

CYTOPATHOLOGICAL ASSESSMENT OF UTERINE CELLS IN *RATTUS NORVEGICUS* DUE TO INDUCED SODIUM FLUORIDE

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ABSTRACT

Sexually matured female Wistar rat of weight ranging from 150-180g were used for the investigation. To observe the cellular changes in uterus, experimental rats were intoxicated with sodium fluoride. Experiments were carried out in four groups, in which first group was considered as normal group of animals, remaining three as experimental which were intoxicated subcutaneously with 30mg NaF/kg bw/day, 45mg NaF/kg bw/day and 75mg NaF/kg bw/day, respectively for continuously 15 days of duration. After completion of exposure period, animals were sacrificed. Desired organ *i.e.* uterus was used to observe normal histology and histopathologically cellular alterations in experimental animal groups by following standard microtechnique procedure. The uterine sections in all groups were stained by Haematoxylin-Eosin staining. The normal cells in uterus were compared with cells in intoxicated group of animals. Observed cellular alterations were discussed with physiology of reproduction.

INTRODUCTION

The well being of human depends on quality of drinking water. Consumption of water containing excess fluoride over a long period of time results in fluorosis. Ramaraju *et al.*, (2000). Investigations of Wood, (1974) showed that, the intake of fluoride is permissible upto 0.5-1.0 mg/L which is beneficial to human health. The maximum allowable concentration for fluoride in drinking water in Indian conditions comes to 1.4 mg/L while as per Indian standard it is 1.5 mg/L. The fluoride concentrations in human being and in animals depends on consumption of water and also changes according to climatic conditions (Suthar *et al.*, 2008). Choubisa, (1999) concluded that excessive intake of fluoride causes dental, skeletal fluorosis which is the chronic diseases manifested by mottling teeth, softening of bones etc. Interpretation of Riggins *et al.*, (1976) was that, the fluoride ion can replace the hydroxyl group in the calcium hydroxyapatite crystal to form calcium fluoroapatite, which alters the normal remodeling cycle of bone.

After attending puberty in animal, reproductive tract plays vital role in the reproductive mechanism. Uterus is considered to be very important part of system which plays role in implantations as well as in nourishment of embryo during pregnancy. Ahmad *et al.*, (2000), found decreased fertility rate due to sodium fluoride toxicity on female rat *Rattus norvegicus*. Biochemical and metabolic disturbances was found in male mice in which ascorbic acid, glycogen and protein content was found to be reduced due to sodium fluoride toxicity, Chinoy and Mehta (1999). Similar result was found in uterus and ovary of mice.

In order to gain further insight regarding effect of sodium fluoride on female reproductive organ, the present study was undertaken to investigate the histopathological changes in

the uterus of *Rattus norvegicus*.

MATERIALS AND METHODS

Sexually matured female Wistar rat *Rattus norvegicus* weighing of about 150-180g were used for investigations. The rats were reared in departmental animal house (CPC SEA 233) Shivaji University, Kolhapur Maharashtra, India. They were housed in aluminum cages consisting two animals within it. They were supplied with Amrut rat feed from Pranav Agro Industry, Kolhapur.

Water miscible Sodium fluoride (NaF) from Qualigens (Ml.Wt. 41.99) was used as toxicant for experimental animals. In whole experiment the rats were divided in four groups in which one as normal group and other three as experimental groups of animals. The first experimental group of animals was injected subcutaneously with sodium fluoride dissolved in sterile water in the dose of 30mg NaF/kg bw/day, second experimental group of animals with 45mg NaF/kg bw/day, and third experimental group of animals with 75mg NaF/kg bw/day dose. All experimental groups were injected its respective dose upto 15 days of experimental duration. After completion of exposure period *i.e.* on 16th day, all the groups of animals including normal, were sacrificed and immediately desired organs as uterus were continued for histological study by following standard microtechnique procedure. H-E *i.e.* Haematoxylin-Eosin staining technique was used to observe histological or cytological alteration in uterus in normal as well as NaF induced experimental rat *Rattus norvegicus*.

RESULTS AND DISCUSSION

Present study reveals with induced histological changes in female reproductive tract. Morphological and Histological changes occurred due to induction of Sodium fluoride varies in

Table 1: Effect of Sodium fluoride on body weight and organs weight after exposure of 15 days duration.

| Parameters | Exposure period (15 days) | | | |
|-----------------------|---------------------------|-----------------------|----------------------|----------------------|
| | Normal | 30mgNaF/ kgbw/day. | 45mgNaF/ kgbw/day | 75mgNaF/ kgbw/day |
| Initial body wt. | 175 g | 173.5 g | 158 g | 162g |
| final body wt. | 177 g | 173.5 g | 155 g | 161g |
| Ovary wt. | 55mg | 45.3 mg | 30 mg | 30mg |
| Uterine branch wt. | 76 mg | 72 mg | 68 mg | 69mg |

in normal and experimental group of rat. The experimental groups of animals showed reduced body weight, hunched posture. The experimental animals in all groups showed slothful behavior as dose of toxicant and exposure period increased. Related to the uterine morphometry as the dose and intoxication period was increased weight of uterus in experimental animals were reduced as compared to the weight of the uterus in normal group of animals. An ovary of experimental rat in all the groups showed similar reduction in the size, shape and weight as compared with the ovary in normal rat. Alterations in the reproductive organs showed inverse proportion to the concentration of toxicant and exposure period (Table 1).

In the normal physiology of female reproductive tract uterus is an extremely important organ. Major portion of the uterus were corpus and uterine cavity. Normal histology of uterus showed basically three layers. Outer formed by epithelial cells with single nucleus stained bluish in H-E staining collectively formed serosa. Histologically middle thick myometrium, layer was marked by smooth muscles, when stained with eosin showed three to four fibrillar layers which were surrounded by connective tissue. Most of the blood vessels was found normal and were intermingled in the layer. Inner endometrium showed regular arrangement of columnar epithelial cells with single nucleus. Some uterine glands were also found in functional layer of endometrium (Fig. 1).

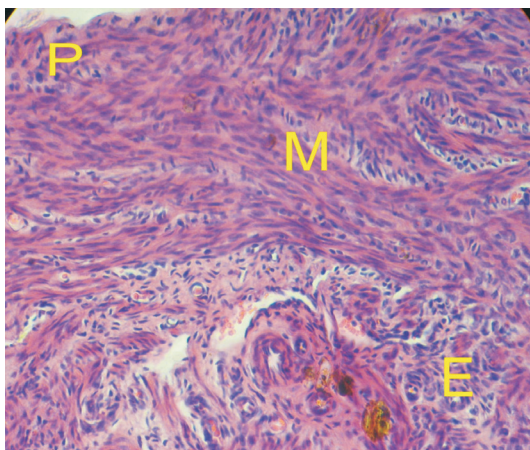


Figure 1: Normal uterine cells stained with HEx400; P= perimetrium; M= myometrium; E=endometrium

Histology of uterine cells and musculature was found disturbed as the concentration and exposure period of toxicant increased, (Fig. 2, 3 and 4). The endometrial cells become large, showed hypertrophy in their size and shape. Hypotrophy was found in inactive spindle shape stromal fibroblast cells resulting in space or lacunae formation. In 30mgNaF/kgbw/

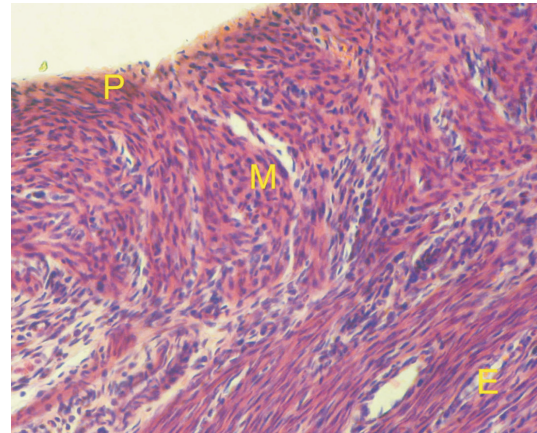


Figure 2: Intoxicated with 30mg NaF/kg bw/day; stained with Hex400; P= perimetrium; M= myometrium; E=endometrium

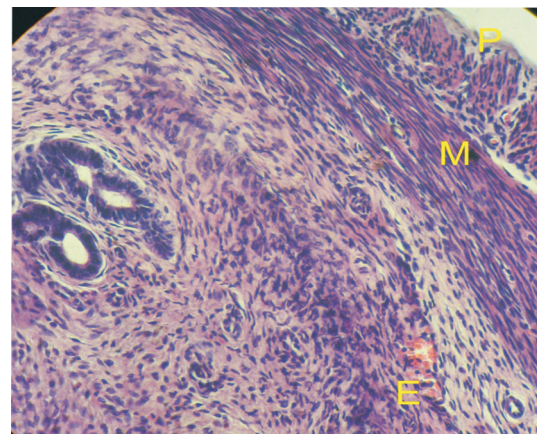


Figure 3: Intoxicated with 45mg NaF/kg bw/day, stained with Hex400; P= perimetrium; M= myometrium; E=endometrium

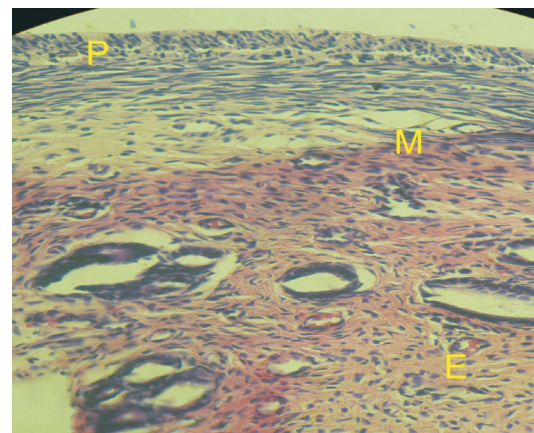


Figure 4: Intoxicated with 30mg NaF/Kg bw/day, stained with Hex400; P= perimetrium; M= myometrium; E=endometrium

day and 45mgNaF/kgbw/day in both the animal groups they showed disruption of endometrium, thickening of endometrial blood vessels as a result of toxic potency to the NaF. The layer of Myometrium showed diffused fibrillar architecture with irregular muscles striation. The perimetrium was found synchronized with internal space. As compare to the above

two groups of uterine cells in the rat exposed to 75mgNaF/kgbw/day showed loose cellular architecture. The endometrial cells was found squeezed, the large space was found in the layer, blood vessels altered their normal shape and size, cellular irregularity were found. Myometrium was found tremendously diffused, fibrillar structure was disconnected showing its limpness, connective tissues was randomly scattered in the layer. Perimetrial layer was irregularly arranged and showed loose connection with each other. Overall there was higher cellular alteration was found in the uterus exposed to 75mgNaF/kgbw/day intoxicated group of animals. These histopathological investigations results that increased concentration and exposure period of sodium fluoride in the reproductive organs of female rat *Rattus norvegicus* has hazardous and lethal effect indicating its toxicity in more concentration and exposure if accumulated in the body.

Further the biochemical estimation of these cells will give clear pathological evidences. Similar results were found in the study of Peter and Vladimir, (1997), where the amount of cadmium altered surface epithelium, glandular epithelium and stroma in endometrium of uterus. Dima *et al.*, (2002) reported that methylene chloride/methanol extract of *Aspilia africana* increases in vitro vascular smooth muscle contraction and mechanism of aortic ring preparations in *Rattus norvegicus*. The inhibitory effect of steroidal saponins on the oestrus cycle has been reported by Tamura *et al.*, (1997). They have been found to reduce fertility in animals upon continuous administration. Oluyemi *et al.*, (2007), observed the effect of *Aspilia africana* on uterus of rat which showed deletion and derangement of the endometrium, endometrial stroma and also thickening of endometrial blood vessels. According to him these effects were suggestive of toxic potency of *Aspilia africana*.

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REFERENCES

- Ahmad, S. Al-Hiyasat, Ahmed, M., Elbetieha and Homa Darmanib. 2000.** Reproductive toxic effects of ingestion of sodium fluoride in female rats. *Fluoride*. **33(2)**: 79-84.
- Chinoy, N. J. and Mehta, D. 1999.** Effect of protein supplementation and deficiency on fluoride-induced toxicity in reproductive organ of male mice. *Fluoride*. **32(4)**: 204-214.
- Choubisa, S. L. 1999.** Some observation on endemic fluorosis in domestic animals in Southern Rajasthan (India). *Vet Res Commun*. **23**: 457-465.
- Dima, T., Tan, P. V., Dongo, E., Kaintchoung, P. and Raktoniria, S. V. 2002.** In Vitro Vascular Smooth Muscle Contractile Activity of *Aspilia Africana* Extract on Rat Aortic Preparation. *Pharmazie*. **57(6)**: 421-3.
- Oluyemi, K. A., Okwuonu, U. C., Baxter, D. G. and Oyesola, T. O. 2007.** Toxic effects of methanolic extract of *Aspilia africana* leaf on the estrous cycle and uterine tissues of wistar rats. *Int. J. Morphol.* **25(3)**: 609-614.
- Peter, M. and Vladimir, U. 1997.** Histological changes in the uterus of rabbits after an administration of cadmium. *J. Environ. Sci. Health, A32. (5)*: 1459-1466.
- Ramaraju, H. K., Madhava, Lakhminarayana, V. and Ranganna, G. 2000.** Impact of tank desilting on fluorosis control - a case study in Bagepalli, Kolar district. Section-6 Limnology, Watershed hydrology and Monitoring.
- Riggins, R. S., Rucker, R. C. and Chan, M. M. 1976.** The effect of fluoride supplementation on the strength of osteopenic bone. *Clin.Orthop*; January-February: 352-7.
- Suthar, S., Vinod, K., Jangir, S., Kaur, S., Goswami, N. and Singh, S. 2008.** Fluoride contamination in drinking water in rural habitations of Northern Rajasthan, India. *Environ. Monit. Assess.* **145**:1-6.
- Tamura, K., Honda, H., Mimaki, Y., Sashida, Y. and Kogo, H. 1997.** Inhibitory effect of a new steroidal saponins, OSW-1, on ovarian functions in rats. *Br. J. Pharmacol.* **121(8)**: 1796-802.
- Wood, J. M. 1974.** Biological cycles for toxic elements in the environment. *Science*. **183**: 1049-1052.

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