Table 6. *F*-values indicating significant difference in herb biomass in canopy types (below-canopy and open) and forest types (eroded and forest sites)

Source of variation	Degree of freedom	*F*-value ($P < 0.01$)
Main effect		
Canopy types	1	181.2
Forest types	1	158.0
Two-way interaction		
Canopy types × forest types	1	185.8

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Clomiphene citrate and its isomers can induce ovulation in laboratory mice

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There is little doubt that clomiphene citrate has emerged as a boon to the specialized area of ovulation induction in the human female. This wonder drug, however, was discovered as an efficient antifertility, antiovulatory drug in laboratory animals. So, the adverse effects observed in clinical use of this compound have not yet been studied well. Numerous uncertainties still remain regarding the mode of action of the compound. One of the worst side effects of this compound is abortion associated with the use of this drug in clinical practice. This aspect is further complicated by the fact that clomiphene citrate is a racemic mixture of two isomers, zuclomiphene and enclomiphene, having individual opposite biological actions. The ovulation-inducing ability was studied in laboratory mice and it was observed that clomiphene citrate and its isomer enclomiphene citrate can induce ovulation and even super-ovulation only in combination with hCG. However, another isomer of clomiphene, zuclomiphene is not capable of inducing ovulation in mice. The standard protocol of ovulation induction for laboratory animals is used and ovulations are checked by oviductal flushing to observe cumulus-bound ova under stereo-microscope.

THE clinical use of clomiphene citrate (CC) had become a major therapeutic breakthrough revolutionizing the science of reproductive endocrinology. It is now often considered as an established therapeutic agent with a well-characterized mode of action in the treatment of anovulatory infertile patients. But still today numerous uncertainties remain regarding the mechanism of action of CC with respect to ovulation as well as the relative role of the other various components of the reproductive axis in this connection. Considerable knowledge gaps still exist regarding the effects of this compound in the female reproduction. This wonder drug was synthesized in 1956 by Palopoli *et al.*[1]. Then all the initial studies were done to establish it as an antifertility agent[2–4]. CC was also reported to decrease the weight of the testis and accessory sex organs in immature and mature male rats[2,5]. In 1961, Greenblatt and coworkers[6] tried to inhibit ovulation in normally ovulating women volunteers by administering this compound, but ovulation was not inhibited. On the contrary, in other trials 28 out of 36 women with oligomenorrhea or secondary amenorrhea responded favourably with CC (ref. 6). Roy *et al.*[7–11] have found that in rats

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CC exerts antifertility, ovulation inhibiting and blastotoxic activities. Docke[12] investigated the ovulation-inducing activity of CC in rats that had been made anovulatory by postnatal androgenization or selective lesions of the suprachiasmatic nuclei. In a different type of study, when CC was administered to intact immature and mature rats for 16 to 39 days, it was observed that CC at 1 mg/kg body weight or higher dose can disrupt the estrous cycle[9]. Roy and coworkers[8,10] were the first to show in animal studies that in lower doses CC increased gonadotropin secretion from the pituitary, whereas in relatively higher doses it suppressed the secretion of gonadotropin. They observed that CC can counteract the ovulation-inhibiting action of estrogen also. But there is no literature available on the ovulation-inducing ability of CC in mice. However in ferret and gerbil, CC suppresses gonadotropin secretions[13,14]. CC consists of two stereochemical isomers, enclomiphene citrate (ENC) and zuclomiphene citrate (ZNC). ENC acounts for its ovulation-induction activity in the racemic mixture and it is more antiestrogenic in comparison to ZNC. ZNC, however, does not have any ovulation-induction property in women[15], but it can get accumulated for a longer time in the human body and it has more agonistic activity than ENC. The racemic mixture of CC used in clinical study contains 38% ZNC and 62% ENC, respectively. It has been noticed that in all the available reports there is no attempt to study the ovulation induction by checking for the ova after treatment with CC or its isomers from the oviductal/uterine flushings. So, it was proposed to study ovulation-induction capabilities of CC and its isomers ENC and ZNC in the mouse model using standard ovulation-induction protocols.

Female virgin adult mice weighing 20–25 g were housed under lighting conditions of 14 h light : 10 h darkness. Only regularly 5-day cycling mice were selected for the experiments. Ovulation inductions were carried out using standard procedures. CC and its isomers were dissolved in DMSO and then diluted in saline. All the three drugs were used at 10 μg or 100 μg dose levels per animal. They were administered as a single injection (i.p.) on D2 of estrous cycle at 6 pm in 100 μl/mice regimen. This was followed by another i.p. injection of hCG (Chorulon, Intervet International B.V. Box meer Holland) at the concentration of 10 IU/mice 48 h later. One group of saline control animals was kept to observe normal ovulation. In another group, five animals were treated with 10 IU hCG 48 h after saline injection on D2 of estrous cycle. The animals were killed by excess ether 16–18 h after hCG injection. After laparotomy, the oviducts were removed and the expanded cumulus masses were released in Medium 199 (Himedia Laboratories, India). The oocytes were dispersed from the cumulus mass with 0.1% hyaluronidase. Then they were counted under a stereomicroscope at low magnification. Ovaries were dissected out and weighed as pairs. Values were expressed in mean ± SEM. They were statistically evaluated by Analysis of variance followed by Schefies F-test. $P < 0.05$ was considered significant.

The vehicle-treated control values showed in the tables were the normal ovulation rates of mice in our colony, which vary from 7 to 10 approximately. CC at the dose of either 10 μg or 100 μg was ineffective in ovulation induction, but along with hCG it could increase the ovulation rate (8.20 ± 0.37 vs 12.00 ± 1.22). CC at 100 μg dose along with hCG resulted in super-ovulation, increasing the number of ova up to 24.20 ± 1.35 (Table 1). ENC showed the same trend as CC when used alone. However, along with hCG, 10 μg dose of ENC could not stimulate ovulation. Yet 100 μg concentration could increase the ovulation when treated along with hCG. ZNC on the other hand, could not produce any increase in ovulation in treated mice (Table 2). The mice treated either with 10 μg or 100 μg ZNC alone or in combination with hCG could not yield any increase in ovulation in comparison to control animals. The specificity of the effects was confirmed when it was observed that hCG alone did not result in any ovulation. CC increased the ovarian weight in all animals irrespective of ovulation or not, except at 10 μg concentrations. ENC in combination with hCG could increase the ovarian weight significantly in comparison to control or ENC alone. ENC at 100 μg increased the ovarian weight non significantly (Table 3). ZUC showed a marginal increase, either alone or in combination with hCG, but it was insignificant because it could not increase ovulation also.

The present data clearly show that CC can induce ovulation at lower doses and super-ovulation in higher doses in mice. This drug is probably the most commonly used ovulatory stimulant in the treatment of anovulatory infertility in human. Paradoxically however, this was originally being developed as an antiovulatory, blastotoxic and contraceptive agent. Originally several investigators observed that CC inhibits reproduction in rats. This inhibition was attributed to the decrease in secretion of gonadotropin[2,3]. Higher doses of CC would cause a suppression of ovarian functions in this manner and it also decreased the content and release of FSH from the pituitary[16–18] and the hypothalamic FSH-RH content. Higher doses of CC caused decrease in plasma LH, pituitary LH and hypothalamic

Table 1. Induction of ovulation by clomiphene citrate (CC) and hCG in mice

Treatment	No. of oocytes per mouse	Ovarian weight (mg)
Control (saline)	08.20 ± 0.37^{a}	04.99 ± 0.23^{b}
10 μg CC	Nil	05.20 ± 0.65^{b}
Saline + 10 IU hCG	Nil	05.22 ± 0.30
10 μg CC + 10 IU hCG	12.00 ± 1.22^{a1}	08.50 ± 0.66^{b1}
100 μg CC	Nil	08.70 ± 0.90^{b1}
100 μg CC + 10 IU hCG	24.20 ± 1.35^{a2}	12.75 ± 0.70^{b2}

Values are mean ± SEM; Values with different superscripts are statistically significant ($P < 0.05$).

LH-RH in rat. Coppola and Perrine[19] reported that high doses of CC suppressed PMSG-induced super-ovulation in rats. They explained this action by possible suppression of LH from pituitary by CC. Docke[12] first reported that rats showing persistent estrus due to induced anovulation showed ovulation with low doses of CC. This result is similar to that of the ovulation induction by CC in anovulatory females. In addition to this evidence for the induction of ovulation, other investigators have shown that CC can stimulate ovulation in pseudopregnant rats. They proposed that the pseudopregnant rat is in a physiological anovulatory state[20,21]. But the experimental protocols of these reports are different from the present study. Our experiments are based on the idea of using CC and its isomers at a very low concentration with a single injection taking into account that lower doses of CC can increase gonadotropin[26] and decreasing the number of treatment days can increase gonadotropin secretion[28]. Initially we have observed that CC showed uterotrophic activity both at 10 and 100 μg concentrations in both the overiectomized adult or immature female mice. When estradiol 17β was combined with similar dose levels of CC, it showed significant antagonistic effects on uterine weight and luminal epithelial cell height[24]. When both the doses were compared, it was observed that 100 μg was more antagonistic than 10 μg in presence of estradiol and similarly the higher dose was more agonistic in the absence of estradiol. So we tried to induce ovulation with both the doses in this study. It is clearly evident from Table 1 that CC alone cannot induce ovulation, but along with hCG it can induce ovulation with 10 μg dose level (12.00 ± 1.22) and super-ovulation (24.20 ± 1.35) with 100 μg dose level. Similarly the ENC isomer which is antagonistic in nature cannot cause super-ovulation to that extent of CC, but 100 μg dose level of ENC shows increase in the ovulation rate (15.20 ± 0.58) significantly (Table 3). ZNC, the agonistic isomer of the CC does not result in any increase in ovulation (Table 2). In another study, it was observed that CC could increase the ovarian weight when treated in unilaterally ovariectomized (ULO) mice at 100 μg concentration in a significant way (intact control : ULO :: ULO + CC at 100 μg :: 1.86 ± 0.69 mg : 4.29 ± 0.52 mg : 6.12 ± 0.55mg) (unpublished data). The data also showed that only at 100 μg dose level could ENC increase the ovarian weight significantly (2.46 ± 0.19 mg vs 4.52 ± 0.18 mg). We tried to measure the FSH level in the blood, however we were not successful enough to quantify it. But we can presume that 100 μg of CC and ENC can raise the FSH level in mice. In this study, induction of ovulation is tried as a single dose administration. So it is a novel way to use CC instead of PMSG in the standard super-ovulation protocol. The mechanism of action of CC in induction of ovulation in mice is not yet worked out[23]. Our results suggest that CC may exert an estrogenic effect on the pituitary and directly stimulates gonadotropin release, which may be independent of GnRH[24,25]. CC at both the dose levels, either alone or along with hCG, could increase the ovarian weights. Present data show an increase in ovarian weight in animals where ovulation occurred. A similar increase of ovarian weight was also noticed in ovaries which did not ovulate. It may be assumed that exposure of CC raised the circulating FSH, which may be manifested in the stimulation of the growing follicles that is reflected in their increase in weights (Table 1). Such prominent stimulation of ovarian weights is not observed in ENC at non-ovulating dose or in ZNC, where ovulation induction is absent. Earlier studies on the direct effect of CC on ovaries are also controversial. Some reports indicate an augmentation of ovarian aromatase activity[27–29]. However, it has been proposed that triphenylethylene drug, CI 628, can decrease the follicular atrasia in rat which is also independent of any increase in gonadotropin[30]. This reduction of atresia by CC may be reflected in an increase in ovarian weight which is observed in this study. However no histological studies are available to justify it.

It may be concluded that clomiphene and its isomer enclomiphene can initiate ovulation even in absence of PMSG, but needs hCG to induce ovulation and super-ovulation due to antiestrogenic and probably progonadotropic activities. Zuclomiphene is unable to induce ovulation due to its estrogenic nature. Further, studies are in progress to evaluate the fertilizability of these ovulated oocytes.

Table 2. Induction of ovulation by zuclomiphene (ZNC) and hCG in mice

Treatment	No. of oocytes per mouse	Ovarian weight (mg)
Control (saline)	8.40 ± 0.50^{a}	5.15 ± 0.23^{b}
10 μg ZNC	Nil	5.34 ± 0.62^{b}
1 ml Saline + 10 IU hCG	Nil	5.36 ± 0.18
10 μg ZNC + 10 IU hCG	8.40 ± 0.24^{a}	6.25 ± 0.55^{b}
100 μg ZNC	Nil	5.80 ± 0.42^{b}
100 μg ZNC + 10 IU hCG	7.20 ± 0.63^{a}	7.62 ± 0.50^{b1}

Values are mean ± SEM; Values with different superscripts are statistically significant ($P < 0.05$).

Table 3. Induction of ovulation by enclomiphene (ENC) and hCG in mice

Treatment	No. of oocytes per mouse	Ovarian weight (mg)
Control (saline)	8.60 ± 0.70^{a}	5.45 ± 0.33b
10 μg ENC	Nil	5.90 ± 0.66^{b}
1 ml Saline + 10 IU hCG	Nil	5.35 ± 0.29
10 μg ENC + 10 IU hCG	10.32 ± 0.89^{a1}	7.80 ± 0.42^{b1}
100 μg ENC	Nil	6.52 ± 0.92^{b1}
100 μg ENC + 10 IU hCG	15.20 ± 0.58^{a2}	10.62 ± 0.40^{b2}

Values are mean ± SEM; Values with different superscripts are statistically significant ($P < 0.05$).

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Divergent structure and composition of the two colliding protocontinents as evidenced from seismic studies

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Coincident reflection/refraction studies across Central Indian Suture (CIS) have yielded crustal structure. The refraction input was confined to a part of the profile, resulting in non-availability of velocity–depth information, an essential component for understanding the composition and rheology of different crustal columns. Using a well-known algorithm of Megallaa, deep reflection data have been utilized in arriving at crustal interval velocity information for the region between Katangi and Kalimati across CIS. It is evident from the present results that the two crustal segments on either side of CIS that were imaged earlier by deep reflection profiling (TWT cross-sections), have significantly different compositions (from velocity information). It is noticed that the north-western crustal segment, belonging to Bundelkhand protocontinent has denser lower crust with a velocity of about 7.0 km/s and relatively normal P_n velocity of 8.1 km/s (for the uppermost mantle velocity). The south-eastern crustal segment belonging to the Deccan protocontinent, while having a similar velocity structure for upper and mid crustal columns (thereby probably similar composition) has a relatively less denser lower crust (with a velocity of 6.7 km/s). It is also interesting to note that the P_n velocity in this segment is only of the order of 7.8–7.9 km/s. Tectonic significance of the results is discussed.

COINCIDENT deep reflection/refraction studies along the 150 km long Mungwani–Katangi–Kalimati profile (Figure 1) have yielded useful information regarding the reflectivity character of the two crustal blocks that are present on either side of the Central Indian Suture (CIS)[1,2]. The limited refraction control with data from two shotpoints, namely SP0 and SP100 has yielded velocity–depth information for the region between Seoni and Katangi. Velocity information in the region between Katangi and Kalimati could not be obtained for want of refraction data. Because of this information gap, even though the divergent reflection fabric on either side of the CIS indicates presence of different crustal segments on either side of the CIS, a meaningful knowledge of the probable composition of different crustal layers (upper, middle and lower) could not be obtained. Since the velocity information is an important input and as the studies across a part of Aravalli

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