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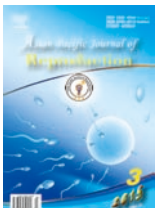

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Antifertility studies of curcumin and andrographolide combination in female rats

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ABSTRACT

Objective: To investigate the potential of curcumin and andrographolide combination by studying its effect on implantation and oestrus cycle. Antigonadotropic and antiestrogenic effects were also evaluated using female rats. **Methods:** Female Sprague Dawley rats (180±30 g) were used for study. They were divided into groups and were treated as per schedule. The antifertility effect of curcumin and andrographolide was evaluated by measuring their effect on implantation and oestrus cycle of rats. Also their antigonadotropic and antiestrogenic effects were evaluated using suitable screening method. **Results:** The combination significantly reduced the number of implants and the size of the litters in rats compared to the normal control group. The combination also significantly altered the durations of each phase of oestrus cycle and synergized the effect to decrease the number of ovarian follicles. **Conclusion:** The results indicate that the combination produces greater antifertility effects and thus can play a vital role in fertility control.

1. Introduction

With the advancement of modern medical science, infant mortality rate has been declined and the average life expectancy is also increased. Progressive increase in birth rate as well as gradual decline in death rate cause a huge population burst in the world. Control on birth rate figures the main basis of the various population control and family welfare programmes[1]. Fertility of humans depends on several factors as nutrition, sexual behaviour, culture, economic condition, lifestyle and emotions[2]. In India, centuries old confidence of rural population in herbal preparation makes a good ground for the use of plant products as contraceptives. Moreover, reports in current literatures regarding the antifertility action of some indigenous plants are quite encouraging[3].

Curcumin is an active constituent found in *Curcuma longa* Linn (Zingiberaceae) (*C. longa*), and is reported to have broad medicinal value and used in the treatment of various ailments. Curcumin has antiovulatory effect probably by its antiestrogenic activity through suppression of negative feedback effect of estrogen on pituitary[1]. On another hand, andrographolide, an active labdane diterpenoid, extremely bitter constituent found in the leaves of *Andrographis paniculata* (Burm. f.) Wall. ex Nees (*A. paniculata*) belonging to family Acanthaceae[4]. Andrographolide can affect the normal growth and development of the ovaries[5,6]. It causes ovarian deformities, variation in the length and size of the ovarioles, ovariole degeneration, oocyte degeneration, resorption and inability of the mature oocytes to oviposite, affects the fertility and the reproductive potential[7].

Report by various workers suggests that both drugs have synergistic effect[8]. Various analytical tests such as UV-Visible, TLC, melting point and FTIR have been performed to establish the interaction. Both were found compatible in combination and have no untoward interaction. The antifertility activity of curcumin is weak and that of andrographolide is good. But curcumin also

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possess anticarcinogenic, antiinflammatory, wound-healing and other activities. Thus, the combination of curcumin with andrographolide may constitute double edged sword to block conception, infection and cancer. So this study was designed to investigate the possible antifertility activity of andrographolide and curcumin combination using female rats. In present study, an attempt has been made to investigate their effect on implantation and oestrous cycle in female rats. Antigonadotropic and antiestrogenic activities of their combination were also evaluated using female rats.

2. Material and methods

2.1. Animals

Female Sprague Dawley rats (180±30 g) were procured from Shree Farms, Bhandara, Maharashtra, India. The rats were acclimatized for seven days, housed in solid-bottomed polypropylene cages and kept under standard husbandry conditions. The rats were fed with standard diet and water ad libitum. The experiments were designed and conducted in accordance with the ethical norms approved by Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India and Institutional Animal Ethics Committee of Institute of Pharmaceutical Sciences, Guru Ghasidas Central University, Bilaspur (994/a/GO/06/CPCSEA, 28/IAEC/Pharmacy/2013).

2.2. Drugs and chemicals

Andrographolide and curcumin were received as gift sample from Dr. V.S. Rana, Senior Scientist, Directorate of Medicinal and Aromatic Plant Research, Boriavi, Gujarat. Alcohol and diethyl ether used were of analytical grade and estradiol (Progynon, Cadila Health Care Ltd, Mumbai, India).

2.3. Pharmacological screening procedure

2.3.1. Anti-implantation effect

Female rats of established fertility were divided in to six groups each consists of six rats, group-I served as control and treated with dimethylsulfoxide (DMSO) as vehicle, group-II and IV were treated with curcumin (10 mg/kg and 20 mg/kg respectively), group-III and V were treated with two different dose of andrographolide (60 mg/kg and 100 mg/kg respectively) and group-VI was treated with a combination of curcumin 10 mg/kg and andrographolide andrographolide 30 mg/kg. Fertility of all the rats were examined by vaginal smear for seven consecutive days for the presence of normal oestrus cycle. At postestrus stage females were paired with males of proven fertility in the ration of 2:1 (female:male). Sign of mating was indicated by the presence of sperm in the vagina in next early morning. Test compounds were administered from first day of pregnancy to 7th day. On 10th day, laparotomy was done and the females were examined for number of implants, number of litters at birth and resorption of fetuses[9].

2.3.2. Pre-implantation effect

One set of experimental pregnant rats were divided into six groups (n=6), and treated with test drugs from day-2 of gestation to day-5. Group-I served as control and treated with DMSO as vehicle, group-II and IV were treated with curcumin (10 mg/kg and 20 mg/kg respectively), group-III and V were treated with two different dose of andrographolide (60 mg/kg and 100 mg/kg respectively) and group-VI was treated with a combination of curcumin 10 mg/kg and andrographolide andrographolide 30 mg/kg. The rats were sacrificed on 10th day of pregnancy, uteri were removed and the number of prominent corpora lutea graviditis and implantation sites were recorded. The frequency of pre-implantation losses was calculated by dividing the missing number of implants (corpora lutea implants) by number of corpora lutea multiplied by 100 [10,11].

2.3.3. Effect on oestrous cycle

Oestrous cycle was determined between 8 am and 10 am using the vaginal smear method. Vaginal secretion was collected with a plastic pipette filled with 10 μL of normal saline. The vagina was flushed three times with the pipette and the vaginal fluid was placed on a glass slide. Different slides were used for each animal. The unstained secretion was observed under a light microscope. After confirmation of regular four day cycle for 2 weeks, the animals were selected for study and divided into six groups and treated with test drugs similar to section 2.3.1. The effect of test drugs on the oestrous cycle was monitored for 28 days [12].

2.3.4. Antigonadotrophic effect

Female rats were studied for five consecutive normal oestrus cycles by vaginal smear method. The rats were anaesthetized using ketamine (60 mg/kg) pretreated with atropine (1 mg/mL), and left side ovariectomy was performed. Left ovary was dissected out carefully from surrounding fatty tissue and dried by soaking on filter paper and weighed. The ovariectomized rats were divided into six groups (n=6), group-I treated with DMSO, group-II and IV were treated with curcumin (10 mg/kg and 20 mg/kg respectively), group-III and V were treated with andrographolide (60 mg/kg and 100 mg/kg respectively) and group-VI was treated with a combination of curcumin 10 mg/kg and andrographolide andrographolide 30 mg/kg. On eleventh day after treatment, the remaining right ovaries of all rats properly dissected out using same anaesthetic condition. Cleaned, dried and their respective

weights were recorded and percentage increase in ovarian weight compared with the weight of the left ovaries were calculated [9]. Percentage increase in the weights of ovary was calculated using the formula [13]:

$$\% \text{ increase in ovarian weight} = \frac{\text{weight of right-weight of left ovary}}{\text{weight of left ovary}} \times 100$$

2.3.5. Antiestrogenic effect

All the rats were ovariectomized by the same methods described in previous procedure and the weight of the ovaries were recorded. The ovariectomized rats were randomly taken and divided in thirteen groups. Except control, other groups were administered with different doses of estradiol (0.1 μg/rat and 1.0 μg/rat) and followed by test compounds respectively for 4 consecutive days. On eleventh day, the rats were anaesthetized using ketamine (60 mg/kg, *i.p.*) and the remaining right sided ovaries were dissected out from all the animals. Properly cleaned, dried and their respective weights were recorded[14]. The ovaries weight variations prior to and after treatment with curcumin, andrographolide and their combination, were calculated. Percentage inhibition of ovarian weight was calculated using the following equation[13]:

$$\% \text{ inhibition in ovarian weight} = 100 \times \frac{1-(XE-C)}{E-C}$$

Where, C indicate mean ovarian weight from rats treated with vehicle, E for estradiol and XE indicates the mean ovarian weight of rats treated with curcumin, andrographolide or curcumin-andrographolide combination and estradiol.

2.3.6. Histopathology

Histopathological studies were performed to measure the effects of curcumin and andrographolide on the number of ovarian follicles. Isolated ovaries were fixed in Bouin's fixative, dehydrated in alcohol. Fixed ovarian samples were embedded in paraffin blocks and five-micron tissue section were cut, mounted on glass slides and stained with hematoxylin and eosin[15]. Samples were evaluated for pathologic changes using conventional light microscopy[16].

2.3.7. Statistical analysis

All the values are expressed as mean ± SEM (n=6). The data of all the groups were analyzed using one-way ANOVA followed by the Dunnett's test. The criterion for the statistical significance was P<0.05.

3. Results

3.1. Anti-implantation effect

Number of litters born in Group-VI and V significantly reduced as (2.16±1.42) and (2.50±1.15) (P<0.01) respectively compared to control. However, group-IV and III respectively produced less significant (P<0.05) reduction in number of litters born, however group-II produced insignificant reduction (Table 1).

3.2. Pre-implantation effect

Group-IV, V and VI significantly inhibited the implantation process by (34.61±1.53)%, (33.41±2.39)% and (37.0±3.75)% respectively. However, group-III and V significantly decreased the number of implants with the mean number of implants of 4.5±2.02 (P<0.05) and 5.66-1.30 (P<0.01) respectively compared to control group (Table 2).

Table 1
Effect of curcumin, andrographolide and their combination on implantation in female rats.

Group	Treatment	No. of implants	No. of litters born	No. of rats showing no implantation sites on day 10	Anti-implantation activity (%)
I	Control (DMSO)	10.16±0.74	10.16±0.75=	0	0.0
II	Curcumin (10 mg/kg)	11.83±1.10	6.66±1.50	0	0.0
III	Andrographolide (60 mg/kg)	6.83±1.44	4.33±1.43*	1	16.7
IV	Curcumin (20 mg/kg)	9.83±2.22	4.66±2.11*	1	16.7
V	Andrographolide (100 mg/kg)	3.83±1.75	2.50±1.15**	3	50.0
VI	Curcumin + Andrographolide (10 mg/kg + 30 mg/kg)	3.33±2.10	2.16±1.42**	4	66.7

Values are expressed as means ± SEM. *P<0.05 and **P<0.01 compared to control group (one-way ANOVA followed by Dunnett's test).

Table 2
Pre-implantation effect of curcumin, andrographolide and their combination in female rats.

Group	Treatment	Mated females	Corpora lutea/ female [A]	Implants/female [B]	Frequency of pre-implantation
I	Control (DMSO)	6	12.66±1.30	11.66±1.17	7.89±1.67
II	Curcumin (10 mg/kg)	6	12.16±2.76	9.33±2.09	23.27±3.03
III	Andrographolide (60 mg/kg)	6	6.00±2.79	4.50±2.02*	25.00±2.64
IV	Curcumin (20 mg/kg)	6	13.00±1.31	8.50±0.84	34.61±1.53**
V	Andrographolide (100 mg/kg)	6	8.50±1.80	5.66±1.30**	33.41±2.39**
VI	Curcumin + Andrographolide (10 mg/kg + 30 mg/kg)	6	12.16±0.70	7.66±0.71	37.00±3.75**

Values are expressed as means ± SEM. *P<0.05 and **P<0.01 compared to control group (one-way ANOVA followed by Dunnett's test).

3.3. Effect on oestrus cycle

The number of days the rats spent in proestrus phase in group II, III, IV and VI was significantly (P<0.01) higher compared to control (group I). Median duration of estrus phase was reduced in groups II, IV and VI (P<0.01). Metestrus phase also declined in groups II, III, IV and V. Diestrus phase was significantly reduced in groups II, IV and VI, but it reduced in groups III and V as compared to group I (Table 3).

3.4. Antigonadotrophic activity

The result indicates that, eleven days after ovariactomy the weight of the right side ovaries of the control group increased to 97.49% compared to left ovaries of control group before treatment. Similarly group-II, III, IV, V and VI produced increase in ovarian weight to 70.81, 50.59, 57.84, 33.39 and 9.2 percent respectively. Group V and VI was found greater significant (P<0.001) declination in the percentage increase of ovarian weight than group II, IV (P<0.05) and III (P<0.01) when compared to control group. Combination dose (curcumin 10 mg and andrographolide 30 mg) showed significant diminution in percentage increase in ovarian weight as compared to all treated group (Figure 1).

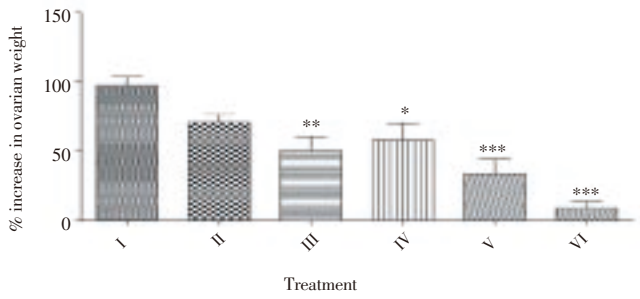

Figure 1. Effect of curcumin, andrographolide and their combination on % increase of ovarian weight in female rats.
Values are expressed as means ± SEM. $^{*}P$<0.05, $^{**}P$<0.01 and $^{***}P$<0.001 compared to control group (one-way ANOVA followed by Dunnett's test).

3.5. Antiestrogenic activity

In this study, effect of curcumin and andrographolide with different dose were administered in combination with different doses of estradiol (0.1 μg/rat and 1.0 μg/rat). Administration of estradiol alone produced significant (P<0.01) increase in the ovarian weight of rats at both doses as compared to control group. Administration of estradiol (0.1 μg/rat) followed by test drugs i.e. group-IV, V, VI and VII produced significant (P<0.01) declination in the increase of ovarian weight, while group-III was found less significant (P<0.05) compared to the increase in ovarian weight produced by group-II alone. The percentage inhibitions produced by group-III, IV, V, VI and VII were 31.72, 80.72, 71.48, 38.95 and 66.66 percent respectively. Similarly, administration of high dose of estradiol (1.0 μg/rat) followed by the administration of same dose of test drugs produced identical level of significance in the reduction of increase of ovarian weight compared with the increase in weight caused by the administration of estradiol (1.0 μg) alone. The percentage reduction in ovarian weight shown by test compounds were 13.22%, 57.41%, 46.45%, 83.57% and 46.77% respectively (Figure 2).

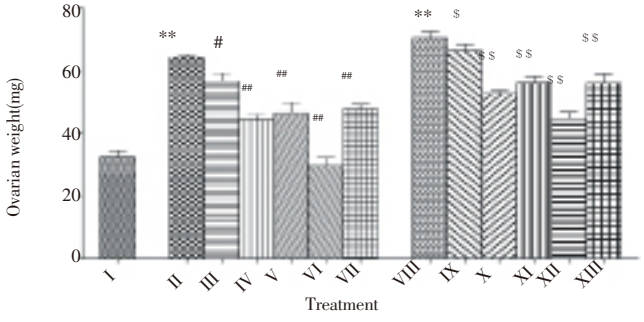

Figure 2. Effect of curcumin and andrographolide treated alongwith estradiol on % inhibition of ovarian weight in female rats. Values are presented as means ± SEM. $^{*}P$<0.05 and **P<0.01 compared with control; $^{\#}P$<0.05 and $^{\#\#}P$<0.01 compared to group-II; $^{\$}P$<0.05 and $^{\$\$}P$< 0.01 compared with E-1.0. Data were analyzed using one-way ANOVA followed by Dunnett's test.

3.6. Histopathology

Number of follicles was counted by using optical dissectors in combination with the fractionator sampling technique. Curcumin at both dose produced significant fall in secondary follicles (P<0.01) while 20 mg/kg dose alone showed significant (P<0.05) decrease in primordial follicles and antral follicles. Andrographolide at dose of 60 mg/kg and 100 mg/kg, produced significant dose dependent reduction in the ovarian follicles (Table 4). However the combination of curcumin and andrographolide showed reduction in primordial follicles (P<0.05) and primary follicles (P<0.01) but didn't produce significant reduction in secondary and antral follicles.

Table 3
Effect of curcumin, andrographolide and their combination on duration of different phases of estrus in female rats.

Group	Treatment	Number of days spent in each phase			
		Proestrus	Estrus	Metestrus	Diestrus
I	Control (DMSO)	2.04±0.10	2.00±0.07	3.49±0.12	9.49±0.18
II	Curcumin (10 mg/kg)	5.38±0.15**	1.47±0.05**	2.88±0.08**	7.33±0.16**
III	Andrographolide (60 mg/kg)	2.58±0.07**	2.05±0.06	1.96±0.10**	10.38±0.16**
IV	Curcumin (20 mg/kg)	6.41±0.07**	1.13±0.05**	2.83±0.08**	7.93±0.14**
V	Andrographolide (100 mg/kg)	1.88±0.05	1.94±0.09	1.41±0.07**	11.74±0.11**
VI	Curcumin + Andrographolide (10 mg/kg + 30 mg/kg)	6.30±0.07**	1.21±0.05**	4.02±0.05**	5.44±0.10**

Values are expressed as means ± SEM. $^{*}P$<0.05 and $^{**}P$<0.01 compared to control group (one-way ANOVA followed by Dunnett's test).

Table 4
Effect on number of ovarian follicles in rats.

Group	Treatment	Primordial follicle	Primary follicle	Secondary follicle	Antral follicle
I	Control (Saline, 1 mL/kg)	24.90±1.88	16.50±1.40	5.60±0.88	13.10±1.36
II	Curcumin (10 mg/kg)	22.00±2.07	16.10±1.47	5.00±0.93**	11.40±0.88
III	Andrographolide (60 mg/kg)	15.50±1.15**	13.30±1.14*	4.90±0.67**	10.30±0.73
IV	Curcumin (20 mg/kg)	17.60±1.75*	15.40±1.37	5.30±0.78**	11.50±0.95*
V	Andrographolide (100 mg/kg)	10.90±1.05**	8.60±1.60**	3.70±0.88**	6.90±1.08**
VI	Curcumin (10 mg/kg) + Andrographolide (30 mg/kg)	14.70±1.45*	12.60±1.46**	5.60±0.37	9.90±0.60

Values are expressed as means ± SEM. *P<0.05 and **P<0.01 compared to control group (one-way ANOVA followed by Dunnett's test).

The administration of estradiol produced dose dependent increase in the number of follicles. Estradiol (0.1 μg/rat) produced significant increase in number of primordial and secondary follicles, but estradiol (1.0 μg/rat) produced highest increase in primordial, secondary, primary and antral follicles. The administration of curcumin to the estradiol treated rats not much affected the follicles count while andrographolide was found effective in reducing the number of follicles which was increased by the administration of estradiol. The combination of curcumin and andrographolide produced significant reduction in the number of ovarian follicle which was increased by the administration of estradiol (Table 5).

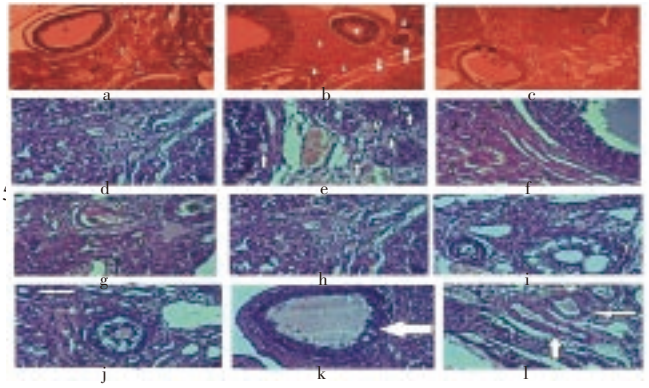

Figure 3. Representation of histopathological study on rat ovaries.

4. Discussion

In present study we evaluated the possible synergistic anti-fertility effect of combination of andrographolide and curcumin using female rats by assessing their anti-implantation and pre-implantation activity, effect on oestrus cycle, antigonadotrophic and antiestrogenic activity.

Normally, the implantation of conceptus occurs on gestation day 4-5 in rodents[17,18]. Chemical insult prior to completion of the implantation process should result in pre-implantation embryonic loss. During this period, a series of changes occur in uterine wall due to synchronized balance of estrogen and progesterone concentration before implantation of fertilized ovum[19]. It is well known that for implantation exact equilibrium of estrogen and progesterone is essential, and any disturbance in the level of these hormones may cause infertility[19,20]. In our study, the combination of curcumin and andrographolide on chronic treatment has shown more anti-implantation potential as significant number of female rats not succeeded to show implantation sites. Their combination significantly decreased the number of implants and litter born as compared to all other treated groups (Table-1), hence we can say that their combination caused significant alteration in the hormonal

Table 5
Effect of curcumin and andrographolide administered with different dose of estradiol on ovarian follicles number.

Group	Primordial follicle	Primary follicle	Secondary follicle	Antral follicle
I	24.90±1.88	16.50±1.40	5.60±0.88	13.10±1.36
II	28.80±1.96*	17.60±1.21	6.40±0.74*	14.60±1.41
III	26.40±1.78	15.30±1.05	6.10±0.76	14.50±1.25
IV	21.60±1.80	13.5±0.94#	5.50±0.71	11.70±0.77
V	16.30±1.26##	11.60±1.06	6.00±0.71	10.30±0.49
VI	14.40±1.29##	11.00±1.81##	4.90±0.99	7.50±0.98#
VII	16.30±0.78##	11.70±0.99	7.30±0.60	8.40±1.02#
VIII	31.20±1.47**	20.90±1.24*	7.60±0.54**	15.90±1.28*
IX	28.00±1.69	17.90±1.02	6.70±0.47	3.4 0±1.02
X	24.40±1.22	14.40±0.89$	5.10±0.62$	11.00±0.91$
XI	20.90±1.45	11.40±0.71	4.10±0.76$$	9.30±1.05
XII	17.40±1.53$$	9.50±1.24$	3.50±1.00$$	9.30±1.29$$
XIII	19.70±1.09$$	10.70±0.63$	2.90±0.83$$	10.10±1.23

Treatments: group I (saline, 1 mL/kg); group II: Estradiol (0.1 μ g/rat); group III: Estradiol (0.1 μ g/rat) + Curcumin (10 mg/kg); group IV: Estradiol (0.1 μ g/rat) + Curcumin (20 mg/kg); group V: Estradiol (0.1 μ g/rat) + Andrographolide (60 mg/kg); group VI: Estradiol (0.1 μ g/rat) + Andrographolide (100 mg/kg); group VII: Estradiol (1.0 μ g/rat) + Curcumin (10 mg/kg) + Andrographolide (30 mg/kg); group VIII: Estradiol (1.0 μ g/rat); group IX: Estradiol (1.0 μ g/rat) + Curcumin (10 mg/kg); group X: Estradiol (1.0 μ g/rat) + Curcumin (20 mg/kg); group XI: Estradiol (1.0 μ g/rat) + Andrographolide (60 mg/kg); group XII: Estradiol (1.0 μ g/rat) + Andrographolide (100 mg/kg); group XIII: Estradiol (1.0 μ g/rat) + Curcumin (10 mg/kg) + Andrographolide (30 mg/kg). Values are expressed as means±SEM. *P < 0.05 and **P< 0.01 compared to control; #P < 0.05 and ##P < 0.01 compared to group-II treated group; $$P$< 0.05 and $$$P$ < 0.01 compared to group-VIII treated group (one-way ANOVA followed by Dunnett s test).

equilibrium. The failure of implantation might be due to antizygotic, blastocytotoxic or anti-implantation activity[21].

We also studied their effect on pre implantation embryonic losses. Our result indicated that higher dose of andrographolide inhibited the corpora lutea formation and number of implants. As gonadotropins are the regulator of follicular development [22] and steroid production in rats [23], so the pre-implantation losses might be due to the direct action of andrographolide on gonadotrophic hormones. Subnormal levels of gonadotropins might have affected oocyte development, maturation, ovulation and corpora lutea formation in rats.

Ovarian hormones are produced by different cell types of the ovary like granulose cells of the mature follicles and the corpus luteum, and imbalance in these hormone leads to irregularity in the ovarian functions and duration of estrus cycle [24,25]. In our result, combination of curcumin and andrographolide produced significant alteration in duration of each phase of oestrus cycle.

The antigonadotrophic effect of curcumin-andrographolide combination was assessed on ovarian weight in unilateral ovariectomised female rats as ovarian hypertrophy might be caused due to direct action of gonadotrophic hormone [5]. Our observations indicated that the treatment with different doses of curcumin and andrographolide significantly decreased ovarian weight in a dose dependant manner. Treatment with combination of curcumin and andrographolide showed synergistic effect on the percent inhibition of ovarian weight as compared to the treatment with curcumin and andrographolide individually. The diminution of ovarian hypertrophy might be due to lesser secretion of gonadotrophins at the level of pituitary or prevention of gonadotrophic hormone release at the target organs.

In another study, antiestrogenic effect of curcumin, andrographolide and their combination on ovarian weight in the estradiol treated rats were evaluated. Prior to ovulation, the role of estrogen is considered to be important in the regeneration and growth of endometrium. The uterine growth responses in rodents are grouped as early and late responses in relationship to a single dose of estradiol [26]. The early responses that usually occur during the first 6 h after administration of estrogen include increase in RNA and protein synthesis as well as water imbibitions. Late estrogen responses include cycles of DNA synthesis and epithelial cell mitosis, which begin 10-16 hour after estradiol administration [14]. Our result indicates that group-III, IV, V, VI and VII, all produced significant inhibition of increase in ovarian weight of rats by estradiol, however andrographolide (100 mg/kg) drastically decreased the ovarian hypertrophy compared to the estradiol treated rats at both dose of estradiol, but the combination (curcumin 10 mg and andrographolide 30mg) produced significant effect at lower dose, showing their synergistic potential to antagonize the effect of estradiol.

During the menstrual cycle, two phases of elevated estrogen concentrations can be distinguished. During the proliferative phase, the growing follicles produce increasing amounts of estradiol that peak at ovulation. Hence it can be said that, the estrogen level depends on the number of ovarian follicles, if number increases the concentration of estradiol increases. After ovulation the corpus luteum continues to produce significant amounts of estrogens, in addition to progesterone [27,28]. Fertilization in female depends on the availability and maturation of the ovarian germ cell, i.e., the oocytes, discrimination and production of ovarian somatic cells, or granulosa and thecal cell[29]. At the time of folliculogenesis, the oocytes grow up and they are bordered by an increasing quantity of granulosa cell layers. From the preantral stage onward, theca cells differentiate outside the follicles[30]. Inappropriate ovarian differentiation or folliculogenesis, due to ovarian regulation defects, results in premature ovarian failure, leading to infertility [31]. The large part of primordial follicles remains inactive, but a few grow and go through conversion into the primary follicle phase. High numbers of preantral and antral follicles would be present from day 9 to 20[32].

Primordial follicles were identified as an oocyte bordered by a sheet of squamous granulosa cells, primary follicles possessed an oocyte bordered by a solo layer of cuboidal granulosa cells. Secondary follicles were surrounded by several of cuboidal granulosa cells, with no visible antrum. Antral follicles possessed a clearly defined antral space[15]. Primordial follicles is required to support normal fertility in the mouse [33,34]. Primary follicles build up receptors to follicle stimulating hormone (FSH) at this moment, but are gonadotropin-independent until the antral stage. Researchers reported that the presence of FSH speed up the follicle growth[15].

We also evaluated the effect on the number of ovarian follicles, and we found that their combination decreased the follicular number with the same level of significance as caused by andrographolide (100mg), this indicates that their combination produced synergistic antiestrogenic and antigonadotropic effect as it has shown the decrease in level of gonadotrophins and estrogen in rats ovary.

Hence we can conclude that curcumin and andrographolide have antifertility potential, however, combination of both drugs has synergistic antifertility effect. The present study confirms that both curcumin and andrographolide play vital role on fertility control in female rats and its combination has synergistic effect in preventing the conception.

Conflict of interest statement

We declare that we have no conflict of interest.

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