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UTERINE PEROXIDASE ACTIVITY DURING PREIMPLANTATION PERIOD IN RAT: EFFECTS OF CENTCHROMAN, A NONSTEROIDAL ANTIESTROGEN

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ABSTRACT

Peroxidase enzyme is a major estrogen–regulated glycoprotein in uterus. It catalyzes conversion of estrogen to catechol estrogens and associated with uterine receptivity. Present study was conducted to determine the uterine peroxidase activity in adult rats (B. Wt. 180-200 g) administered centchroman (CCN; 1.25 mg/Kg; p. o.) or a vehicle on day 1 *post-coitum (p.c.)* and autopsied between 10:00-11:00h from days 2-6 *p.c*. Results on cellular localization of uterine peroxidase activity showed strong staining intensity in endometrial exogenous eosinophilic leucocytes and blood vessels on day 2 *p.c*., which increased further from days 3-5 *p.c*., with maximal enzyme activity on day 5 *p.c*. in entire endometrium (including uterine exogenous/endogenous cells and blood vessels). Biochemical estimations of uterine (soluble/Ca2+-extracted) peroxidase activity revealed an increasing trend from days 3-5 *p.c*., with its maximum on day 5(10:00 h) *p.c*. in control rats. In contrast, enzyme activity (in uterine frozen sections/Ca2+-extracted fraction) declined following post-sensitivity period (on day 6 *p.c*.). Post-coital treatment of CCN (1.25 mg/kg) produced a localized strong staining reaction in endometrial eosinophilic leucocytes on day 2. But, caused significant decrease in uterine (sections/tissue extracts) peroxidase activity on days 3, 4 and 6 as compared to corresponding controls. Uterine peroxidase activity did not show any significant change on days 5 and 6 (in soluble fraction) except its insignificant increase on day 5 coinciding with eosinophilic leucocytes activity and plasma peroxidase level in treated-rats. In contrast, uterine ligation (on day 1 *p.c*.) caused significant inhibition in Ca2+-extracted peroxidase activity on days 5 and 6. In immature ovariectomized (OVX) rats. CCN

treatment (0.25 or 1.25 mg/kg for 3 days) in combination with E2 (1 or 10µg), caused significant decrease in uterine Ca2+-extracted peroxidase activity as compared to E2 treated rats. Findings indicate- (1) Highest peroxidase activity in relation to maximal endometrial sensitivity, (2) Correlation between uterine soluble peroxidase activity and localized staining intensity in endometrial eosinophilic leucocytes related to non-genomic estrogen action, whereas, Ca^{2+}-extracted peroxidase activity of endogenous (stromal/epithelial) cells related to genomic response, (3) Inhibition of uterine (fractions/ sections) peroxidase activity on days 3, 4 (pre-sensitivity) and 6 (post-sensitivity) by CCN treatment may be due to inhibition of estrogen action. Its insignificant increase on day 5 (10:00 h) coinciding with increased plasma peroxidase level in treated-rat, may be due to weak inherent estrogenicity of this compound, (4) Inhibition of enzyme activity by uterine ligation may suggest the role for local embryonic estrogen/peroxidase activity in uterine sensitivity.

KEYWORDS: Peroxidase activity, endometrial sensitivity, centchroman, Rat.

INTRODUCTION

Blastocyst-implantation, one of the crucial phenomenon in mammalian reproduction requires timely endometrial sensitization responsive to blastocyst signal(s) for decidualization.[1-4] Hormonal (estrogen and progesterone) control of uterine growth and proliferation, and differentiation into decidua has been shown to be mediated by cytosol-nuclear–receptor system (a genomic response) which induces a cascade of events that exert changes at morphological, physiological, biochemical, histochemical and molecular levels during pre-implantation and early pregnancy period in uterine (stromal/epithelial) compartments of mammals including rodents.[5-16] On the other hand, nongenomic (early) response of estrogen action has been shown to be mediated by eosinophil-estrogen-receptors and evidenced by the accumulation of exogenous eosinophilic leucocytes in uterus.[17-19] This uterine accumulation of eosinophils has been further demonstrated to be controlled by estrogen-regulated genes[20] or chemo taxis.[17,21]

Uterine peroxidase is a major estrogen–regulated glycoprotein in uterine fluid and epithelium as evidenced by immunofluorescence[22], histochemical, biochemical[23-29] and Electron microscopy studies.[26] Further, it has been considered as ‘marker’ protein for mapping of estrogen action in endometrial exogenous eosinophilic leucocytes cells. Its release into uterine stroma functions for remodeling of endogenous uterine stromal cells in immature and mature rats.[18,23,28,30-32] Moreover, peroxidase enzyme has been shown to play a role as a

catalyst in the conversion of estrogens to catechol estrogens which are involved in uterine vascularity[33] and in embryo-implantation processes.[34] The antiestrogens have been shown to inhibit the estrogen (E2)-induced uterine peroxidase activity indicating possible inhibition in non-genomic and genomic responses of estrogen action.[9,28,35,36] Inhibition in uterine peroxidase activity by receptor antagonists are estrogen–receptor-mediated biological activity has been demonstrated in short term in vivo assays for estrogenicity in rat and mouse.[37]

Present study was undertaken to determine the effects of Centchroman (CCN, 1.25mg/kg; p. o., on day 1 *p.c.*), on uterine estrogenic 'marker' enzyme, peroxidase during pre-sensitivity (day2-3 *p.c.*), maximal endometrial sensitivity (day 5 *p.c.*) and post-sensitivity (day 6 *p.c.*) periods so as to explore the role of this enzyme activity in endometrial receptivity to blastocyst-implantation. CCN, a triphenylethylene antiestrogen with weak inherent estrogen agonistic activity[38,39] has been shown to inhibit endometrial sensitivity and blastocyst-implantation via inhibition of estrogen action but not the secretion of nidatory estrogens between day 4(22:00h)-day 5(10:00h) in rats.[40] In addition, uterine peroxidase activity was also analyzed in ligated rats to explain more precisely the role of local estrogen of blastocyst-origin in blastocyst-uterine-attachment reaction. Estrogenic/antiestrogenic mode of action of CCN was also studied in immature OVX rats using peroxidase enzyme as a 'marker' enzyme activity for estrogen action.

MATERIALS AND METHODS

Chemicals and Reagents

All the require chemicals for histological, histochemical and biochemical studies were purchased from Sigma Chemical Co., USA; BDH and Qualigen Fine Chemicals, Bombay, India. Indigenous chemicals were of analytical grade.

Animals and tissue collection

Adult Sprague-Dawley rats (body weight 180-200 g) received from Institute's breeding colony were maintained under standard conditions (at 22 ± 1^0C; 12h light: 12h dark periods). Females caged overnight with coeval males of proven fertility (3:1) and vaginal smears were examined following next morning. Rats with sperm-positive smears considered as day 1 *post-coitum (p.c.)* were randomized, treated with a single anti-implantation dose of CCN (1.25 mg/Kg, p.o.)[40] or vehicle (oral suspension of gum acacia in distilled water) on day 1 *p.c.* Animals were fed with a standard diet supplied by Lipton India Ltd., Bangalore, India and

free access to tap water. Autopsy was done between 10:00-11:00 h on days 2, 3 and 4 (pre-sensitivity), 5 (maximal endometrial sensitivity) and 6 (immediately post-sensitivity) *p.c*. The uteri from individual rats were dissected out, washed immediately in chilled physiological saline, freed off connective tissues and blood clot, and kept at -70^0C until biochemical assay. For histochemical and histological studies the middle portions (5-7mm) of the uterine horns were cut and fixed immediately in Cryoform embedding medium (IEC, USA) at -20^0C and in Bouin's fluid (24h), respectively. Total of 150 rats were used in Experiment I, containing 15 rats each day, out of which 12 rats (6 rats/day for control and treated groups) were used for biochemical estimations, and 3 rats for histochemical localization of peroxidase enzyme activity.

In second experiment, 27 rats were ligated bilaterally at utero-tubal-junction (UTJ) on day 1 *p.c*. to prevent entry of native embryos into uterine horns[7] under light anesthesia (solvent anesthetic ether) and 9 rats/day (out of which 3 rats used for histochemistry) autopsied on days 4, 5 and 6 *p.c*. so as to explore the role of local embryonic steroid (estrogen) involved in implantation process using peroxidase activity as 'marker' for estrogenic action.

In third experiment, immature rats were bilaterally ovariectomized (OVX) surgically in sterile conditions under light anesthesia (solvent anesthetic ether). After a rest period of 7 days, rats were randomly divided into various groups. Total of 72 (36 for biochemical analysis, 36 for localization studies) rats were divided into six groups containing 6 rats each and treated with estradiol (E2; 1 or 10µg/rat, i.m.), and CCN (0.25 or 1.25 mg/kg, i.m.) alone or in conjunction with estradiol for 3 days. Determination of uterine peroxidase activity was carried out during the estrogenic and antiestrogenic mode of action of this compound.

Localization of peroxidase activity

Cryostat (-20^oC) sections from immature and adult rats uteri cut at 8 µm were mounted on microscopic glass slides and incubated in a solution containing 2.7mM 3,3'-diaminobenzidine (DAB Sigma, USA) and 8.8 mM H_2O_2 in 0.1M Citrate Buffer (pH 5.6) at 20^0C for 1h and lightly counterstained with 0.1% methyl green as per method described.[28] Control sections were incubated with substrate-deficient medium served as standard (Std.) control.

Extraction of uterine peroxidase activity

Uterine horns from individual rats were immediately frozen in liquid nitrogen, pulverized to a fine powder and subsequently homogenized (100mg/ml) in ice-cold Tris-HCl buffer (pH7.2) using glass homogenizer. Homogenates were centrifuged (Beckman's ultracentrifuge model L8-70MR, USA) at 40000xg for 40 minutes at 4^0C and the supernatant (soluble fraction) separated out. Pellets were rehomogenized in the same buffer containing 0.5M $CaCl_2$ to solubilize the peroxidase present in the particulate fraction and centrifuged at 100,000xg for 40 min at 4^0 C, the supernatant represented Ca^{2+}-extracted fraction.

Ortho-Phenylenediamine assay for peroxidase activity

Uterine peroxidase activity was determined as per method described by Farley et al.,[33] using the substrates H_2O_2 and O-phenylenediamine. Briefly, Uterine fractions (soluble and Ca^{2+}-extracted) adjusted to 0.25M $CaCl_2$ I^{-1} immediately before assay and aliquots of 100 µl were mixed in a reaction mixture containing [650 µl of 154 mM sodium citrate buffer (pH5.0), 200µl O-phenelynediamine solution (75mM l^{-1}), and 50µl H_2O_2 (100mMl^{-1}) to make a volume of 1ml. Reaction mixture was vortexed and incubated for 30 minutes at 25^0C. Then the reaction was stopped by adding 1ml of 2M HCl before reading the absorbance (at 496nm) in spectrophotometer (Spectronic 3000, Model CM-335, Beckman's Instruments, Roy Co., and USA). The uterine extracts in each rat were assayed in duplicate at three dilutions. A Standard Curve was obtained with Horseradish peroxidase to determine the enzyme activity; protein was estimated[41] in different uterine fractions and results expressed as horseradish peroxidase (ng)/mg protein.

Determination of peroxidase activity in Blood plasma

5ml of blood was collected by injecting the sterilized syringe into the heart in lightly anesthetized (solvent ether) rats. Out of which, 0.5 ml of blood was used for WBC counting and remaining blood (4.5 ml) allowed to stand for 30 minutes and centrifuged at 2000xg for 5 minutes. Peroxidase activity[33] and proteins[41] were determined in the supernatant fraction of peripheral blood.

Statistical analysis

Statistical analysis was done by Student's "t" test for significance level between control- and treated-animals and values are expressed as Mean ± SEM.

RESULTS

Peroxidase activity in adult rat

Pre-sensitivity period: Localized deposition of DAB-H_2O_2 reaction product for peroxidase activity in uterine fresh frozen sections indicated strong staining intensity in endometrial exogenous eosinophilic leucocytes and blood vessels, whereas, weak staining intensity was observed in endogenous stromal cells/uterine (luminal and glandular) epithelium on day 2 *p.c.* (Fig. 1A), which increased further on days 3(Fig. 1C) and 4 (Fig. 1E) *p.c.*, with maximal staining intensity in stromal eosinophilic leucocytes on day 4 (11:00h). Peroxidase staining intensity in uterine epithelium, stromal blood capillaries and leucocytes cells in muscularis (serosa and myometrium) region also showed increase on days 3 and 4 (Fig. 1 C & E) in vehicle control rats.

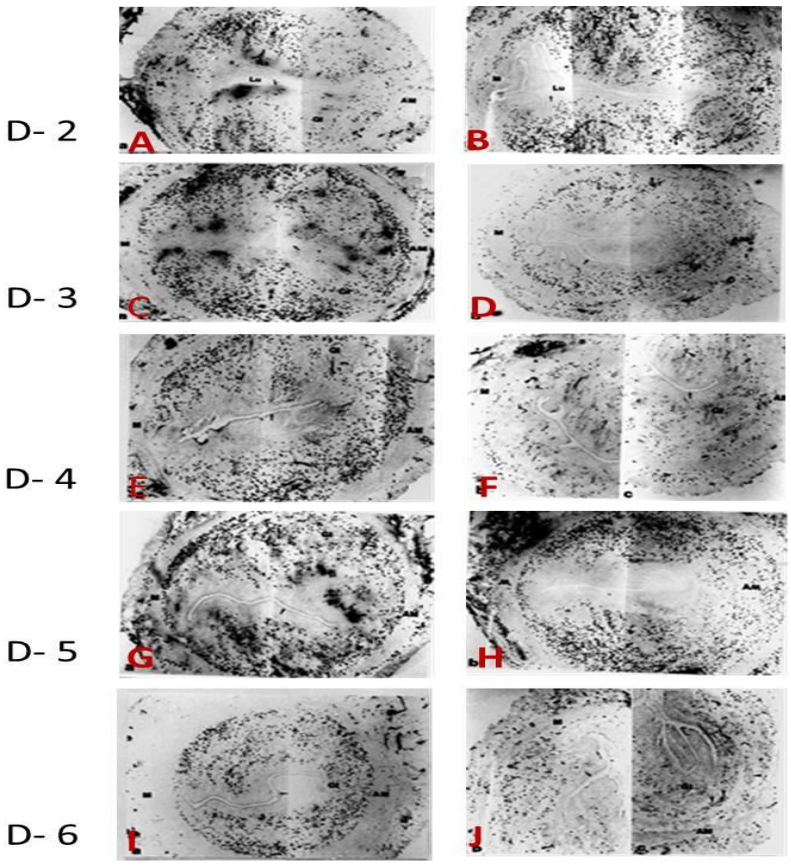

Figure 1: Localization of peroxidase enzyme activity in uterine fresh frozen sections (8µm) from days 2-6 (D2-D6) *p.c.* in rats. Note the strong staining intensity in uterine eosinophilic leucocytes and blood vessels, and weak in uterine epithelium on day 2 *p.c.* (A). Note the further increase in enzyme intensity on days 3(C) and 4(E), with maximum intensity on day 5(G)p.c. On day 6 (I) enzyme activity decreased as compared to day 5(G) in control rats. In CCN-treated rats, note the strong staining intensity in

endometrial eosinophils on day 2(B). But, decreased staining intensity can be seen on days 3(D), 4(F) and 6(J) in CCN-treated as compared to control rats. On day 5, CCN treatment (H) shows peroxidase positive uterine eosinophils similar to control. LU: Luminal epithelium, Gl: Glandular epithelium, BV: Blood vessel, AM: Antimesometrial and M: Mesometrial sides of uterus. [DAB-H_2O_2/ methyl green staining; All figures are microphotographed at x 40 magnification].

Period of maximal endometrial sensitivity: Period of maximal endometrial sensitivity (day 5 *p.c.*; 10:00h) was characterized by a marked increase in staining intensity of peroxidase activity in endometrial endogenous- and exogenous leucocytic-cells, blood vessels/capillaries and in muscularis region in control rats (Fig. 1G). While, in uterine (glandular and luminal) epithelium displayed weak peroxidase staining intensity on day 5 than in day 4 *p.c.*

Post-sensitivity period: In rats autopsied immediate to post-sensitivity on day 6 (11:00h) *p.c.*, revealed decreased enzyme activity in endometrial stroma than that of day 5 *p.c.* except positive staining in leucocytic cells and uterine epithelium (Fig. 1I). By day 7, enzyme activity decreased in entire endometrium (in inter-implantation and implantation sides) than in day 6 *p.c.* rats.

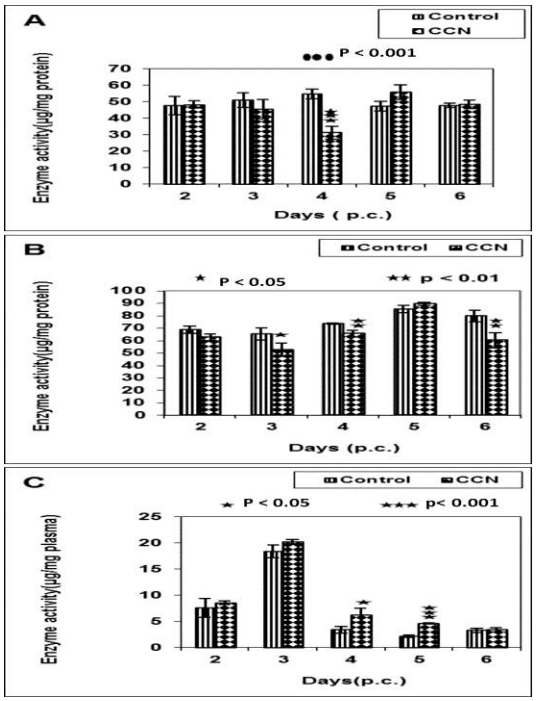

Figure 2: Uterine peroxidase activity in soluble (A) fraction, microsomal(Ca^{2+}-extracted) (B) fraction, and in blood plasma (C) in rats (control and CCN-treated) autopsied on days 2, 3, 4, 5 and 6 p.c. (* p< 0.05; ** p< 0.01; *** p< 0.001).

Biochemical results obtained by o-phenelynediamine assay using Horseradish peroxidase (HRP) as a standard, showed an increase in uterine (soluble/Ca2+-extracted fractions) peroxidase activity from days 3-5 *p.c.*, with maximum activity on day 5 (10:00h). In contrast, enzyme activity decreased following post-sensitivity (day 6 *p.c.*) period in both soluble (Fig. 2 A) and Ca2+-extracted (Fig. 2 B) fractions in control rats. In blood plasma, peroxidase enzyme activity was observed to be increased on day 3 *p.c.* as compared to day 2, but further declined on days 4, 5 and 6 *p.c.* in control rats (Fig. 2 C).

CCN treatment

Post-coital treatment (1.25 mg/Kg; p.o., on day 1 *p.c.*) of centchroman exhibited localized increase in staining intensity of peroxidase activity in uterine exogenous leucocytic cells on day 2 (Fig. 1B), but caused significant decrease in its activity on days 3 and 4 as evident in uterine frozen sections (Fig. 1 D, F) or in uterine (soluble/Ca2+)-extracts (Fig. 2A, B) as compared to controls. Uterine epithelium (glandular and luminal) did not show any significant change in its staining intensity on days 2-4 (Fig. 1 B, D, F) in treated rats. Inhibition of maximal endometrial sensitivity on day 5 (10:00h) by this antiestrogen, did not cause any significant change in uterine peroxidase activity but, displayed increased staining intensity in exogenous leucocytic cells/endogenous epithelial cells (Fig. 1H), and insignificant increase in its activity in both uterine (soluble/Ca2+) fractions (Fig. 2A, B). In contrast, enzyme activity declined on day 6 (10:00h) in endometrial stroma (Fig. 1J) or in Ca2+-extracted fraction in treated-rats (Fig. 2B). In blood plasma, peroxidase enzyme activity increased significantly on days 4 and 5 in treated-rats (Fig. 2C) as compared to control rats.

Uterine ligation

In ligated (at utero-tubular-junction, on day 1 *p.c.*) rat, a localized decrease in peroxidase enzyme activity was observed in uterine stromal exogenous eosinophilic leucocytes but, exhibited strong staining intensity in uterine (luminal/glandular) epithelium on day 5 (Fig. 3B) as compared to control (Fig. 3A). Enzyme activity estimated in uterine soluble fraction show insignificant decrease on this day. But, caused significant decrease in Ca2+-extracted enzyme activity on days 5 and 6 (Fig. 4 A, B). While, peroxidase staining intensity in endometrial leucocytes cells (Fig. 3 D) and uterine soluble peroxidase activity (Fig. 4A) showed increase on day 6 in ligated rat. On day 4 of pre-sensitivity period, uterine ligation caused a significant increase in uterine Ca2+-extracted peroxidase activity; it did not cause

any significant change at blood plasma peroxidase level from days 4-6 as compared to controls (Fig. 4C).

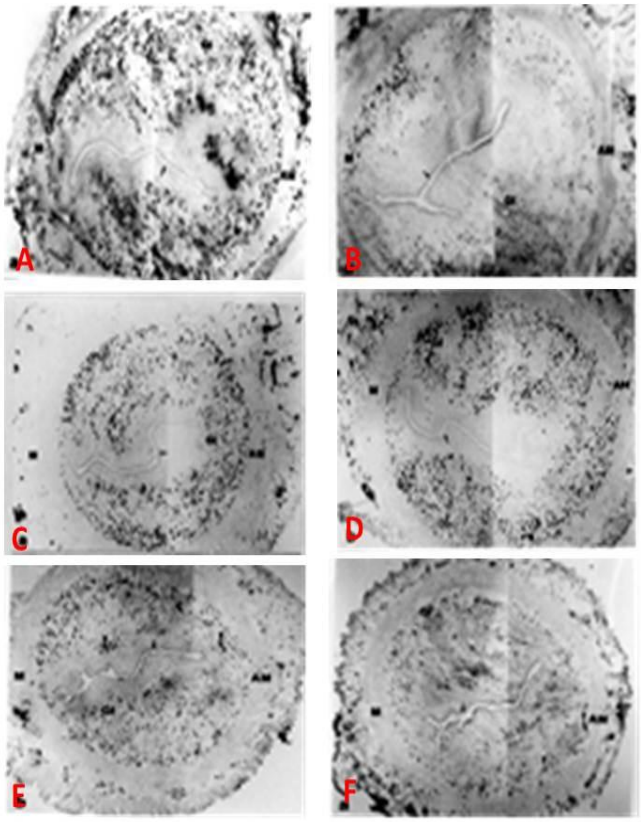

Figure 3: Uterine cross section of ligated rats showing decreased peroxidase staining reaction on day 5(B) as comapared to control (A). On day 6(D), it did not show any marked change in enzyme intensity as compared to control (C). However, on day 7, ligated rat uterus (F) showed decreased enzyme activity compared to non-ligated uterine horn (E). [DAB-H_2O_2/ methyl green staining; All figures are microphotographed at x 40 magnification.

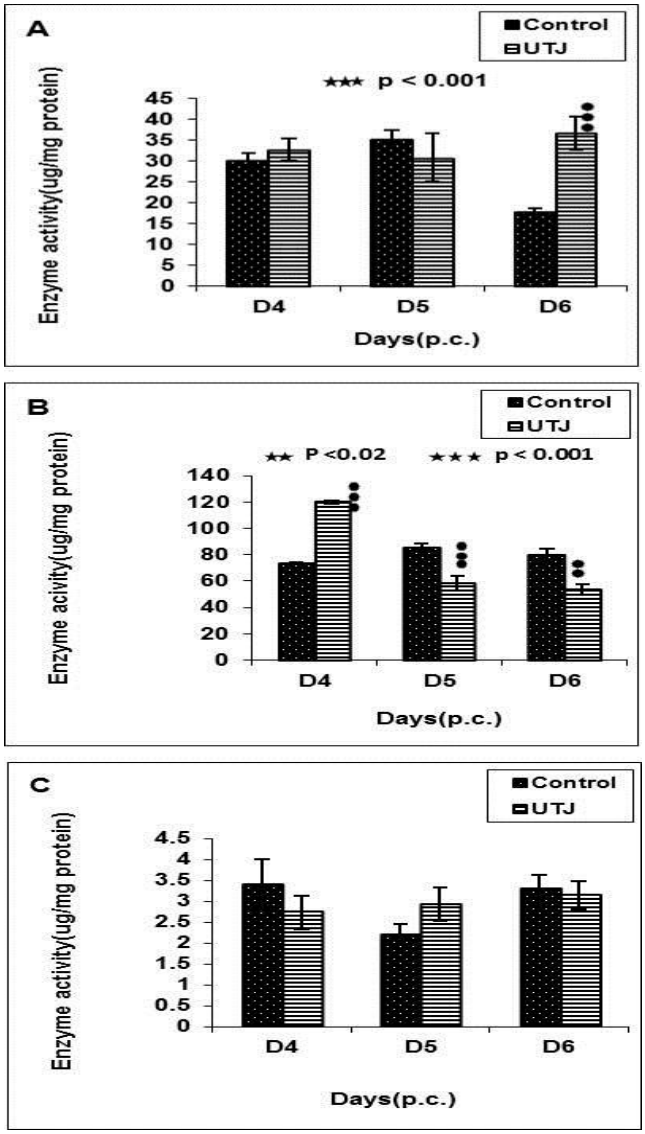

Figure 4: Peroxidase activity in soluble (A) - and Ca^{2+}-extracted (B) - fractions of uterus and in blood plasma (C) on days 4, 5 and 6 in ligated rats.

Enzyme activity in immature rats

In immature OVX rats, E2 (1 or 10 µg/rat, i.m. for 3 days) treatment caused an increase in uterine peroxidase activity both in extracts (Fig. 6) or in frozen sections (as evident by its localization in exogenous eosinophilic cells, blood vessels and endogenous epithelial cells) (Fig. 5). CCN treatment at the dose of 0.25mg/kg (Fig. 5D) or 1.25mg/kg (Fig. 5G) for 3 days did not cause any significant change in uterine peroxidase activity in frozen sections or in soluble/ca2+-extracted fractions (Fig. 6A, B)] as compared to OVX-control (Fig. 5 A & Fig. 6A,B). But, caused significant decrease in Ca2+-extracted (Fig. 6, B) peroxidase activity or localized enzyme activity (Fig. 5F, I) in rats administered CCN (0.25 or 1.25 mg/kg) in conjunction with E2 (10µg), as compared to E2 alone-treatment.

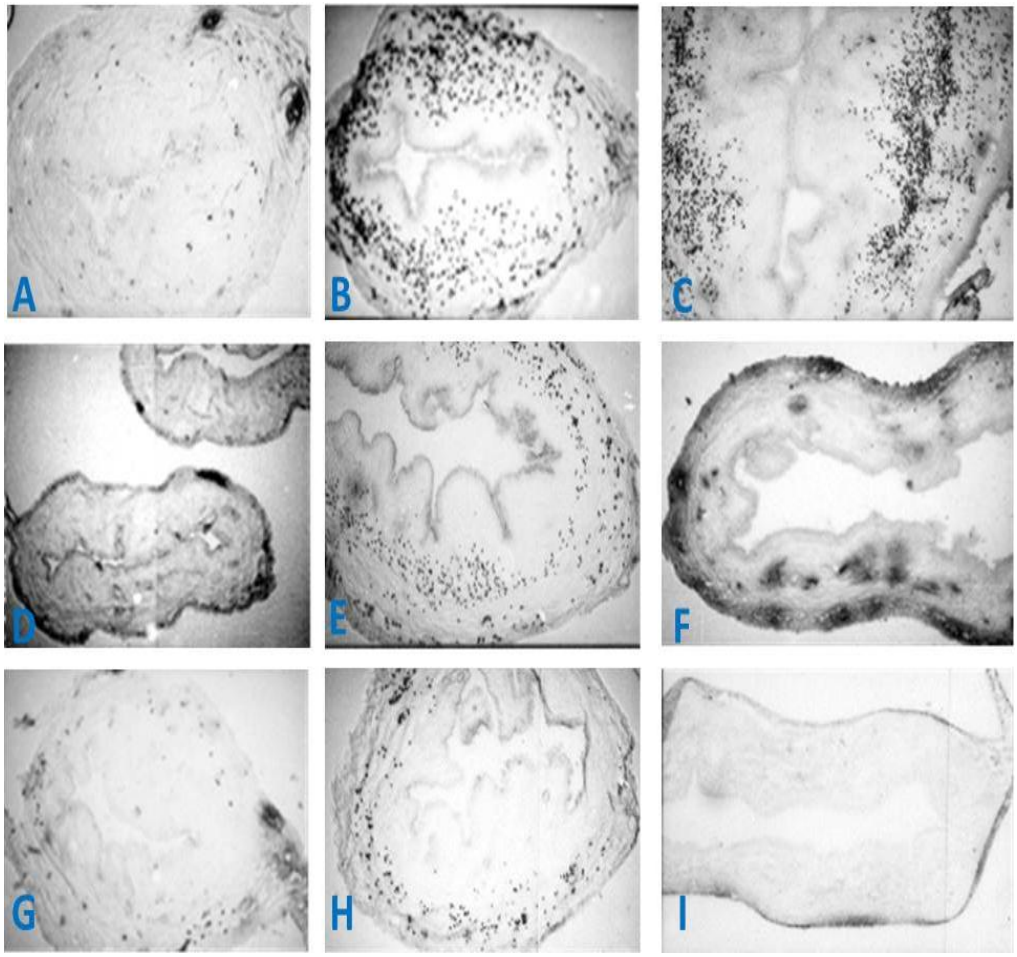

Figure 5: Uterine cross sections showing peroxidase enzyme reaction in immature OVX-C rat (A). Rats injected with E2 at the dose of 1µg/rat (B) or 10 µg/rat (C) intra muscularly (i. m.), showing increased activity than in ovx-c rat. Treatment of CCN at the dose of 0.25 mg/ kg (D) or 1.25 mg/kg (G) showing slight staining reaction, whereas in E2(1 µg) + CCN(0.25mg/kg)-treated(E) or E2(1 µg) + CCN(1.25mg/kg) treated (H)-treated rats showing positive peroxidase activity but less than E2 treatment. Treatment with E2 (10µg/rat) + CCN (0.25mg/kg (F), and E2 (10 µg/rat) + CCN (1.25mg/kg (I) for 3 days caused marked decrease in peroxidase enzyme activity than in E2 treatment. OVX-C: Ovariectomized control, E2: Estradiol, CCN: Centcroman. Magnification for all microphotographs is x100.

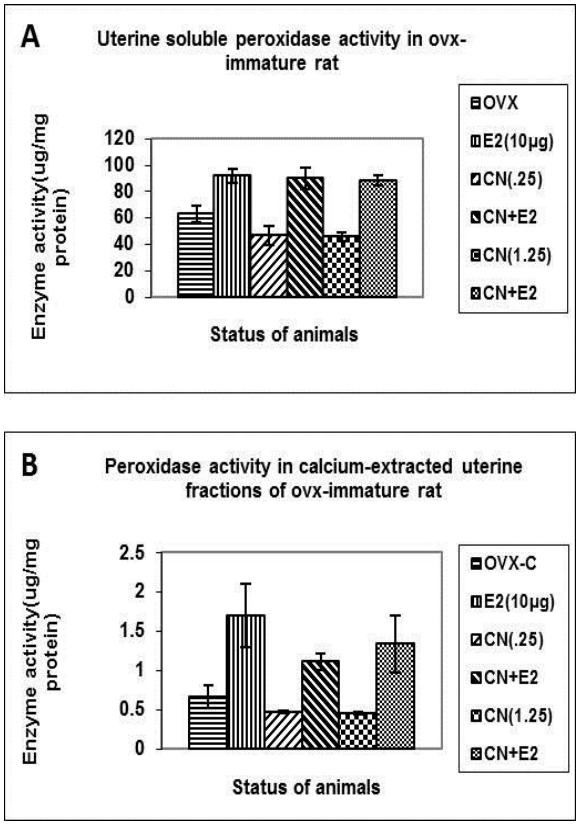

Figure 6: Peroxidase enzyme activity in uterine soluble (A) and Ca2+-extracted (B) uterine fractions in OVX immature rats administered CCN(0.25 mg/kg or 1.25 mg/kg) alone or in combination with E2(10µg/rat, i.m.) for 3 days.

DISCUSSION

Present study demonstrates the effects of CCN, a nonsteroidal antiestrogen on uterine peroxidase activity "marker" for estrogenic action in rat. Results of the study show the stimulation of peroxidase enzyme activity in uterine extracts or in tissue sections (mainly in endometrial eosinophils, blood vessels and epithelium) in immature OVX-rats administered E2. In adult rats**,** localized strong staining intensity in endometrial eosinophils and blood vessels observed on day 2 *p.c.*, showed further increase in number of endometrial eosinophils and uterine soluble/Ca2+-extracted peroxidase activity on days 3 and 4, with maximal eosinophilic activity on day 4 *p.c.*, may be due to increased peripheral estrogen level.[9] In contrast, marked increase in uterine peroxidase activity on day 5(10:00h) *p.c.* as evidenced in uterine frozen sections (in exogenous/endogenous cells, blood vessels and epithelium) or in tissue extracts (soluble/ca2+-extracted) similar to the previous investigation of Baiza-Gutman et al[42], demonstrate the role of this enzyme in uterine receptivity.[3,5,40,43,44] In contrast, decreased uterine peroxidase enzyme activity in ligated rats on day 5, indicate that the local steroid (estrogen) from blastocyst-itself may be involved in endometrial sensitization

receptive to blastocyst signal(s).[40,43] Moreover, the role of catechol estrogens in blastocyst-activation have been demonstrated[34] where peroxidase enzyme plays a significant catalytic role in conversion of estrogen to catechol estrogen.[33] While, decreased enzyme activity on day 6 *p.c.* was concluded to be due to progesterone–estrogen interaction.[42]

The infiltration of uterine eosinophils, 'marker' for early (nongenomic) response to estrogen action, has been used as an index of uterine peroxidase activity possibly because of the release of peroxidosomes-specific granules which contain a basic protein with peroxidase activity.[45] It is capable of stimulating early uterotrophic events and/or remodeling processes in endogenous uterine stroma.[28,30,46-48] Inhibition of migration of these cells in endometrium and uterine peroxidase activity (in frozen sections or in soluble/Ca2+-extracted fractions) during pre-sensitivity (days 3 and 4) and post-sensitivity (day 6) periods by CCN is attributed to the direct interference with the estrogen-dependent eosinophilic chemotaxis, eosinophil-estrogen receptor system and cytosolic-estrogen receptor mediated estrogen action.[5,18,28,49-56] Previous studies have been shown significant inhibition in uterine cytosolic or total estrogen receptors by CCN in adult[57,58] and immature[59] rats in spite unaffected plasma level of estrogen and progesterone. Results in immature OVX rats too, indicate an inhibition of uterine Ca2+-extracted peroxidase activity or its uterine localization by CCN administration in conjunction with estradiol. Therefore, this decrease in peroxidase localization in uterine sections and in endometrial eosinophilic leucocytes in association with decreased uterine soluble and Ca2+-extracted peroxidase activity may indicate the inhibition of estrogenic action via these non-genomic and genomic responses by CCN at uterine level.

Inhibition of endometrial sensitivity (day 5, 10:00h) by post-coital treatment (1.25 mg/Kg; p. o., on day 1 pc) of CCN[40,57] did not cause any significant change in uterine (soluble and Ca2+-extracted) peroxidase activity as compared to control rats. But, exhibited localized increase in staining intensity in uterine exogenous leucocytic cells, and epithelium associated with insignificant increase in uterine soluble and Ca2+-extracted peroxidase activity in treated rats on day 5. Exogenous leucocytic cells scattered throughout the muscularis (myometrium/serosa) region and in adjacent stroma as in estrogen–stimulated rats have been shown to contribute to the soluble peroxidase activity.[6,60,61] On the other hand, Ca2+-extracted peroxidase activity of endogenous (stromal/epithelial) cells have been shown to be contributed to the total pool of the uterine enzyme activity.[23,60-62] In immature OVX rats, CCN treatment alone did not cause any significant change in uterine soluble/Ca2+-extracted

peroxidase activity or its uterine localization as compared to OVX controls but, causes inhibition in Ca2+-extracted peroxidase activity in conjunction with estradiol. Thus, insignificant increase in uterine peroxidase activity and in exogenous eosinophilic leucocytes coinciding with increased plasma peroxidase level on day 5 in treated rats may be due to the weak inherent estrogenicity of CCN as reported to induce uterotrophic events.[38, 59] Reported studies have been also shown that CCN did not inhibit the secretion of nidatory estrogen but, causes an inhibition in action of estrogen at uterine level via inhibition of cytosolic estrogen receptors despite stimulation in nuclear estrogen receptors as reported in adult and immature rats.[57, 59] Moreover, increased uterine 17β-Hydroxysteroid steroid dehydrogenase (type 2) enzyme activity by CCN during pre-implantation period in rat uterus have been demonstrated, due to the inactivation of potent estrogen, E2 to its weak form, estrone, leading to inhibition of estrogen action in relation to inhibition of endometrial preparation for its sensitization.[7,58]

CONCLUSION

Findings indicate that: – 1) Correlation between uterine soluble peroxidase activity and localized staining in endometrial eosinophils related to non-genomic estrogen action, whereas, Ca^{2+}-extracted uterine (endogenous stromal/epithelial cells) peroxidase activity related to genomic response; 2) Significant inhibition of uterine peroxidase activity during pre (days 3 and 4) - and post (day 6)-sensitivity periods by centchroman may be due to inhibition of estrogen action. 3) Its insignificant increase on day 5(10:00h) in treated-rats coinciding with endometrial eosinophilic leucocytes and increased plasma peroxidase level, may be due to weak inherent estrogenicity of this compound.

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