* seeds p.o.
- **Group IV:** 500 mg/kg b.w. of *Cicer arietinum* seeds p.o.

After 30 minutes of the treatment to the Groups I, II and III, 2gm/kg body weight glucose was given orally to the animals. Blood samples were collected from tail just prior to glucose administration and at 60, 120 and 180 minutes after glucose loading. The glucose levels were estimated for all the three groups by lateral tail vein puncture method using Accu-check glucometer.

Statistical analysis

Results were expressed as Mean ± SEM. Statistical analysis were performed with Graph pad prism 5 software using one-way analysis of variance (ANOVA) followed by Dunnett's t test. P values less than *p<0.05, p**<0.01, p***<0.001 was considered to be statistically significant, when compared with control and standard group as applicable (Diabetes and Metabolism 1989).

Acute Toxicity Study (OECD Guideline 423)

Animals were fasted prior to dosing, food but not water was withheld overnight. Following the period of fasting, the animals were weighed and test substance was administered. After the substance had been administered, food was withheld for further 3-4 hours. As a dose was administered in fractions over a period, it was necessary to provide the animals with food and water depending on the length of the period. (Ghosh MN, 1984; Turner R, 1965)

Three animals were used for each step. The dose level of the extract to be used as the starting dose was selected from one of the four fixed doses levels 500, 1000, 1500 and 2000mg/kg body weight (Lorke D, 1983). The starting dose levels such that which was most likely to produce mortality in some of the dosed animals. After administration of the test sample, the animals were observed continuously for first four hours for behavioral changes and at the end of 48 hour for mortality, if any.

Result

Table 1: Oral glucose tolerance test

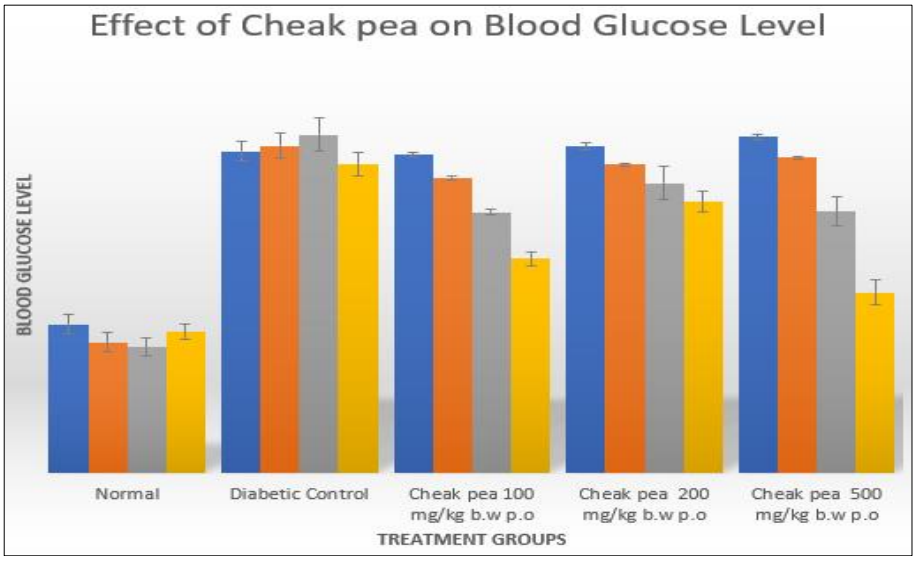
Treatment Groups	Blood Glucose Levels (mg/dL) at			
	O min	60 min	120 min	180 min
Normal Control	93.42±3.87	154.49±3.78	184.64±2.94	241.56±2.44*
Cicer Arietinum 100 mg/kg b.w p.o	86.12±5.92	129.27±6.87**	110.48±7.81**	101.87±4.85**
Cicer Arietinum 200 mg/kg b.w p.o	90.47±4.63	139.61±7.62**	117.32±5.83**	98.23±9.78**
Cicer Arietinum 500 mg/kg b.w p.o	99.83±4.39	129.27±6.36**	107.48±2.93**	96.34±3.91**

Values are expressed as Mean±SEM (n=5) *P<0.05 *P<0.01 was considered significant with respect to control group using ANOVA followed by Dunneet's t-test.

Table 1: Effect of *Cicer arietinum* on blood glucose level

Treatment Groups	Blood Glucose Levels (mg/dL) on			
	Day 1	Day 7	Day 14	Day 21
Normal	120.04±7.40	105.52±8.25	101.31±7.39	113.69±6.39
Diabetic Control	259.09±8.24	263.56±9.84	272.47±13.81	248.59±9.84
Cicer Arietinum 100 mg/kg b.w p.o	256.23±1.69	237.58±2.05*	210.02±2.54**	172.56±5.79**
Cicer Arietinum 200 mg/kg b.w p.o	262.88±2.86	248.21±0.94*	233.22±13.45**	218.48±8.23**
Cicer Arietinum 500 mg/kg b.w p.o	270.48±2.05	253.61±0.90*	210.42±11.89**	145.54±9.62**

Values are expressed as Mean±SEM (n=5) *P<0.05 *P<0.01 was considered significant with respect to control group using ANOVA followed by Dunneet's t-test.



Graph 1: Effect of *Cicer arietinum* on blood glucose level

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, pcoumaric acid, pangamic acid, ciceritol, pseudouridine, pantothenic acid, riboflavin, vit B6, β sitosterol & volatile components has been reported in the seeds**.** The constituents like starch, glucose, fructose, polysaccharides, levulose, γ – galactan, betaine, choline, adenine, inositol, phytin, saponin and citric and oxalic acids are also reported**.** OGTT study indicated that *Cicer arietinum* enhanced glucose utilization in nondiabetic & diabetic mice. Administration of *Cicer arietinum* 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*, *Ficus religiosa* and Psidium guajava 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, starch, sugars, adenine, choline, inositol, phytin, saponin and citric and oxalic acids. In glucose loaded animals, the *Cicer arietinum* 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 *Cicer arietinum* possesses antihyperglycaemic activity. Further study is required to isolate the active constituent responsible for antihyperglycaemic activity.

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