DISCUSSION
Plants and plant products find extensive application for their therapeutic properties and development of new and better drugs. Global resurgence required exploration of traditional Indian medicinal plants using modern scientific tools (Singh, 2010).

An impressive array of health promoting, disease preventing and life prolonging properties of *Ocimum sanctum* Linn. have been described and documented over five millennia. In the last few decades, many of these benefits have been investigated and verified by modern scientific research. Several literatures have also shown that this herb could influence reproductive function of mammals (Vohora *et al.*, 1969; Batta and Santhakumari, 1970; Kasinathan *et al.*, 1981; Khanna *et al.*, 1986; Reghunandan *et al.*, 1997; Sardessai *et al.*, 1999). But no detailed work on female reproductive system has so far been reported on antifertility effect of *O. sanctum* leaves.

In view of the above mentioned facts the present study was pursued to understand in some detail the modulation of the fertility control function of *O. sanctum* in experimental rats. The studies have been made in (1) Reproductive behavioural pattern and (2) Endocrine aspects of physiology for fertility regulation. As a consequence determination of *O. sanctum* toxicity, understanding of the LD$_{50}$ dose of the *O. sanctum* extract used and reflection of the doses on some liver enzymes have been studied.

Instead of alcoholic extract water soluble extract was used as it contains easily digestible water soluble constituents of *O. sanctum* leaves. The result with non toxic graded doses (50, 100, 200 and 400 mg/kg body weight/day) of aqueous extract of *O. sanctum* showed no change in gross behaviour i.e., motor activity and open field test improving haemoglobin % and body weight. Therefore *O. sanctum* also enhanced general health and well-being having positive overall effects on the body. (Logambal *et al.*, 2000; Halim *et al.*, 2001). These dose response of the experiment was also supported by Kantak and Gogate (1992). A higher dose of 600 to 800 mg/kg/day produced different toxicity of treated animal.

LD$_{50}$ of the extract for the present experiment was ranged between 720 – 753 mg/kg/day for repeated administration of 14 days. Therefore, the selection of experimental doses were within maximum tolerated doses.
Duration of the treatment for 14 to 20 days were used because the extract showed a minimum lag period for 14 days to show the effects in nonpregnant female rat. As the reproductive changes during gestational period and maternal behaviour continued for 20 to 21 days the drug were administered throughout this period.

Animal behaviour is an integrated phenomena comprising participation of neural, neuroendocrine, endocrine and humoral factors. As the intention of the present work was to understand the effect of the herbal material on fertility control, the reproductive behaviour was also studied as a part of it. Most easy phenomena to be studied is the reproductive behaviour of the experimental rat, which received the herbal extract.

Reproductive organs possess two functional components. One component secrete hormones and other produces gammet. Interstitial cell of Leydig produce testosterone in the male and in female ovarian follicles secrete estrogen, 17β-hydroxy progesterone. Variation of progesterone secretion depends upon the time of ovarian cycle. The small amount of progesterone which is present in the female blood are formed mostly by peripheral conversion from androstenedione which is secreted by ovary. Reproduction depends upon hormonal control in all aspects. Most of the functioning of the hypothalamo pituitary system control the gonadal development and generation of cyclic fashion of reproductive rhythms. This cyclic fashion provides conditions suitable for conception and implantation. Moreover when the function of placenta is established the foetal development become independent of pituitary and ovarian support. But menstrual cycle (Estrous cycle in rodents), ovulation, implantation and early development of zygotes are controlled by the hormones. Reproductive behaviour for example, mating (copulatory) behaviour, pregnancy behaviour and maternal behavioural changes also influenced by neuroendocrine system (LH, FSH, Prolactin etc.).

In the present study treatment with graded doses of aqueous leaves extract of *O. sanctum* significantly increased the duration of estrous cycle with prolonged diestrous and metaestrous stages in normal matured albino rat.

After withdrawal of the extract reversibility of the normal duration of estrous cycle indicating reversible nature of estrous modulatory activity of the *O. sanctum* extract. It is suggested that changes of estrous behaviour after *O. sanctum* extract
administration in presence of ursolic acid probably influence ovarian steroidal hormonal level of experimental rat (Circosta, 2001; Prakash and Gupta, 2005; Wango, 2005).

The *O. sanctum* extract inhibited the copulatory behaviour (Lordosis response) after administration of *O. sanctum* leaves extract to female adult experimental rat and simultaneously decreased reproductive hormones estrogen, progesterone, FSH, LH level. Khanna *et al.* (1986); Kantak and Gogate (1992) suggested that this decreased mating behavioural score in *O. sanctum* treated male was due to reduced gonadal hormone level. Different stages of copulatory (Lordosis) behaviour are a sequence of sensorimotor reflexes and dopamine system in striatum and forebrain (Baum, 1999). Sardessai and coworkers (1999) supported this reduced Lordosis response and suggested that low gonadal hormone on dopamine system suppress this Lordosis behaviour in *O. sanctum* treated animal.

Further, Khanna *et al.* (1986) suggested that inhibitions of the mating response after *O. sanctum* extract administration may result in inhibition of LHRH and LH release, as well as inhibition of progesterone synthesis which was necessary for bringing about mating response. This disturbance in LHRH surge could be responsible for prolongation of estrous cycle and lack of sexual receptivity.

Effective change of the reproductive behaviour for example estrous cycle and copulatory behaviour were observed with 100, 200 and maximum at 400 mg/kg/day doses. Therefore these graded doses were used throughout the biochemical and other experiment work for reproductive functional analysis.

Simultaneous decrease of FSH and LH, regulated by LHRH and estradiol. All reduced substances able to inhibit this release which could provoke estrous cycle blockade, decreased mating behaviour and ovulation disruption by decreasing number of mature follicles in ovaries of treated female rat (Gallo, 1981; Hasimoto *et al.*, 1987; Dalkin *et al.*, 1990; Kage *et al.*, 2009).

The effect of this extract upon cholesterol utilization of ovarian enzymes like \( \Delta^5 \)-3\( \beta \)-hydroxysteroid dehydrogenase and 17\( \beta \) - hydroxysteroid dehydrogenase to synthesize steroid hormones cannot be ignored. Moreover, positive quantitative correlation has been observed between these enzymes activity and steroid hormone
production (Kage et al., 2009). The present result is reflected in significant decrease in enzyme protein level and its activities in ovaries after treatment with graded doses of O. sanctum extract. Simultaneously the decreased level of gonadal hormone as well as LH, FSH could be explained by decreased activities of these enzymes. Ghosh et al. (1990) supported the diminished activities of ovarian enzymes after administration of O. sanctum, may be brought about by reduced synthesis of enzyme protein.

In the present experiment simultaneous decrease in estrogen, progesterone with low ovarian weight and reduced corpus luteum count were observed in O. sanctum treated non-pregnant animal. (Photograph IV.1c). Both volume and weight of uterus significantly increased with deposition of adipose tissues. (Photograph IV.1a and IV.1b).

Luteolysis degeneration of the extract treated ovaries evident by low ovarian weight, low Δ5-3β-HSD and 17-β HSD enzyme activities.

Microscopical structure of treated ovaries showed increased atretic follicles in comparison to control ovaries which also indicating O. sanctum extract induced luteolysis (Photograph IV.2e and IV.2f). Microscopical structure of uterus with folding in lumen lining, congestion, oedema, small non-secretary endometrial glands (photograph IV.2b) with compact stroma indicating low ovarian hormonal influence in endometrium of O. sanctum treated animal.

Ursolic acid, one of the major constituents of O. sanctum leaves has been suggested to poses antifertility effect in rats of both sexes and in male mice. Anti-estrogenic effect of ursolic acid reduces spermatogenesis and causes a decrease in gonadal cell counts (Rajeshwari, 1992). This supports the degeneration of corpus luteum in O. sanctum treated ovaries.

Observation also conducted with O. sanctum extract for normal reproductive function on pregnant albino rat. Result indicated the graded doses (100, 200, 400 mg/kg /day) of aqueous leaves extract of O. sanctum administration in pregnant albino rat produced anti-implantation and abortifacient activity. Graded doses 100, 200, 400 mg/kg /day of O. sanctum administration initiated 16.66%, 33.33% and 50% suppression of pregnancy accordingly and delivered decreased number of Low Birth Weight (LBW) pups. For the ‘interceptive’ effect of the extract autopsy of pregnant uterus on 14th and 20th day of gestation were done. The result indicated utero resorption maximum at 3rd
week, 33.3% and 50% with 200 and 400 mg/kg/day doses of extract administration. Before the onset of parturition low level of progesterone and estrogen probably induced undisturbed parturition. There were no abnormalities in surviving LBW litters. The result indicated that the extract may act as potential antifertility agent rather than its toxic effect (Chattopadhyay et al., 1984).

Simultaneously decreased level of serum estrogen, progesterone, LH, FSH hormones and gonadal Δ5-3β-HSD, 17β –HSD enzymes activity were observed in graded doses of *O. sanctum* extract treated pregnant animal. The pregnancy interceptive effect of *O. sanctum* leaves extract can be interpreted as due to imbalance of steroid for example, estrogen and progesterone in treated pregnant animal. Yadav and Jain (1999), Vasudeva and Sharma (2008) explained that direct command of estrogen-progesterone interplay at cellular level and a slight disbalance of these hormonal level may result in an unfavourable endometrial environment. Observation of Denker (1993), Haimovici and Anderson (1993) supported this antiimplantation effect of *O. sanctum* and suggested that preimplantation losses can also arise due disruption of events that are prerequisite for fertilization or an impairment in the production of cytokines, growth factors and various adhesion molecules either by the developing blastocyst or by uterine epithelium around the site of implantation.

According to early relevant literatures (Rao et al., 1973; Leavitt et al., 1977) estrogen has been credited with its ability to produce uterine progesterone receptor synthesis. So it is assumed that the extract primarily acts in inhibiting uterine uptake of estrogen. Interference with uterine estrogen utilization under influence of the extract is followed by impairment in the estrogen mediated progesterone receptor formation which eventually leads to lower uptake of progesterone (Kabir et al., 1984).

All these reports indicated the possible antiimplantation effect of *O. sanctum* extract in albino rat.

On the basis of the low level of progesterone and decreased corpus luteum count of treated group luteal regression may be held at least as one of the factors responsible for reduction of ovarian weight. Further, significant decreased activity of dehydrogenase enzymes in ovary during pregnancy may be considered as an additional proof supporting functional refractoriness of the luteal tissue. So, it is assumed that extract induced
reduction of corpus luteum may be considered as causal factor behind interceptive effect of the extract (Pakrashi et al., 1986; Kage et al., 2009)

In the present study prolactin level reduced with graded doses of *O. sanctum* extract administration in different reproductive phases i.e., non-pregnant, pregnant and lactating animals. It is suggested that *O. sanctum* extract potentiate dopamine receptor sensitivity which initiate antistress action. This increased dopamine level inhibited prolactin synthesis in *O. sanctum* treated animal (Maity et al., 2000; Ravindran et al., 2005). Hadley (2004) indicated that luteotropic prolactin may act on LH receptor and stimulate corpus luteum for biosynthesis and secretion of progesterone. This low level of prolactin along with reduced FSH and LH level may be associated with decreased progesterone synthesis during different reproductive phases.

Moreover, it has been indicated that prolactin affects the ovarian steroidogenesis by increasing the precursors for androgen biosynthesis, perhaps by regulating the activity of cholesterol ester synthetase (Ghosh, 1991). In the present experiment serum cholesterol level significantly decreased after administration of *O. sanctum*. It is suggested that hypolipidemic action of *O. sanctum* extract may be due to the presence of a major component essential oil eugenol (Rai et al., 1997; Halim et al., 2001). Low level of prolactin in *O. sanctum* treated rat induced reduction of cholesterol level in experimental animal.

In the present study a gradual increase in ascorbic acid content and weight of adrenal gland observed with increased *O. sanctum* doses. This rise is possibly due to antistress property of the herb, which depress ACTH release from anterior pituitary, which in turn decrease corticosteroid and increase ascorbic acid accumulation in adrenal gland. The result also supported by Chattopadhyay et al., 1984 and Sembulingam et al., 1997.

Testosterone is produced in the adrenal cortex of women and is the precursor to the estrogen (Chatterjee, 1991; Hadley, 2004). Result shows serum level of testosterone are equally well suppressed as the level of estrogen during different reproductive phases with graded doses of *O. sanctum* treatment. This supports the observation of increasing level of ascorbic acid in adrenal gland after extract treatment.
In the animal models, the onset of maternal behaviour towards young is not a spontaneous event but rather requires hormonal induction specially estrogen. In rats and other mammals changes in the hormonal milieu during pregnancy have been shown to stimulate the onset of maternal behaviour at parturition (Bridges and Dunckel, 1987; Roy et al., 1999; Champagne et al., 2001).

Female rats, unless they are in late pregnancy or lactating, generally show an aversion toward pups. In normal condition, the relevant hormonal events occur in the latter phase of pregnancy and include an increase in estrogen level at a background of low progesterone level reduces the fear of novelty and facilitates the expression of maternal behaviour in the rat. Estrogen and progesterone exposure during pregnancy sensitize the neural substrate to the subsequent actions of oxytocin and prolactin which act in the brain to induce maternal behaviour (Pederson et al., 1982; Champagne et al., 2001; Aggrawal et al., 2010).

Estrogen is a potent stimulator of prolactin secretion (Amenomori et al., 1970). Further Bridges and Ronsheim (1986) suggested that treatment of steroid-primed female rats with dopamine agonist suppress endogeneous prolactin secretion, which blocks the rapid onset of maternal behaviour. This result indicated that a major behavioural function of estrogen exposure during pregnancy and specially prepartum is to stimulate prolactin secretion. These data suggest that prolactin has a much or more of a direct action than does estrogen in stimulating the rapid onset of maternal behaviour at parturition. Present result showed that low estrogen level in O. sanctum treated rat stimulate dopamine level which cause the decreased production prolactin.

Prolactin also has a role to increase activity of Δ5-3 β-HSD and 17 β-HSD (Ghosh, 1991). So, the protection of ovarian Δ5-3 β -HSD and 17 β-HSD activities were hampered which causes reduced production of estrogen and progesterone throughout lactation period.

Therefore, the O. sanctum treated animals showed no facilitation of maternal behaviour. Score of these behaviour (i.e., pup retrieval and grouping, crouching over young, alertness on hearing squealing of pups, nursing and biting tendency of cage wire etc) reduced 7.11%, 10.05%, 21.32% at first week and 4.46%, 28.77% and 31.85% at 3rd
week of lactation with graded doses of 100, 200 and 400 mg/kg/day of *O. sanctum* extract.

Maternal behaviour although influenced by hormonal condition but sensori stimuli provided by the offspring are also important for its maintenance (Sosa, 1980). Indeed the maintenance of maternal behaviour is thought to become hormonally independent overtime. What is interesting to consider here is the potential influence of the behaviour on the neural mechanisms that maintain maternal behaviour (Rosenblatt, 1994).

Maternal licking/grooming is associated with increased activity in the mesolimbic dopamine system (Shahrokh *et al.*, 2009). In the present study depression of ‘nursing posture’ and aversion towards pups provided reduced suckling reflex, which cause disruption of lactation process during postpartum period and progressive low weight gain and muscle wasting of the litters during 3 weeks of lactation.

Therefore all the reproductive changes mentioned in the present study is due to depressed steroidogenesis as well as prolactin synthesis in female albino rat with *O. sanctum* treatment. The molecular mechanisms by which this drug brings about decline in pituitary gonadotropins other than negative feedback mechanisms is yet to be investigated. It may be assumed that the indigenous herbal leaf extract of *O. sanctum* could modulate the synthesis and release of these gonadotropins and alter the reproductive behavioural function i.e. estrous cycle, copulatory behaviour, gestational behaviour, maternal behaviour and the level of hormone and enzyme activities.

All these data considered together suggest a possible role of *O. sanctum* in fertility control and reproductive behavioural homeostasis. This noble approach in the fertility research needs further follow up.