

## Formulation, Development and Evaluation of Anti-Implantation Activity of Intrauterine System of Centchroman (Saheli) using Wistar Albino Rats

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### ABSTRACT

Centchroman – A Non-steroidal, post-coital anti-implantation drug which is generally popular as SAHELI is one of the best agents available. The fundamental aim of this study was to formulate such an Intra-uterine System of Centchroman which should be safe and acceptable. This new drug delivery system surpasses the first pass metabolism, thus increasing the bioavailability and reducing the drug load. This formulation delivered the drug upto 21 days. Polylactic-co-glycolic acid served as the best fit polymer for delivering the drug. The best optimized formulation was selected and subjected to stability studies. The effect of the foreign bodies was also observed on the fertility, mating behaviour and estrous cycle of the rats along with anti-implantation activity of the IUS. This formulation was tested on *Wistar* albino rats and is proved to be safe at the optimized dose level. The formulation exhibited pre-implantation loss of 100% and post-implantation loss of 0% in *Wistar* albino rats at a particular dose when delivered on 1<sup>st</sup> day and 4<sup>th</sup> day post-coital. Experimental part on animals showed that any foreign body, if put inside the uterus would not affect the fertility unless the size is more than 6mm. Results showed that 3mm objects had no effect on fertility. The foreign bodies and the IUS showed neither any alteration in the estrous cycle nor on the mating behavior of the animals.

**Keywords:** Centchroman, Intrauterine System, Development and Evaluation of Anti-Implantation Activity

### INTRODUCTION

In the recent years, considerable attention has been focused on enhancing the drug absorption and efficacy by redesigning the delivery process rather than the drug molecules or chemical entities. This has led to the development of **Novel Drug Delivery System**<sup>[10]</sup>. There are several advantages of Intra-uterine delivery as opposed to oral delivery, for e.g. drug avoid the hostile environment of the GIT (the action of pH and enzyme) and avoid first pass metabolism (the drug is directly been delivered to the uterine cavity and thus to systemic

circulation). In addition, drug delivery by the IUS can show sustained plasma profile over long period of time. This could minimize the risk of fluctuation of drug plasma level. Other attractive feature is that it can be inserted once and can be terminated when the lady wants to conceive. The contraceptive options present today are oral pills, IUDs, condoms, vaginal creams etc. Amongst them the most acceptable method of choice is either the oral pills or intrauterine devices<sup>[8]</sup>; still there lies a large unmet need for contraception.

Thus our fundamental aim for carrying out this research work was to formulate such a **novel delivery system** which should be safe and acceptable<sup>[4]</sup>. For that purpose we have chosen centchroman<sup>[9]</sup> which is a Non-steroidal post-coital contraceptive agent of established anti-implantation activity<sup>2</sup>. This drug candidate served a great fit to our set criteria for research work. This property has boosted its efficacy with minimizing side effects. Using centchroman in the IUS form is a novel thought and delivering the same for 21 days was an add-on feature to the formulation. For the better safety and use the drug was dispersed in the polymer PLGA uniformly. PLGA is a biodegradable polymer, therefore the device need not to be removed from the body when not required further, thus increasing patient compliance. Moreover the polymer was found to be highly compatible with the drug. This formulation is tested on *Wistar* albino rats and is proved to be safe at the optimized dose level. The efficacy and safety of the IUS was compared with the oral drug<sup>[5]</sup>. During the Pharmacodynamic evaluation of the IUS the effect of presence of foreign bodies was also figured out through the same<sup>[1]</sup>. The present study was undertaken to evaluate the effect of IUS on the fertility. The study was done to determine the effect of time, site and length of foreign bodies on fertility. As foreign bodies, Nylon thread, surgical suture and leaf petiole were selected. These were placed in the lower segment of the right uterine horn. The distance of the vagina from the cervix was optimized previously. The piston was allowed to push the patch upto the lower segment of the horn. The length of the foreign agent taken ranged from 3mm to 6mm.

## MATERIALS AND METHODS

**Animals:** *Wistar* albino female rats having weight between 150-200 g were used in the present study. Our Protocol No.7 (IAEC) of the year 2006-07, was approved by "Institutional

animal ethics committee" (IAEC), as per the norms of **CPCSEA** (Committee for the Purpose of Control & Supervision of Experimentation on Animals). Healthy albino rats were obtained from animal house, of DIPSAR, Pushp Vihar, New Delhi (Registration no. 215/CPCSEA 1<sup>ST</sup> June 2000). All the animals were kept under identical conditions of food, water, and temperature at DIPSAR animal house.

**Materials:** The drug sample of centchroman was obtained as gift sample from Hindustan Latex Ltd. Batch No. CENT F002, Manufacturing date Oct /2006. **UV** spectroscopy, **FTIR** and **DSC** authenticated the drug sample. PLGA used was provided by Supreme combine Bombay. Animal ultrasound machine was used during the study for evaluating the implants of the animals.

**Methods:** Centchroman sample was authenticated by **UV**, **FTIR** and **DSC**. Standard curve of Centchroman was prepared and the absorbance was obtained at 278nm. Matrix type IUDDS was prepared using solvent casting technique in which the drug was dispersed uniformly in the polymer that is PLGA<sup>3</sup>. 2% propylene glycol was added to the formulation as a plasticizer. The formed film containing the dispersed drug was stripped off and kept in oven at 40°C for 3-4 hrs. Total of five formulations were prepared, out of which one was optimized which showed the highest release rate among all. The formulation was tested for physical parameters like thickness, weight, area volume and uniformity assay. The dissolution studies were carried out in 230 ml of phosphate buffer pH 7.2 at a temperature of 37°C at a constant speed of 50 rpm. The developed film was subjected to accelerated stability testing according to the guidelines prescribed by **International Conference on Harmonization** (ICH). The films were kept at 40°C temperature and 75% relative humidity. The films were packaged with butter paper and aluminum foil (Blister pack). These packs were

then put into a resealable plastic bag and subjected to 40° C at 75 % RH and 25° C at 65 % RH in an incubator. The films were analysed for Drug release profile and Polymer-drug interaction/Polymer-polymer interaction (FTIR & DSC)

Further *in-vivo* animal study was conducted which accounted for the efficacy and safety of the system formulated. In addition a partner study was also conducted to determine the effect of time, site and length of foreign bodies on fertility. As foreign bodies, Nylon thread, surgical suture and leaf petiole were taken. These were placed in the lower segment of the right uterine horn. The patch was delivered through the vagina of the animal. For that a polyethylene tough tube of diameter 2mm was used inside which the patch was rolled in. The patch was then pushed through another tube which acted as a piston. The distance of the vagina from the cervix was optimized previously which is about  $2.8 \pm 0.5$  cm. The piston was allowed to push the patch upto the lower segment of the horn. The position of the patch was confirmed by looking inside the uterus of the few animals slaughtered after insertion. The length of the foreign bodies varied from 3mm to 6mm also.<sup>6,7</sup>

## RESULTS

Mean of readings of the same specimen was taken as actual value. Data were presented as mean  $\pm$  SEM. One-way ANOVA followed by Tukey test was used to compare mean values of the groups.

The dissolution of the optimized formulation showed a release of 5.12, 19.31, 45.66, 80.96, and 98.66 % of the drug in 1, 18, 108, 263 and 479 hr respectively. The regression coefficient value of Higuchi's plots was calculated to be 0.9944. Thus the formulation was following Higuchi's release pattern giving it a confirmation of sustain release. (fig-1)

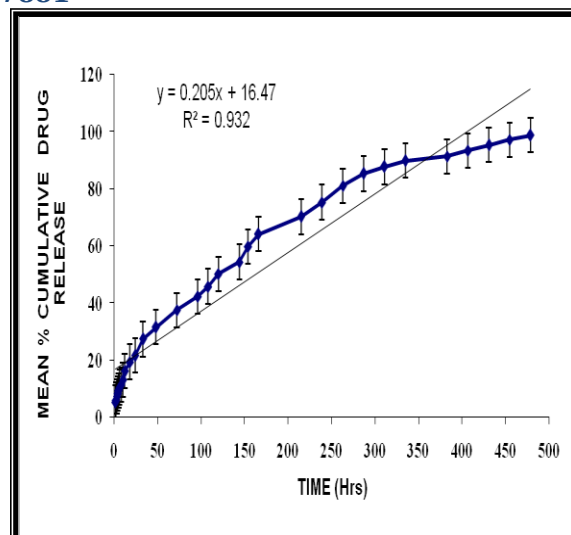


Figure 1 Depicts mean % cumulative release of the optimised formulation

For carrying out animal studies 3 groups were made which were classified into orally treated, Foreign body inserted and IUD inserted. Each group comprised of 6 animals. Oral control groups were administered with 2% w/v gum acacia solution while uterine controlled groups were inserted with blank polymeric films. The test group was inserted with the drug loaded patch. The dose volume employed for the oral dosing was 3.1mg/kg. Dosing was done taking into consideration the individual weight of the animals. The drug solution was introduced into the lower part of the esophagus using an oral feeding catheter. The effect of foreign bodies on pregnancy was also seen by inserting various agents like nylon thread, leaf petiole and suture. The uterine control group of animals, in which blank patch was inserted before pregnancy and was kept for mating the same day showed % pre-implantation loss of  $23.06\% \pm 4.97$  and % post-implantation loss of  $20.48\% \pm 5.71$  while the uterine control group of animals, in which drug loaded patch was inserted before pregnancy and was kept for mating the same day, showed % pre-implantation loss of 0 % and % post-implantation loss of 0%.



Fig 2 Depicts implants on 10<sup>th</sup> day of pregnancy



Fig 3 Depicts effect of IUS inserted on 10<sup>th</sup> day of pregnancy

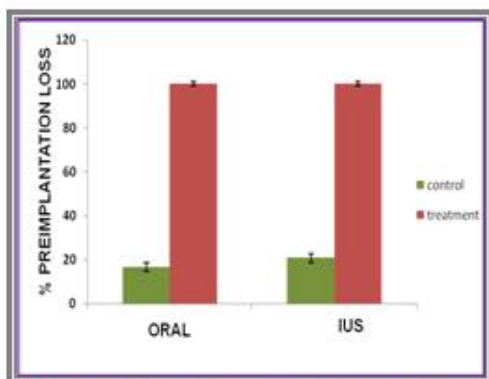


Fig 4. Depicts % pre-implantation loss after oral and IUS treatment on 1<sup>st</sup> day of pregnancy

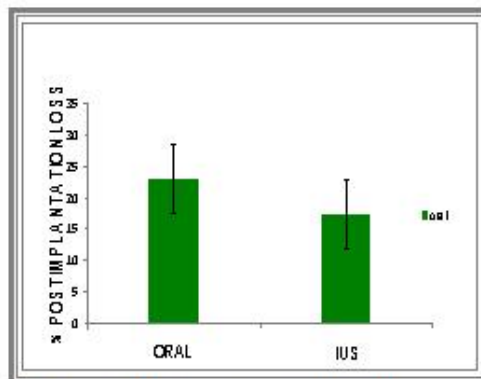


Fig 5. Depicts % post-implantation loss after oral and IUS treatment on 1<sup>st</sup> day of pregnancy

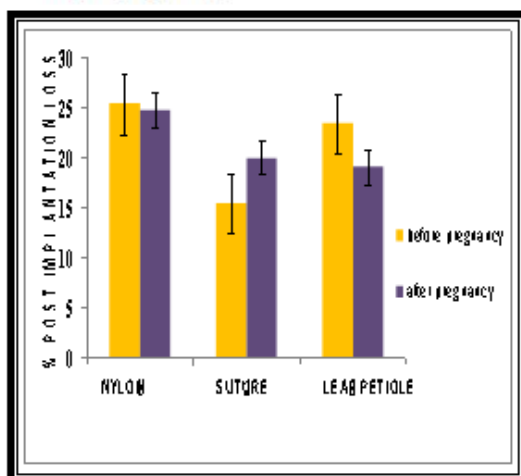


Fig.6 Shows comparison between before and after pregnancy %pre-implantation loss when nylon thread, surgical suture and leaf petiole were inserted inside the uterus as foreign bodies

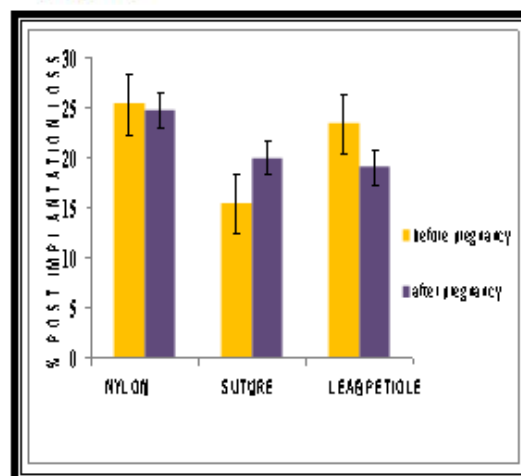


Fig.7 Shows comparison between before and after pregnancy %post-implantation loss when nylon thread, surgical suture and leaf petiole were inserted inside the uterus as foreign bodies

The Analysis of Variance calculation for the *in vivo* study of Centchroman resulted  $F_{(5,66)}$  value of 0.035 and was found to be less than that of tabulated value of  $F_{(0.05)}$  ( $P > 0.05$ ) which was 0.06 therefore, statistical significance in the results of intrauterine administered Centchroman was observed. Tukey test between the drug loaded patch of centchroman and blank patch resulted in a P-value less than 0.05 and was considered very significant i.e. there was very significant difference between the two groups.

### DISCUSSION

The main aim of this research work was to formulate and evaluate the matrix type Intrauterine drug delivery system for Centchroman using a biodegradable polymer PLGA. The polymer offers several advantages of being biocompatible, biodegradable, non-toxic and cost effectiveness along with displaying suitable controlled release characteristic of the controlled drug delivery system. The pharmacodynamic effects of the optimized patch shows an anti-fertility activity of 100%. The effect

of foreign bodies on the fertility depends on many factors. The most vital amongst them is the size of the foreign body and its location inside the uterus. It has been seen during the study that a foreign agent of 3mm does not produce any antifertility effect but of 6mm can probably affect the pregnancy. Similarly the position of that body inside the uterus affects the fertility accordingly. There was no effect on the estrous cycle and mating behaviour of animals when foreign agents were inserted. The same treatment i.e. control and test was given on the 4<sup>th</sup> day (day of implantation) just to ensure that the IUS is going to affect the implantation site leading to abortion.

### CONCLUSION

Considering the results of the present work, it can be concluded that the formulated IUS containing half the oral dose of centchroman has the advantages of lesser dose, less frequent administration and better user compliance. It has been estimated to be of great success if used commercially, as it reduces user compliance too.

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