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Effects of Halofantrine on Oestrous Cycle, Ovulation and Microscopic Structure of the Ovary in Cyclic Sprague–Dawley Rats

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ABSTRACT: This study was designed to examine the effects of Halofantrine on oestrous cycle, ovulation and microscopic structure of the ovary in female Sprague–Dawley rats. Thirty virgin cyclic female rats divided into six experimental groups of five rats each were used for the ovulation studies. The first 3 groups received 24mg/kg of Halofantrine at 1000h, 1400h and 1800h respectively.

Three groups of rats totalling, twenty-four were used for the experimental study on oestrous cycle; while ten rats were used for the effects on the ovary. Results showed reduction in number of ova produced in rats given 24 mg/kg of the drug and a more significant reduction ($P < 0.01$) in those that were administered 48 mg/kg body weight. The effect on oestrous cycle showed a distorted cycle that is reversible and dose–dependent. No histological abnormality of the ovary was seen when compared with the control group. Halofantrine could be developed as a female contraceptive in developing countries.

Key words: Oestrous cycle; Ovulation, Ovary, Contraception.

Introduction

Many antifertility agents alter the ovulation process through direct effect on ovarian Graffian follides. Contraceptive drugs alter the normal cyclic hormonal balance and thereby achieve contraception.

Non-steroidal anti-inflammatory agents interfere with ovulation by reducing the number of ova released (1). The principle behind their action lies in the fact that follicular maturation is similar to an inflammatory reaction.

Interestingly, chloroquine, a similar anti-malaria to Halofantrine blocked ovulation at 40mg/kg body weight given intraperitoneally (IP) at 1000h proestrous to cyclic female rats (2).

Halofantrine is a phenanthrenemethanol anti-malarial that is effective against asexual forms of multi-drug resistant plasmodium falciparum malaria. It has no action on gametocytes or hypnozoites in the liver (3).

Besides a few nuances of kinetic nature observed in pregnant women, a good knowledge of teratogenous or embryotoxic effects is necessary. But this remains fragmentary (4).

Because of lack of experimentation of this drug on pregnant women and also its effects on this study was carried out to see the effects of the drug on oestrous cycle, ovulation and ovary of lower primates before determining its embryotoxic or teratogenous effects.

Materials and Methods

A total of sixty-four Sprague – Dawley rats weighing 135 – 175g were obtained from the Animal House of the Faculty of Science of the University of Ilorin.

They were acclimatized for one week in a well ventilated house at the Department of Anatomy. They had access to feeds and water *ad libitum*.

Their oestrous cycles were established by daily vaginal smear taken with separate pipettes containing physiological saline (0.9 NaCl.).

The smears were examined under light microscope to determine the epithelia cells predominantly present in the vaginal lavage. The predominant presence of uniformly large nucleated cells indicate pro-estrous stage; cornified cells indicates estrous (ovulation) and predominant presence of leucocytes with or without epithelial cells indicates the diestrous I or II stages. Rats which exhibited two or more consecutive four – day oestrous cycle were used.

For determining the effects on ovulation, rats were divided into seven groups. Each group contains five rats (Control, test groups N, - 1000h, 1400h, 1800h, N_2-1000h, 1400h and 1800h).

The treatments were as follows:

Group 1	24mg/kg body weight of oral Halofantrine at 1000h proestrous
Group 2	24mg/kg body weight or oral Halofantrine at 1400h proestrous
Group 3	24mg/kg body weight of oral Halofantrine at 1800h proestrous
Group 4	48mg/kg body weight of oral Halofantrine at 1000h proestrous
Group 5	48mg/kg body weight of oral Halofantrine at 1400h proestrous
Group 6	48mg/kg body weight of oral Halofantrine at 1800h proestrous.

Control rats had no treatment.

The rats were smeared until the day of oestrous at 9000h daily after which they were sacrificed by decapitation. The fallopian tubes were opened and examined for ova after which they were fixed in Bouin's solution. The ovaries and those of other rats treated with on dioestrous I and II with the two respective dosages were removed and fixed.

They were dehydrated in graded series of alcohol, cleared in chloroform and embedded in paraffin wax at 60°C. Sections were stained with haematoxylin and eosin.

Results

(A) Effect of Halofantrine on Oestrous Cycle

(i) Treatment on Proestrous (N)

Three groups of rats were treated with 24mg/kg body weight of the drug at 1000h, 1400h and 1800h proestrous, respectively.

All the three groups continued into oestrous (c) the following day. There is no alteration of the oestrous cycle at this stage.

A group treated with double dose, 48mg/kg body weight, at 1000h, had complete alteration of their oestrous cycle. There was prolongation of proestrous for about two days in most members of the group after which some continued into oestrous while others skipped oestrous maintaining diestrous II for about three days.

The second group later returned to proestrous and attained normal oestrous cycle about 8 days after treatment.

(ii) Treatment of Diestrous II and Proestrous (LN)

A dose of 24mg/kg body weight of the drug was given to members of this group for two days consecutively after which their oestrous pattern was monitored. After the first dose, half of the members of the group had normal oestrous cycles while half had prolonged diestrous II for one day before attaining proestrous and oestrous respectively.

(iii) Treatment Diestrous I and II

This group received 24mg/kg body weight of the drug and showed a prolongation of diestrous II in all members of the group, some for one day and others for two or more days not attaining estrous.

(B) Effect on Ovulation

The fimbrial end of both fallopian tubes of each rat were excised and ova counted under light microscope. Presence of transparent regions signifies the present of ova.

The results were as follows:

Table 1: Effects on Ovulation

Group	Sample number (n)	Treatment dose	Mean No. of Balloons (Mean ± S.D.)	Mean No. of Ova (Mean ± S.D.)
N1 – 1000h	5	24mg/kg	1.5 ± 0.5*	1.5 ± 0.5*
N1 – 1400h	5	24mg/kg	2.5 ± 0.5*	8.0 ± 1.0*
N1 – 1800h	5	24mg/kg	2.0 ± 1.0*	6.5 ± 0.5*
N2 – 1000h	5	48mg/kg	0.5 ± 0.5*	0.5 ± 0.5*
N2 – 1400h	5	48mg/kg	1.0 ± 1.0*	3.0 ± 1.0*
N2 – 1800h	5	48mg/kg	1.5 ± 0.5*	1.5 ± 0.5*
Control	5	Nil	2.0 ± 1.0	9.0 ± 1.0

*$P < 0.01$ when compared with corresponding controls.

Effect on the Histology of the Ovary

Administration of 24mg/kg of Halofantrine on proestrous (N) did not alter the histological pattern of the ovary. When the dose was also doubled, there was no histological abnormality after recovery.

The ovaries of rats treated on diestrous I and II also appeared to be like those of control group when the rats fed with 24mg/kg and 48mg/kg of Halofantrine respectively.

Discussion

This result of this study has showed that Halofantrine altered the oestrous cycle. This distortion is dose dependent and reversible. It also reduced the number of ova produced at a clinical dose of 24mg/kg body weight. A more significant reduction was observed with a dose of 48mg/kg body weight. However, no abnormality was noticed on the microscopic structure of the ovary.

Although, when nutritional, endocrine and genetic factors have been known to disrupt ovarian function and the oestrous cycle, several drugs can affect oestrous cycle by acting at different levels at the hypothalamic – pituitary axis or at the ovarian level to inhibit ovulation (2).

Effect on oestrous cycle was assessed by daily monitoring of the vagina lavage for the predominant presence of leucocytes, nucleated cells or cornified cells (1,5,6). The effect on ovulation was assessed by counting the ova in the excised fimbria ends of the tube underlight microscope (2). In this present study, the above procedures were also used.

As shown in Table 1, a significant reduction ($P < 0.01$) was observed in the number of ova produced in rats fed on 24mg/kg body weight of Halofantrine at proestrous 1000h, 1400h and 1800h. A more significant reduction was observed with a dose of 48mg/kg body weight. The reduction was most marked when the rats were fed at 1000h. This is similar to the observation of chloroquine studies (1,2). Disruption of the estrous cycle which was found to be dose-dependent and reversible was also observed with chloroquine (2).

In conclusion, Halofantrine could be developed as a female contraceptive in developing countries.

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