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EVALUATION OF THE ABORTIFACIENT AND ESTROGENIC ACTIVITY OF *CICER ARIETINUM* LEAVES ON FEMALE ALBINO RAT

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ABSTRACT

There is a growing demand for the discovery of new phytoestrogens to be used as safe and effective hormonal replacement therapy. *Cicer arietinum* is an indigenous herb found abundant in India. It is traditionally being used as an abortifacient. Aqueous, alcoholic and chloroform extract of this herb were tested for abortifacient activity in female albino rat from day 11 to 15 of pregnancy at the dose level of 100, 200 and 400 mg/kg body weight. The aqueous extract at a dose of 400mg/kg was found to be most effective in causing significant abortifacient activity. Similarly it was also found to increase the reproductive organ weight and possess estrogenic activity when tested in immature ovariectomised female albino rats suggesting the antifertility activity of the extract.

***Keywords*:** *Cicer arietinum, Abortifacient, Estrogenic activity, Albino rat.*

Number of Table: 03 **Number of References: 22**

INTRODUCTION

The control of human fertility, in the sense of its limitation is the most important and the urgent one amongst all bio-social and medicinal problems, confronting mankind today. Birth-control is a regimen of one or more action, devices, or medication followed, in order to prevent deliberately or reduce the likelihood of a woman becoming pregnant or giving birth. Methods and intentions, typically termed for birth-control may be considered a pivotal ingredient to family planning. Family planning has been promoted through several methods of contraception, but due to serious adverse effects produced by synthetic steroidal contraceptives, attention has now been focused on indigenous plants for possible contraceptive effect. Hence, there is a need for searching suitable product from indigenous medicinal plants that could be effectively used in the place of synthetic pills. Phytoestrogens are plant- derived xenoestrogens functioning as the primary female sex hormone, not generated within the endocrine system but consumed by eating phytoestrogenic plants also called "dietary estrogens," they are a diverse group of naturally occurring nonsteroidal plant compounds that, because of their structural similarity with estradiol (17-B estradiol), have the ability to cause estrogenic or/and antiestrogenic effects (Yildiz, 2005). The plant *Cicer arietinum* Linn. belonging to family Fabaceae is largely cultivated in most parts of India. The plants seed is aphrodisiac, antihelementic and tonic, enriches the blood, and cures skin diseases, inflammation and diuretic. The leaves of this plant are sour, astringent, improves taste and appetite, cures bronchitis, cause flatulence (Kritikar and Basu, 2005).Despite traditional claims no in depth scientific study has been performed regarding its antifertility and estrogenic activity. Thus, the aim of the present study was to validate scientifically the claimed antifertility activity of *Cicer arietinum* leaves extract at different doses.

MATERIALS AND METHODS

Collection of plant material

Fresh plants were collected from adjoining areas of Amravati district and was identified by botanist in the Department of Botany, Bhartiya Science Mahavidyalaya, Amravati and the specimen sample of the same was deposited at the Botanical Survey of India, Pune region, India for authentication and with the voucher accession number (MAWCIA2) for future reference.

Preparation of extract

The plant material were collected, shade dried, powdered and subjected to soxhlet extraction successively with distilled water, alcohol, and chloroform for about 36 hours. The extract was evaporated to near dryness on a water bath. Extract was weighed and kept at 4^0c in refrigerator until the experimental testing.

Phytochemical screening

The presence of various plant constituent in plant extract was determined by preliminary phytochemical screening method as prescribed by Thimmaiah (2004).

Procurement and rearing of experimental animals

Albino rats (Wistar strain) used in the present investigation were procured from Institute of Pharmacology Education and Research Wardha (M.S). The rats were acclimatized for 15 days to the best laboratory condition (prior to experiment). Sexually matured healthy, colony-bred female albino rats, weighing 150- 200gm were used for determining the abortifacient activity, while immature colony- bred female albino rats 21-23 days old, were used for the study of estrogenic activity. The rats were housed in polypropylene cages measuring 12"x10"x8". Animal room was well ventilated with a temperature range of 25-27^{0}C underday/night 12-12 hr photoperiodicity and maintained on balanced diet (Trimurti lab feeds, Nagpur). Water was provided *ad libitum.*

All experimental protocols were subjected to the scrutinization and approval of Institutional Animal Ethics Committee [registration number 1060/ac/07/ CPCSEA (IAEC/7/2009)].

Acute toxicity study

Acute toxicity was carried out as described by Turner (1971). Adult albino female rats were divided into four groups containing five animals in each group. The rats were fasted for 18hrs with water *ad libitum*. The prepared drug was administered orally at three different doses 1000, 2000 and 4000 mg/kg body weight, respectively to different groups of rats separately. Control rats received the vehicle (distilled water) only. The animals were observed for 72 hrs for behavioural changes and mortality.

Abortifacient activity

The plant extract were tested in female albino rats for abortifacient activity by the method described by Khanna and Chaudhary (1968). The vaginal smears of caged female rats of known fertility were monitored daily. Rats found in the proestrous phase of cycle were caged with males of proven fertility in the ratio of 2:1 in the evening and examined the following day for the evidence of copulation. Rats exhibiting thick clump of spermatozoa in their vaginal smear were separated and that day was designated as 1st day of pregnancy. These rats were randomly distributed into 4 groups, a control group and 3 experimental groups of 6 animals each. On the 10th day of pregnancy animals were laparotomised under light anesthesic ether using sterile conditions. The two horns of uteri were examined to determine the implantation sites. The extracts to be tested were then fed to operate pregnant rat's i.e. aqueous extract, alcoholic extract and chloroform extract of experimental plants at dose of 100, 200 and 400 mg/kg body weight daily by an intragastric soft rubber catheter from day 11 up to the 15 day of pregnancy. The animals were allowed to go full term. After delivery the pups were counted and the abortifacient activity of extract was evaluated and litters were examined for any malformation.

Study of estrogenic and antiestrogenic activity

For determine potential estrogenic and antiestrogenic activity the uterine weight and vaginal cornification method was employed for this assay. Colony-bred immature female albino rats, 21-23 days old, weighing between 35 and 45 gm were bilaterally ovariectomised by dorsolateral approach under light anesthetic ether and semi–sterile conditions. They were divided into four groups, consisting of six rats

each. The first group served as a control and received vehicle only (olive oil). The second group received ethinyl estradiol in olive oil (1 ug/rat per day) orally. The third group received the most effective extract only. The fourth group received in addition to ethinyl estradiol, a test dose of effective extract. All the above treatments were given for 7 days. On the 8th day, the rats were sacrificed, the uteri dissected out and surrounding tissue removed. The uteri were blotted on filter papers and weighed quickly on a semi-microbalance. Estrogenic and antiestrogenic activity was assessed according to the method of Edgren and Calhoun (1957) by considering uterine wet weight, opening of the vagina and cornification of vaginal epithelial cells as the points of evaluation. Additionally, the uterine tissue of rats from each group was fixed in 10% formalin for 24 hrs. The tissues were dehydrated and embedded in paraffin. The paraffin sections were cut at 6 um and stained with hematoxylin – eosin for histological observation. The diameter of the uteri and thickness of the endometrium were measured using an ocular micrometer.

Effect on body weight and reproductive organ weight

To study the effect of maximum effective extract on reproductive organ weight and body weight, adult female rats (110-140 gm) were used for the experiment. The animals were divided into two groups consisting of six animals each. Before starting the experiment, weight of rats were noted. Group I served as control and received vehicle only (distilled water). The effective abortifacient drug was administered to rats of group II for 30 days. On the 31st day the final body weight were recorded and the animals were further sacrificed by deep anesthesia, and their reproductive organs i.e ovary and uterus were dissected out and freed from surrounding tissues, blotted on filter paper and weighed quickly on a semi-microbalance.

Statistical analysis

All the data was analyzed to determine the significant difference of results between treated and control groups. The results were subjected to one way analysis of variance (ANOVA) and paired and unpaired student's t-test (Mahajan, 1997).

RESULT & DISCUSSION

Phytochemical screening

Preliminary phytochemical screening of dry powder of *Cicer arietinum* (leaves) confirmed the presence of alkaloids, saponins, flavonoids, steroids and tannins.

Acute toxicity study

Clinical toxicity symptoms such as respiratory distress, salvation, weight loss and change in appearance of hair as well as maternal mortality were not observed at any period of the experiment. Similarly no mortality and changes in the behavior were observed in all treated rats upto a dose of 4000 mg/kg body weight. Hence one-tenth of the doses were used for further testing.

Abortifacient activity

Administration of different extracts of *Cicer arietinum* caused pregnancy interceptive activity in rats. Aqueous extract at a dose of 100mg/kg and 200mg/kg body weight showed 29.54% and 56.86% abortifacient effect respectively, while administration of aqueous extract at a dose of 400mg/kg body weight showed maximum abortifacient activity i.e 83.67%

(Table 1). Similarly the alcoholic extract and the chloroform extract also exhibited significant abortifacient activity but it was less as compared to that of aqueous extract. Significant abortifacient activity was observed at higher dose of all the extracts as compared to lower dose. All the treatments reduced the number of litters born significantly confirming the abortifacient activity of the plant used. The litters born to the experimental animals did not show any morphological defects hence, it can be stated that the treatment does not exhibit any teratogenic effect.

Estrogenic and antiestrogenic activity

The effect of aqueous extract of *Cicer arietinum* leaves on the immature rats uterus is shown in Table 2, 3. Oral administration of the aqueous extract at 400 mg/kg body weight caused a significant increase in uterine weight ($P<0.001$) as compared to control in immature ovariectomised rats. Simultaneous administration of aqueous extract at 400 mg/kg body weight and ethinyl estradiol caused highly significant increase in the uterine weight as compared to control. The degree of uterotrophic response was greater than that produced by ethinyl estradiol alone (Table 2). It also caused highly significant increase in the diameter of the uterus and thickness of the endometrium as compared to the control ($P<0.001$). The uteri of these rats were inflated and fluid filled, resembling the proestrus and estrous uterus. The epithelium of the endometrium was loose and edematous and consisted of spindle shaped cells. The vaginal smear showed predominantly cornified and nucleated epithelial cells. The number of cornified cells in the vaginal smears was considerably higher than that of control, but noteably less than that of the ethinyl estradiol treated animals (Table 2).

Effect on body weight and reproductive organs

A significant increase in the weight of uterus ($P<0.001$) and ovary ($P<0.001$) while a non significant change in the body weight of the animals was observed on administration of 400mg/kg body weight aqueous extract of *Cicer arietinum* leaves for 30 consecutive days (Table-3).

Table 1: *Effect of aqueous, alcoholic and chloroform extract of Cicer arietinum (leaves) on fertility of female rats when fed orally from day 11 to 15 of pregnancy*

Treatment Groups	Body weight (gm)	Drug Dose (mg/kg of body weight)	No. of foetus in individual rats on day 10	No of litters delivered	No of resorption in individual rats	No of resorption in mean ± S.E.	% abortifacient activity
Group- I Control (Vehicle)	150-200	-	7,7,9,8,7,4	6(7,7,9,8,7,4)	0,0,0,0,0,0	0	Nil
Group- II Aqueous extract	170-200	100	8,6,9,8,6,7	6(5,4,6,6,4,6)	3,2,3,2,2,1	2.16±0.31***	29.54%
	150-200	200	9,9,8,10,6,9	6(3,4,2,5,3,5)	6,5,6,5,3,4	4.83±0.48***	56.86%
	140-210	400	10,8,9,7,6,9	6(2,1,2,0,0,3)	8,7,7,7,6,6	6.83±0.30***	83.67%
Group- III Alcoholic extract	190-200	100	8,9,9,7,6,8	6(7,8,8,6,4,7)	1,1,1,1,2,1	1.16±0.16***	14.89%
	150-200	200	8,7,9,9,10,8	6(6,5,8,7,8,6)	2,2,1,2,2,2	1.83±0.16***	21.56%
	140-200	400	9,8,8,9,9,9	6(5,4,5,5,6,4)	4,4,3,4,3,5	3.83±0.30***	44.23%
Group-IV Chloroform extract	150-220	100	9,8,9,8,7,6	6(9,7,8,8,6,5)	0,1,1,0,1,1	2.00±0.21***	8.51%
	140-200	200	8,9,9,7,6,5	6(7,7,8,5,5,4)	1,2,1,2,1,1,	1.33±0.12***	18.18%
	160-220	400	9,10,9,8,9,8	6(6,8,6,6,6,6)	3,2,3,2,3,2	2.5±0.12***	28.30%

Values are from 6 animals in each group, P values: *<0.05, **<0.01, ***<0.001, when compared within group.

Table 2: *Effect of aqueous extract of Cicer arietinum on uterine weight, diameter of uterus and thickness of endometrium*

Treatment Groups	dose (mg/kg B. Wt)	Uterine weight (mg) Mean±S.E	Vaginal cornification (vaginal opening)	Diameter of uterus (um) Mean ± S.E)	Thickness of endometrium (um) Mean ±S.E
I	Control	54.48 ± 1.47	Nil (closed)	353.33±1.36	58.83±2.23
II	Ethinyl estradiol (1ug/rat)	140.33 ± 3.00***	+ + + (open)	636.00±5.66***	244.00±5.75***
III	Aqueous extract (400mg/kg)	106.83 ± 2.9***	+ to + + (open)	583.17±5.02***	96.50 ±2.28***
1V	Ethinyl estradiol) (1ug/rat) +Aqueous extract (400mg/kg)	210.33 ± 3.58***	+ + + (open)	922.50±7.72***	276.50±5.31***

+ nucleated epithelial cells; + + nucleated and cornified cells; + + + cornified cells.

Values are from 6 animals in each group, P values: *<0.05, **<0.01, ***<0.001, when compared between group.

Table 3: *Effect of aqueous extract of Cicer arietinum leaves at dose of 400 mg/kg on body weight and reproductive organs of female albino rats*

Treatment groups	Dose (mg/kg)	Body weight (gm)		Reproductive organ weight	
		Initial	Final	Ovary (mg)	Uterus (mg)
Group- I control	Vehicle	112± 1.83	119 ± 1.85	43.67±2.52	117.33 ± 2.28
Group- II Aqueous extract	400	130.33 ± 1.67	139.33±1.54 ***	59.33 ± 1.41***	210.33 ± 4.92***

Values are from 6 animals in each group, P values: *<0.05, **<0.01, ***<0.001, when compared within group.

Cytotoxic agents can disrupt pregnancy possibly by interfering with the mitotic division of the fetus, chemical insults both before and after the implantation process can result in pre and post implantation embryonic loss (Elbetieha, *et al*., 2000; Goonasekera, *et al*., 1995). Therefore, the increase in the number of dead fetus as well as reduced survival ratio is an indication of the abortifacient activity of the extract. In the present work a high number of dead fetuses suggest a more potent abortifacient activity of the extract. The implantation index, resorption index and post implantation loss are useful indices for evaluating the number of blastocyst implanted in the uterus and the underdeveloped (Almelida and Lemonica, 2000). Therefore, the increase in the resorption index by the extract is an indication of failure in the development of the embryo, a rate which was seen to be dose dependent. Such occurrence of fetal resorption suggests that interruption of pregnancy occurred after implantation (Elbetieha *et al*., 2000).All these are indications of pregnancy terminating potential of the extract of *Cicer arietinum* leaves and may lend credence to the folk reputation of the plant as an abortifacient. Similar observation of resorption of foetus was reported by Shibeshi, *et al*. (2006) following the administration of methanolic extract of *Achyranthes aspera* leaves to pregnant rats and Koneri, *et al*. (2006) on administration of ethanolic extract of roots of *Momordica cymbalaria* Fenzl in rats.

A non significant increase in body weight and significant increase in ovarian and uterine weight can be attributed to the estrogenic activity of the plant extract. (Thakur, *et al*., 2009).

Typical estrogenic compound possess ability to increase the uterine wet weight and induce cornification and opening of vagina in immature rats (Turner, 1971a). It has been reported by Jonathans, *et al*.(1995) that ingestion of phytoestrogenic substances produce effect to those of gonadal steroid 17b-estradiol on reproductive tract of mature and immature rats. Oral administration of 400mg/kg body weight extract of *Cicer arietinum* leaves in immature ovariectomised rats tended to increase uterine weight, indicating that the extract contain estrogen like compounds which also increased other parameters like diameter and thickness of the endometrium as compared with the control.

Safranski, *et al*. (1993) found that vaginal smear characterized by full cornification of

vaginal epithelial cells requires a higher surge of estrogen level. This could explain the appearance of fully cornified cells in smear of rats treated with ethinyl estradiol alone. In the present study it was observed that the alcoholic and aqueous extract potentitated the action of ethinyl estradiol indicated by the significant increase in diameter of uterus and thickness of endometrium. This suggests presence of some components in *Cicer arietinum* leaf extract couldinduce estrogen related changes. Our findings agree with that of Padmashali, *et al*. (2006) and Vasudeva, *et al*. (2008) on administration of *Balanites roxburghii extract* and *Hibsicus rosa-sinesis* root extract respectively on immature ovariectomized rats.

Flavonoids have been reported to possess antifertility activity (Bhargav, 1984; Khanna and Choudhary, 1968; Pincus, 1966; Anderson, *et al*., 1972; Hafez, 1970). In the present study the antifertility activity of the extract may be due to the presence of flavonoids or other constituent in it. On the basis of these observations it may be concluded that aqueous extract of *Cicer arietinum* showing to its estrogenic nature may have altered the biochemical milieu of the uterus which lead to a change in the normal status of reproduction in female reproductive tract of rats and thus produced significant antifertility activity.

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