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Oestrus cycle pattern in hexavalent chromium toxicity in female wistar rats

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Abstract
An experiment was conducted for a period 4 months in 120 female wistar rats to study the oestrus cycle pattern in hexavalent chromium toxicity. Experimental rats were divided into six groups with four replications containing five rats in each. These were fed with control and experimental feeds and rats and were subjected to examination of oestrus cycle pattern by vaginal smear examination for cellular composition. Studies revealed that there is an extreme variation in its regular cycles which was varied from its physiological limits in toxic groups while distinct amelioration has been observed in ameliorative groups.

Keywords: Female wistar rats, oestrus cycle pattern, hexavalent chromium toxicity, vitamin C, *Emblica officinalis*

Introduction
Chromium is the sixth most abundant element in the earth's crust. It is used in three basic industries: metallurgical, chemical and refractory. Hexavalent chromium is a primary contaminant due to its toxicity to humans, animals, plants and microorganisms. It is more toxic than trivalent chromium because it readily enters the cells producing various pathological conditions, including reproductive dysfunction. High concentration of chromium (40-50 ppm) have been reported in the effluents of the industries. Chromium exposure through drinking water has been shown to impair ovarian follicular maturation and differentiation. Chromium is a reproductive metal toxicant that can traverse the placental barrier and cause a wide range of fetal effects including ovotoxicity. Recently it was reported that lactational exposure of hexavalent chromium induces delay /arrest in follicular development at secondary follicular stage. Hence the present investigation has been undertaken to study the toxic effect of hexavalent chromium on oestrus cycle pattern in female wistar rats and its amelioration with Vitamin C and *emblica officinalis*.

Materials and Methods
All rats were handled in accordance with the guidelines of Institutional Animal Ethics Committee (IAEC) (No 1/4/14,date 27.11.2014).

Determination of oestrous cycle
There are four phases of oestrous cycle (Pro-oestrous,oestrus,metoestrus and dioestrus).These phases of estrous cycle were determined by vaginal smear examination for cellular composition [1, 2]. The smears were examined under light microscope (LM) to determine the epithelial cells predominantly present in the vaginal lavage. It was performed daily for a period of 3 cycles for 3 weeks.

Experimental design

Group	No of Rats	Type of treatment / diet
I (Control)	20	Basal diet
II (Toxin control)	20	Basal diet + Potassium dichromate 500 parts per million(ppm) in drinking water orally for 3 months
III (Vit C control)	20	Basal diet + vitamin C @ 100 milligram (mg)/kg body weight(kg.b.wt) orally for 3 months.
IV(*Emblica officinalis* control)	20	*Emblica officinalis* powder given @ 2 % in feed for 3 Months
V (Chromium VI + Vitamin C control)	20	Basal diet + Potassium dichromate 500 ppm in drinking water orally for 3 months + vitamin C @ 100 mg/kg b.wt orally for 3 months.
VI (Chromium VI + *Emblica officinalis*)	20	Basal diet +potassium dichromate 500ppm in drinking water orally for 3 months+ *Emblica officinalis* powder given @ 2% in feed for 3 months.

Results

An increase in the length of total oestrous cycle(4-5 days) was observed in toxin fed group rats among which the oestrous period for (24 hours), and dioestrous period is for 32 hours), when compared to control, protective and ameliorative group rats. Other phases where as pro-oestrous and met estrus were not affected during its total length of oestrus cycle.

Oestrus cycle pattern under microscopic studies was as follows

Dioestrus: During this phase scanty material was available for microscopic examination, which was collected and examined under light microscope and detected traces of secretary material with cellular debris, few intact cells and few leucocytes.

Early pro-oestrus: The representative smear is very "clear" with less mucous and parabasal cells when compared with intermediary cells. Cell debris was not observed in this phase which were showed in fig: 1.

Pro-oestrus: Microscopic pictures of this phase reveals a predominant intermediary cell number and few parabasal cells and leucocytes are hardly seen in Fig: 2.Transition phase from pro-oestrus to oestrus: Fig 3 shows a clear cellular domination which consists of intermediary cells, superficial cells and anuclear or keratinized cells.

Oestrus: Microscopic examination of the smear during oestrus phase consists of keratinized superficial cells which are single during early stage and were in groups as the oestrus phase progresses, at the end of this phase these cells were resulted in formation of large flakes and few intermediate cells were appears to be intacted nuclear material may occasionally seen. The transition from oestrus to metoestrus: In this phase flakes of keratinized cells are seen and it is characterized by the presence of leucocytes and to a lesser extent intermediary cell.

Metoestrus: The microscopic picture of metoestrus phase dominated by leucocytes in large numbers and few intermediary cells were also found. As the stage progresses, more intermediary cells appear which are often small and dark. Few parabasal cells, large intermediary cells and leucocytes are seen. In this stage the smear is characterized by the reduction in total cell number and the reappearance of mucous, often in thin strands was observed. As the stage advances, the mucus becomes progressively more visible and cell numbers more declined. The characteristic fern pattern appearance (Fig 4) of mucous is evidenced.

Anoestrus: This stage is resembles to the transition from dioestrus to pro-oestrus and the microscopic picture is darker due to more amount of mucus and rare appearance of cells (Fig :5). The transition from pro-oestrus to oestrus sows a characteristic early oestrus development after a period of anoestrus, and in some cases the vaginal smear show fewer cells.

Figures

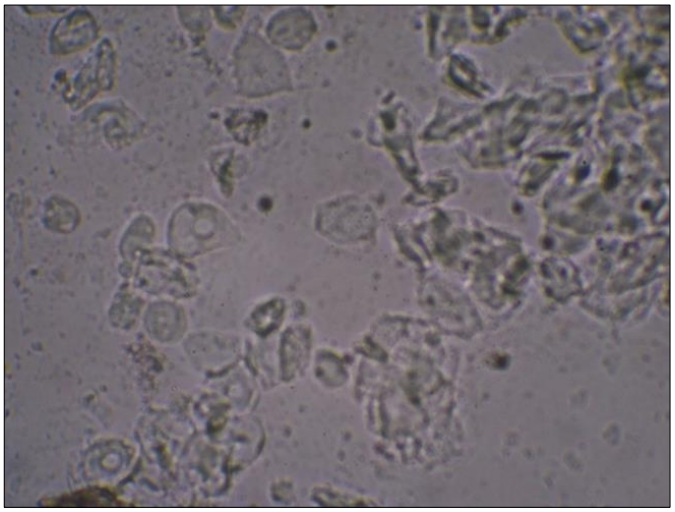

Fig 1: Note the early pro-estrus stage of estrus cycle showing very "cleaner" with less mucus, para basal cells are very clear in comparison with intermediary cells and cell debris cannot be observed in toxin group (group II) of rats x 20.

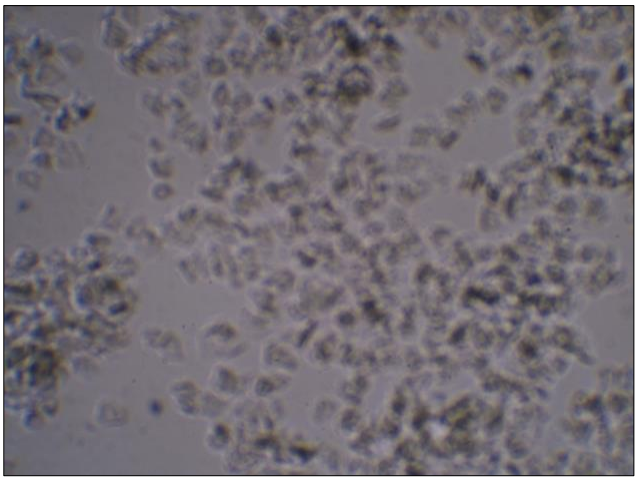

Fig 2: Note the Microscopic picture of pro-estrus stage of estrus cycle revealing a predominant intermediary cells and rare or few parabasal cells and hardly seen leucocytes in the toxin group (group II) rats x 20.

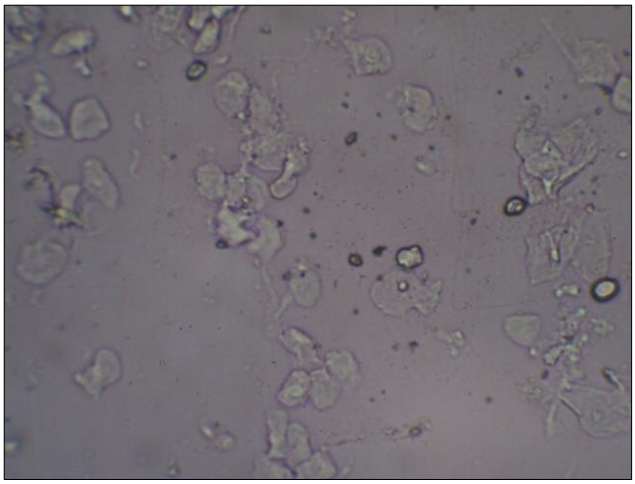

Fig 3: Note the microscopic picture of transition phase of from pro-estrus to oestrus stage of the oestrus cycle showing a clear domination of cellular content consisting of intermediary cells, superficial cells and a nuclear or keratinized cells in the toxin group (group II) rats x 20.

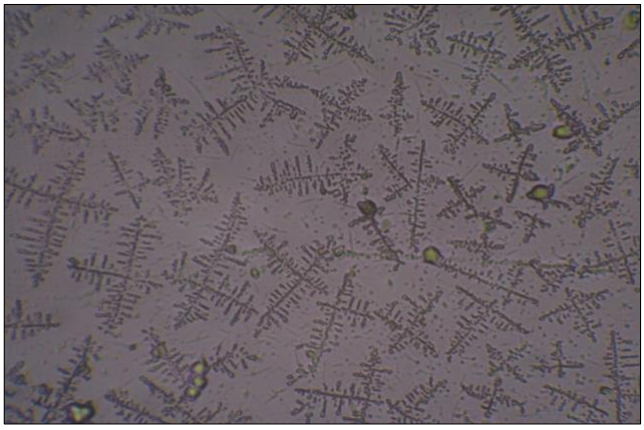

Fig 4: Note the transition phase from metoestrous to dioestrus of oestrus cycle characterized by the decline in cell numbers, reappearance of mucus often in thin strands as the stage advances, the mucus becomes progressively more visible with characteristics fern pattern appearance in the toxin group (group II) rats x 20.

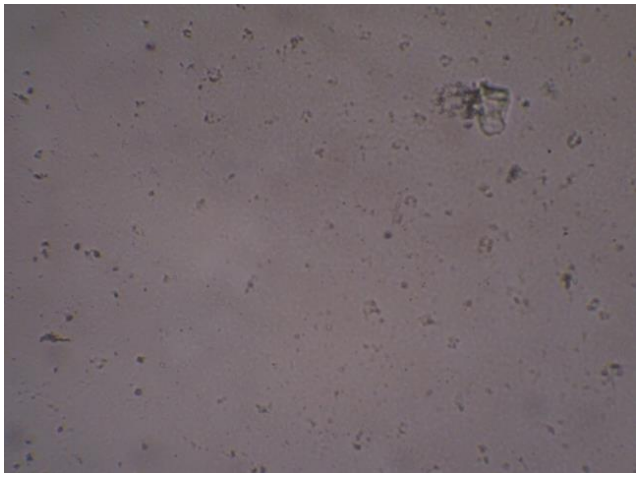

Fig 5: Note the anestrus is characterized by the lot of mucus and cellular debris and rarely appear intact cells in the toxin group (group II) of rats. Generally the rat'sestrus cycle may halt at this stage x 20

Discussion

The prolonged oestrus cycle especially dioestrus phase may be due to chromium toxicity. The estradiol is responsible for changes in the reproductive tract, mammary glands and regulation of gonadotropin. The stages of oestrus cycle and their inter conversions are mainly governed by the hormones viz., estrogens and progesterone. Any change in these hormones would lead to changes in the cyclicity and impaired fertility. Hence, the lengthening of phases of oestrus cycle in the chromium treated rats could be correlated with decreased estradiol levels.

Conclusion

In the present study the toxin group rats reflected an extreme variation in its regular cycles which was varied from its physiological limits. There is a significant lengthening of estrous cycle specifically pro-oestrus and dioestrus phases in the toxin (group II) group rats compared to control (Group I,III,IV),protective(group V) and ameliorative (Group IV) group of rats. The lengths of other phases of oestrus cycle were not affected.

References

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