Evaluation of Antifertily effect of Barleria prionitis roots on female Albino rats.

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Abstract:- Herbal contraceptives are a catch all category for herbs that have an anti-fertility effect. There are many different ways in which herbs can impair fertility. Roots of *Barleria Prionitis* extracts is prepared by using maceration method and checked antifertility activity. In phytochemical activity Barleria prionitis roots show present of alkaloids, glycosides, protein, fats, carbohydrate and tannins. The comparative study on the plant *Barleria prionitis* root for Antifertility effects on female rats were performed. The plant experiment that barleria prionitis are possessing better antifertility activity. The above studies of the root extract of *Barleria prionitis* Linn had a very good Antifertility activity in male albino rats with special reference to testicular cell population.

Key words: Barleria prionitis, Antifertility, Hormone,

Introduction Numerous herbs have been used historically to reduce fertility, and modern scientific research has confirmed anti-fertility effects in at least some of the herbs tested[1]. Herbal contraception may never reach the level of contraceptive protection as the pill, but it offers alternatives for women who have difficulty with modern contraceptive options or who just want to try a different a different way. Very little is known about many of the herbs, or about long term side effects or safety concern[2].

Herbal contraceptives are a catch all category for herbs that have an anti-fertility effect. There are many different ways in which herbs can impair fertility. Some herbs may effects ovary, while others act upon the uterus, affect normal hormone premergency or block certain hormones, of others we really do not understand their action. Some herbs have the ability to interfere with implantation; these herbs can be taken on as needed basis, and are useful as an emergency contraceptive. [3]

MATERIAL AND METHODS

Collection of plant: Roots of *Barleria Prionitis* were collected/ purchased from Ayurvedic Snjeevani Garden Bhopal (M.P). It was identified and Authenticated by DR. Pramod Patil, Professor & Department of Botany of Govt. M.L.B. Girls Autonomous College, Bhopal (M.P.).

Drying and size reduction of plant: The roots were thoroughly washed under running tap water so as to remove any type of contamination. Then air dried in the shade, powdered in grinder and passed through sieve of mesh size no-40.

Extraction of plant material: The Root of *Brleria prints* was collected and shade dried. Coarse powdered and the powder was packed in to soxhlet column and extracted successively with 95% ethanol (75 – 80°c) . The ethanol was concentrated and dried extract was stored in airtight container in refrigerator below 10°c used for fertility model and toxicity study[5].

Fig. No. 9: Dried Root extract of *Barleria prionitis* .

Thin Layer Chromatography: The pharmacological evaluation of antifertlity activity, ethanolic extract was found the most active extract. The activated plate is kept at room temperature and the spot of the Ethanolic extract was applied to one end precoated silica gel plate. Which was eluted by Petroleum ether : Diethyl ester (1:1) Mobile phase for Flavonoids. Developed plates were kept in 10-15 minutes. Now the plate was sprayed with suitable spray reagent for developing the spotes after visualized and scanned under UV light[6].

Than we are calculated the Rf value for Flavonoids: $Rf\ value = \frac{Distance\ traveled\ by\ solute}{Distance\ traveled\ by\ solvent}$

$$= \frac{4.8}{6.2} \qquad = 0.77$$

Fig No.10: TLC of Flavenoids

Phytochemical investigation :

The chemical tests were performed for different chemical groups present in extract[7].

A. Alkaloids

i. Mayer's test

To 2-3 ml of filtrate, few drops of the Mayer's reagent was added. Formation of cream precipitate indicated the presence of alkaloids.

ii. Hager's tests

To 2-3 ml of filtrates, few drops of Hager;s reagent was added. Formation of yellow precipitate indicated the presence of alkaloids.

iii. Wagner's test

To 1-3 ml of filtrate, few drops of Wagner's reagent was added. Formation of reddish brown precipitate indicated the presence of alkaloids.

B. Glycosides

i. General test

Test A: 200 mg of extract were diluted with 5 ml of dilute sulphuric acid by warming on a water bath and filtered it. Then the acid extract was neutralized with 5% solution of sodium hydroxide. Then 0.1 ml of fehling's solution A and B were added until it became alkaline and heated on a water bath for 2 minutes. Noted the quantity of red precipitate formed and compared with that of formed in test B.

Test B: 200 mg of extract was diluted with 5 ml of water instead of sulphuric acid. Then equal amount of water (as used for sodium hydroxide in the above test) after boiling was added. Then 0.1 ml of fehling's solution A and B were added until it became alkaline (test with pH paper) and heated on a water bath for 2 minutes. Noted the quantity of red precipitate formed. The quantity of precipitate formed in test B was compared with that formed in test A. If the precipitate in test A was greater than in test B then glycosides may be present. Since test B represent the amount of free reducing sugar already present in the crude drug, whereas test A represent free reducing sugar plus those related on acid hydrolysis of any glycoside in the crude drug.

ii. Baljet's test

One ml of extract, and added two drops of picric acid. Make it alkaline with sodium hydroxide solution.

iii. Borntrager's test (Anthraquinone)

1ml of benzene and 1ml of 10% ammonia was added with 0.5 ml of extract. Presence of anthraquinone was observed by the formation pink, red or violet color in the lower phase of ammonia.

C. Flavonoids

i. Alkaline reagent test

Take 1ml of extract in a test tube and to this add alkaline solution, formation of yellow colour indicated the presence of flavonoids.

ii. Shinoda's test

In a test tube containing 0.5ml the extract, a small piece of magnesium was added. Then few drops of conc. Hydrochloric acid was added. Formation of pink colour indicated the presence of flavonoids.

D. Carbohydrates

i. Molish's test (General test)

In a test tube containing 2ml of extract, 2 drops of freshly prepared 10% alcoholic solution of α-naphthol was added. Then it was shaken and 2ml of conc. Sulphuric acid was added slides of the test tube. So the violet ring was formed at the junction of two liquids, indicated the presence of carbohydrates.

ii. Fehling's test

To 2ml of extract, equal volume of mixture of equal parts of Fehling;s solution A and B were added and boiled for few minutes in boiling water bath. Formation of red or brick red coloured precipitate indicated the presence of reducing sugars.

iii. Benedict's test

Equal volume of benedict's reagent and test solution were added in a test tube and boiled for 5min in a water bath. Formation of green, yellow red colured precipitate depending on amount of reducing sugar present in test solution indicated the presence of reducing sugar.

E. Foam test (Saponin)

To 1ml of extract was added with 2ml of distilled water in a test tube. The solution was vigorously shaken and observed for the stable froth persistence.

F. Proteins

Biuret's test (General test)

To 1ml of test exract, 4% of sodium hydroxide solution and few drops of 1% copper sulphate solution were added. Formation of a violet red colour indicated the presence of proteins.

G. Tannins

Ferric chloride test

Extract solution were treated with 5% Ferric chloride solution. Formation of blue colour indicated the presence of hydrolysable tannins and formation of green colour indicated the presence of condensed tannins.

H. Fixed oils & Fats

Paper test

Paper gets permanently stains with oil.

5.2.6 Animals

An Albino Wistar femal rats of 150-200gm was used throughout the experiments. The animals were procured from experimental lab. All animals were housed in rectangular polypropylene cages (32×24×16cm, four per cage) kept on racksbuilt of slotted angles and the cage were provided with dust free paddy husk as a bedding material. The animals were housed in environmentally controlled condition of temperature (24 ±1 0 C), relative humidity

(65±10%), light and dark cycle (14:10 h) and fed with standard pellet food and water ad libitum. The norms of Good Laboratory Practice (GLP) were followed for care of laboratory animals.

5.2.7 Acute toxicity: Acute toxicity study of ethanolic extract of *Barleria prionitis* Linn were carried out in rats according to OECD guidelines. Extract at different doses up to 2000 mg/kg, p.o. was administered and animals were observed for behavioral changes, any toxicity and mortality up to 48 h. There was no toxic reaction or mortality, and found to safe. Based on acute toxicity result we have selected 150 mg/kg and 300 mg/kg for antifertility evaluation[8].

Antiimplantation activity

Female rats of proestrus phase were kept with male rats of proven fertility in the ratio of 2:1. The female rats were examined in the following morning for evidence of copulation. The animal which showed thick clumps of spermatozoa in vaginal smear were separated from the male partner and divided into 3 groups (n=6). Animals in the groups I given vehicle only and serve as control. Ethanolic extract of *Barleria prionitis* at 150 mg/kg and 300 mg/kg were administered to group II and group III respectively from day 1 to 7 of pregnancy. The day when spermatozoa detected in vaginal smear was considered as day 1 of pregnancy. All the animals were sacrificed under light ether anesthesia and laprotomy was performed to determine the number of implantation sites on the both uteri horn and the number of corpora lutea on the both ovaries. The fertility rate was calculated by the percentage of implantation per number of corpora leutea (representing number of eggs ovulated)[9].

The percentages of anti-implantation activities were calculated by using following formula:

$$\%\text{of anti-implantation activity} = 100 - \frac{\text{No. Of Implantation}}{\text{No. of Corpora lutea}} \times 100$$

Estrogenic and antiestrogenic activity:

Colony-bred immature female albino rats (Wistar strain), 25 days old, weighing between 30-35 g were bilaterally ovariectomised by dorsolateral approach under light ether anaesthesia under semi-sterile conditions. They were divided into four groups consisting of six rats each. The group I served as control and received vehicle only (olive oil). The group II received ethinyl estradiol in olive oil (1ug/rat/day) orally. The group III received most effective extract at the dose of 300 mg/kg and 150mg/kg body weight. The group IV received, in addition to ethinyl estradiol a test dose of effective extract. All the above treatments were given for 7 days. On day 8, the rats were sacrificed, the uteri were dissected out, and surrounding tissue was removed. The uteri were blotted on filter paper and weighed quickly on an electronic balance and fixed in Bouin's fluid for 24 hours. The tissues were dehydrated and embedded in paraffin. The paraffin sections were cut at 5µm and stained with Haematoxylin-eosine for histological observations of uterus by the methods.

Estrogenic and antiestrogenic activity was assessed according to the method of Edgren and Calhoun, by considering uterine wet weight, opening of the vagina, and cornification of vaginal epithelial cells as the points of evaluation[10].

Identification of Vaginal Smear

The **estrous cycle** (also **oestrous cycle**; derived from Latin **oestrus** and originally meaning sexual desire) comprises the recurring physiologic changes that are induced by reproductive hormones in most mammalian therian females. Estrous cycles start after sexual maturity in females and are interrupted by anestrous phases or pregnancies.

Vaginal smears taken on consecutive days over a period of time can provide detailed information on the oestrous cycle. The normal oestrous cycle in the rat usually follows a 4-day pattern and the varying characteristics of the cells in the smear allow the days of the cycle to be classified relative to the predicted time of ovulation. Ovulation occurs at approximately midnight after the pro-oestrous stage, when the females become receptive to the male. The classic stages of the rat oestrous cycle can be designated as oestrus (E), metoestrus (M), di-oestrus (D) and pro-oestrus (P). The stages of the vaginal cell cycle will normally correlate with changes in the female reproductive organs e.g. ovary and uterus[11].

Pipette smear technique

This method consists of flushing cells from the vaginal lining by introducing a small amount of fluid into the vagina using a pipette and placing one or two drops of the resulting cell suspension onto a slide. A small amount (approximately 0.2 ml) of saline is drawn up into the pipette tip. The rat is held around the thorax, ventral surface uppermost, with one hand whilst the hand holding the pipette is used to restrain the tail, to provide additional support and help prevent the animal struggling. The tip of the pipette is pushed gently into the entrance of the vagina to a depth of 2-5 mm and the fluid is flushed into the vagina and back up into the pipette two or three times by gently squeezing and releasing the bulb of the pipette. A small amount of the cell suspension is then expelled onto a labelled glass slide. cell suspension can be examined without further preparation but the use of cover slips. Examine the vaginal smear under the microscope to know about the proper stage of oestrus cycle. If the rat is not in oestrous , inject 0.1mg/kg of ethinyl estradiol and wait for 24hr[12].

Evaluation of Antiestrogenic activity

Biochemical analysis

The biochemical analysis in adrenal gland and uterus of the treated rats were carried out to know the effect of flavonoid extract on the total protein content, and total cholesterol content of both organs. The total protein and cholesterol content of both organs were estimated[13-19].

Histopathology

The uterus of one side of each animal was fixed in bouins fluid dehydrated by using various grade of alcohol, cleared in toluene or xylene. Embedded in paraffin wax and sectioned at 5µm thickness. The paraffin sections were stained with haematoxyline and eosin for histopathological study[20-22].

Results

Extraction of Plant material

Dried powder of *Barleria prionitis* root's is extracted with ethanol by using soxhlet's apparatus and ethanol was concentrated.

Table No. 1: Percentage of extraction value

S. No.	Colour	State	Weight of dried extract	Etraction value
1	Dark brown	Semi solid	14.68 gm	8.53% W/W

Phytochemical analysis

S.No.	Test	Inferences	Result
Alkaloids			
1	Mayer's test	Cream colour	Alkaloid Present
2	Hager's test	No Yellow colour	Alkaloid absent
3	Wagner's test	Reddish colour	Alkaloid Present
Glycosides			
1	General test	Amount of reducing sugar	Presence of sugar moiety
2	Baljet's test	No Red colour	Cardiac Glycoside absent
3	Borntrager test	Pink Red colour	Anthraquinone Present
Flavonoids			
1	Alkaline reagent test	Yellow colour	Flavonoids Present
2	Shinoda test	Red colour	Flavonoids Present

Carbohydrates			
1	Molish's test	Blue colour ring	Carbohydrate Present
2	Benedict test	Green colour	Carbohydrate Present
3	Fehling's test	No Red Precipitate	Carbohydrate Absent
Saponins			
1	Foam test	Stable foam	Saponin Present
Proteins			
1	Biuret test	Silver mirror precipitate	Protein Present
Phenolic compound (Tannins)			
1	Potassium per magnate test	Give red colour	Tannin present
2	Ferric chloride test	Dark green blue colour	Tannin present
Fixed oils & Fats			
1	Paper test	Paper stain with oil	Oils & Fats Present

Antiimplantation activity

Table No.3: Effect of Root extract of *Barleria prionitis* on anti-implantation activity:

Group and Treatment	Animals used	No. Of Implantation	No. Of Carpora lutea	% of Anti - Implantatio activity
1. Control (Vehicle)	6	12 12 10 12 11 13	13 14 15 12 12 14	12.5%
2. 95% ethanolic Extract 300mg/kg	6	6 3 5 5 4 6	10 6 8 10 7 9	42%
3. 95% ethanolic Extract 150mg/kg	6	5 4 5 6 6 4	6 6 8 7 6 7	25%

Estrogen and Antiestrogenic activity

Table No. 4: Vaginal smear cell types and numbers during the oestrous cycle of rat

Stage	Typical of numbers					Reliability*
	Leucocytes	Neucleated epithelials	Cornified	Non nucleated epithelials	Totals	
Oestrous	-	-/+	+++	+/-	+++	+++
Metaestrous	+++	-	-/+	+	+++	++
Diestrous	++	+/-	+/-	+/-	+/++	+++
Proestrous	-	++	-/+	-	+/++	++

* Probability of stage being seen on expected day in smears taken between 08:00 and 10:00 from females in room with 12: 12 light: dark cycle and lights on at 06:00 GMT

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None of very few

+ Low

++ Moderate

+++ High

1 Based on pipette smears; swab smears often show fewer leucocytes and more cornified at M and D than indicated in this table.

Table No. 5: Estrogenic activity of Root extracts of *Barleria prionitis* in immature female rats:

Values are the Mean S.E.M. of six rats / treatment

Significance $P<0.01$ (n=6) $P<0.001$ (vs. Control).

+ = Nucleated epithelial cells

++ = Nucleated epithelial cells & cornified cells

+++ = Cornified cells

CONCLUSION AND DISCUSSION

The comparative study on the plant *Barleria prionitis* root for Antifertility effects on female rats were performed. The plant experiment that barleria prionitis are possessing better antifertility activity. The above studies of the root extract of *Barleria prionitis* Linn had a very good Antifertility activity in male albino rats with special reference to testicular cell population dynamics which can be attributes the peresnce of phytochemicals such as flavonoids, steroids, saponin, tannins, phenolics and carbohydrates compounds.

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