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Effect of formaldehyde on ovaries of female albinorats– Amorphological&histological study.

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ABSTRACT: Background:Formaldehyde is recognized as toxic agent, resulting adverse effects on people who work closely with it. Embalmers, anatomists, pathologist, technicians and medical students are among the people who have high exposure to formaldehyde. It is also a suspected to be a deleterious agent.

Aim: To analyze the gross and micro-anatomical changes in ovaries along with hormonal imbalance in wistar albino rats after formaldehyde exposure through inhalation process.

Material Methods: The animals required for the study were procured from Institutional Animal House, GovtKilpauk Medical College, at Chennai. 20 Wistar albino rats, average weighing 110 to 120 gms were used for the study. Animals were divided into four groups of five rats in each group. Group I (Control group), which were not exposed to formaldehyde, while the remainders regarded as the experimental group, exposed to formaldehyde for 15 days (FAT 15), 30 days (FAT 30) and 60 days (FAT 60). The blood samples were drawn by cardiac puncture at the end of experimental period.The blood collected was centrifuged and serum was separated and used for hormone assay, Oestrogen, Progesteron, Follicular stimulating hormone, Luteinizing hormone were estimated. After respiration ceased, the animals was trans-cardially perfused using formal saline. ThenOvarian tissues were dissected out and post fixed for histological study.

Results: All the rats in Formaldehyde treated groups (FAT 15, FAT 30 and FAT 60) showed increased body weight than the control group. In FAT 30 and FAT 60 groups, morphological changes in ovaries were noticed indicating, Shrinkage, darkening and decreased in volume. Oestrogen level was significantly increased in FAT 30 and FAT 60 groups, with a gradient decrease in FSH and LH level in the FAT 15, 30 and 60 group. There was a significant decrease in Progesterone level in the experimental group compared to control. Mean volume of ovary, cortex and medulla, corpus luteum and graffian follicles was decreased in the FAT group. Also there was a significant increase in total volume of atretic follicles with prominence of connective tissue fibrosis, congestion of blood vessels and condensation of connective tissue around the vessels was seen. Irregularfollicle and hydropic degeneration in follicular granulose were also detected. These changes were more pronounced in FAT 60 days group.

Conclusion: Formaldehyde inhalation with mentioned concentration and duration can induce hormonal and histopathologic changes in ovary of female albino rat. Empirical evidence shows a strong association between formaldehyde exposure and reproductive toxicity.

KEYWORDS: Formaldehyde, Toxicant, Oestrogen, Progesteron, Ovary, Atretic, Fibrosis.

I. INTRODUCTION

50% of infertility cases are due to the female infertility factors. Female infertility could be due to problems with ovulation, damage to fallopian tubes or uterus, or problem with cervix. Hormone imbalance is one of the major factor leading to ovulatory problems . The process of ovulation largely depends upon Follicle stimulating hormone (FSH), oestrogen, luteinizing hormone (LH), and progesterone

Substances or agents that affect the reproductive health of women or men or the ability of couples to have healthy children are called reproductive hazards. Emerging evidence supports an association between formaldehyde exposure and multiple adverse health effects [1]. A reproductive hazard could cause one or more health effects, depending on when the woman is exposed.Reproductive impact of formaldehyde in humans was unlikely at occupational exposure levels, with increased risk of spontaneous abortion (SAB) of formaldehyde exposed workers [2]. Exposure to harmful substances during the first 3 months of pregnancy might cause a birth defect or a miscarriage. During the last 6 months of pregnancy, exposure to reproductive hazards could slow the growth of the fetus, affect the brain development, or cause premature labor.

Formaldehyde (FA) is a flammable,

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colorless substance, which is readily polymerized as a gas atnormal room temperature [3]. It is water-soluble, whose pure form is irritant anditssolidstateiscalledParaformaldehyde.Formaldehydeconcentrationisgenerallyexplainedaspartspermillion(ppm;1ppm1⁄41.25mg/m^3), and40%–50% of its aqueoussolutioniscalled formalin [4, 5].FA is also a classified carcinogen and ubiquitous environmental contaminant, widely used in the construction, textile, furniture, medical, chemical, and pharmaceutical industries [6].Chronic exposure may result to rhinitis, hyperplasia and squamous metaplasia of the ciliated and non-ciliated nasal respiratory epithelium, headache, fatigue, neurobehavioral symptoms [7].

FA can affect the female reproductive system, which could be menstrual problems, altered sexual behavior, infertility, change in puberty onset, altered length of pregnancy, lactation problems, change in menopause onset and pregnancy outcome.It also results in chromosome and DNA damage (genotoxicity), oxidative stress, altered level of enzymes, hormones and apoptosis [6].It could also adversely impact the reproductive system through stress-induced effects on the HPA gland axis, endocrine or other regulatory systems [8].

In the dissection lab, and during cadaver's dissection, instructors of anatomy and medicalstudents are exposed to formaldehyde vapor derived from fixed cadavers [9, 10]. This study was designed to determine the hist-morphological changes of ratovary tissue after formaldehyde exposure of 3 hours/day for 60 days.

II. METHODS

The animals required for the experiment were procured from Institutional Animal House, GovtKilpauk Medical College, at Chennai after getting clearance from institutional ethical committee. Female Wistar albino rats (Rattusnorvegicus), average weight 110 to 120 Gms were used for thestudy.

Animals were maintained under controlled conditions [room temperature (23 ± 2^{o} C), humidity (50 ± 5%) and light/dark cycle] at animal house and were fed with High fat diet and drinking water ad libitum.Animals were grouped under IV groups: I Control, II Formaldehyde Treated (FAT15) for 15 days, IIIFormaldehyde Treated (FAT30) for 30 days, and IV Formaldehyde Treated (FAT60) for 60 days.

Formaldehyde Treatment Procedure:

Wistar albino rats of experimental groups were exposed to 10 parts per million of FA 3 hours/day for 15,30 and 60 days. The exposure was carried in a closed chamber with height 34cm, width36cm and length 55cm. The chamber was modified with openings for light and ventilation at different levels

Follow - up Investigative Procedure:
Euthanasia and Tissue Harvesting:

Animals were euthanized at the end of intended experimental period of 60 days, by administering over dose of chloroform. After respiration ceases, the animals was trans-cardially perfused using formal saline. Then Ovarian tissues were dissected out and post fixed for histological studies in freshly prepared 10%formalin.

Morphological Observation:

Ovary morphology was analysed by weighing with balance, Volume with water replacement method, and measurements like length, breadth and width was also observed.

Histo-pathological Study:

Tissues were processed for paraffin sectioning. They were briefly dehydrated in graded alcohol series, cleared in chloroform andxylene, and embedded in paraffin wax. Tissues were sectioned at 5 micron thickness and stained with Haematoxylin and eosin, Masson's trichrome stain

Hormone Assay:

In the intended period the blood samples were collected by cardiac puncture. The blood collected was centrifuged and serum was separated and used for hormone assay. Hormone estimation wasdone by radio-immunoassay technique. Oestrogen, Progesteron, Follicular stimulating hormone, Luteinizing hormone were estimated.

III. RESULTS AND DISSCUSSION

All the rats in Formaldehyde exposed groups (FAT 15, FAT 30 and FAT 60) showed increased body weight with colour change from white to yellow. Ovaries of experimental group had morphological alterations. After detailed gross necropsy examination, ovaries were trimmed of fat and blottedon filter paper and weighed. In 30 daysand 60days FAT group the ovary was shrunken, decreased in weight & dark in colour indicating ovarian damage.

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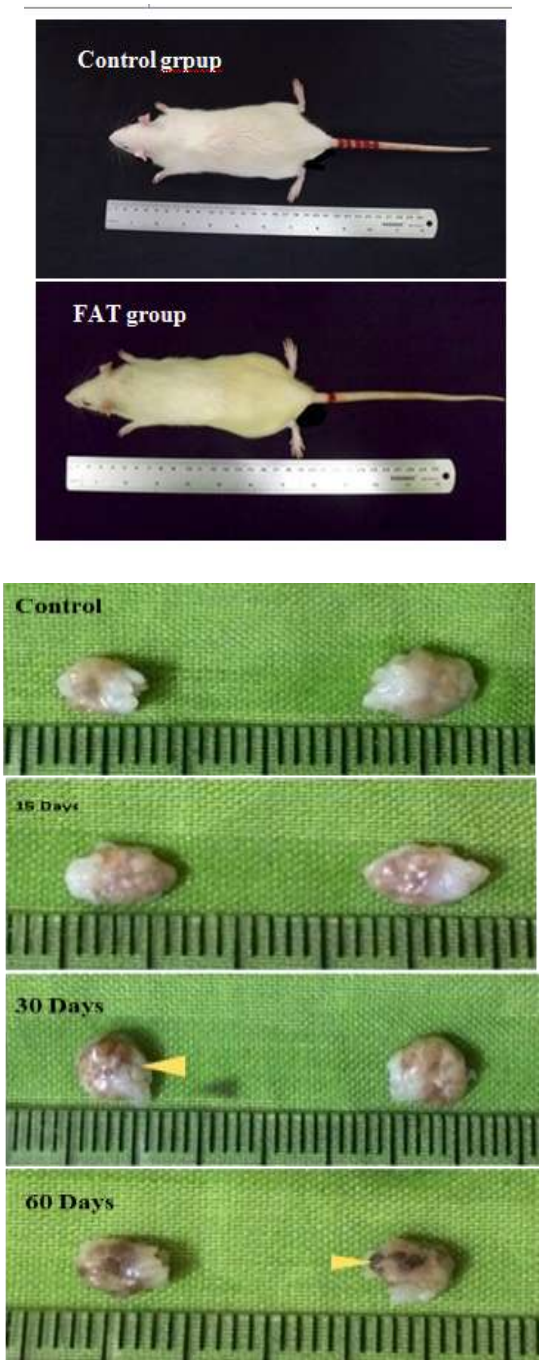

Plate 1: Photograph showing the morphology of ovary in various experimental groups. Note the arkening of the organ.

Formaldehyde inhalation resulted in reduction of the total number of primordial, primary and graffian follicles. Mean volume of ovary, cortex and medulla, corpus luteum and graffian follicles was decreased in the FAT group. Also there was a significant increase in total volume of atretic follicles. On light microscopy, thickening of tunica albugenia, prominence of connective tissue fibrosis, congestion of blood vessels and condensation of connective tissue around the vessels is seen. Irregular follicle and hydropic degeneration in follicular granulose were alsodetected using hematoxylin and eosin.

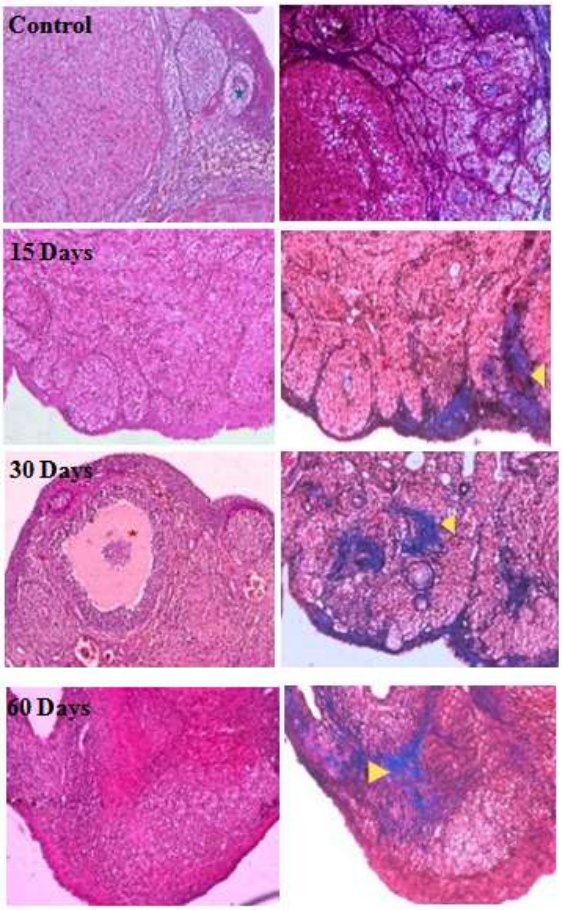

Plate 2: Microphotograph showing the histological changes in ovary of control and various experimental groups.Stained with H&E and Trichrome. Magnification 10x. Arrow indicates collagen deposition in all FAT groups. Blue asterisks indicate primary follicles and red asterisks, non-ovulated abortive follicles.

FAT group resulted in follicular atresia, with apparent reduction in the number and types of follicles and corpora lutea. The collagen deposition was observed using masson'strichromein formaldehyde treated groups owing to fibrosis which always follows the cellular loss. The entire FAT group showed germ cell loss with inflammatory cytokines and ROS activity thus showing collagen deposition. These changes were more pronounced in 60 days exposure group.
Stressors generally induce depression of hypothalamo-pituitary-ovarian system, mediated by activated hypothalamo-pituitary-adrenocortical

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system, resulting in fall in plasma LH and FSH levels. Formaldehyde induces the release of endogenous opioids from hypothalamus, which along with corticosteroids suppresses the secretion of hypothalamic gonadotrophin releasing hormone (GNRH), which makes it difficult for information to be passed between the brain, pituitary gland, and the ovaries. Suppression in secretion of GNRH causes reduced secretion of LH & FSH from pituitary. Since secretion of progesterone is dependent on luteinizing hormone, the reduced luteinizing hormone leads to decreased progesterone level. The drop in progesterone production, prompts the body to increase estrogen levels to compensate this decline.

Oestrogen level was significantly increased in formaldehyde exposed groups when compared to thecontrol, were as progesteron was vice-versa to oestrogen which was significantly decreased. FSH and LH level was reduced comparatively

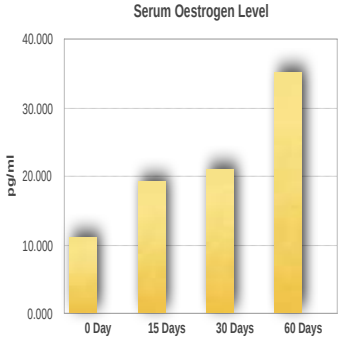

Fig. 1 illustrates serum FSH level of all the experimental groups. Results were expressed as mlU/ml.

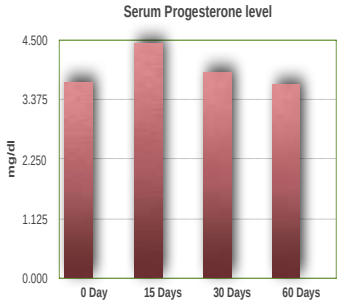

Fig. 2 illustrates serum Progesterone level of all the experimental groups. Results were expressed as mlU/ml.

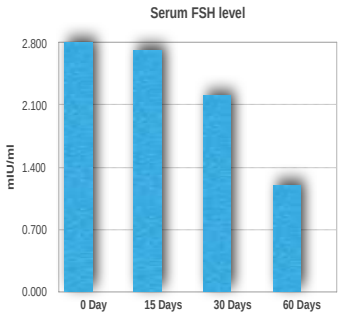

Fig**. 3** illustrates serum FSH level of all the experimental groups. Results were expressed as mlU/ml.

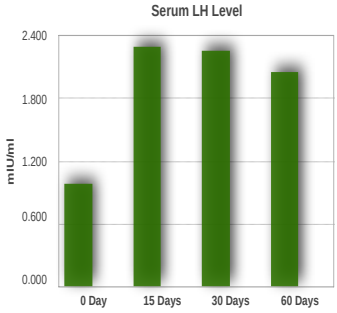

Fig**. 4** illustrates serum LH level of all the experimental groups. Results were expressed as mlU/ml.

IV. CONCLUSION

According to the current study, the formaldehyde inhalation with mentioned concentration and duration can induce hormonal and histopathologic changes in ovary of female albino rat. Empirical evidence, showing a strong association between formaldehyde exposure and reproductive toxicity. In conclusion, the present study was aimed at creating awareness that female personnel in the Anatomy and Histopathology laboratories should avoid the prolonged exposure to FA (specifically at high concentrations) in the working environment, in order to reduce the health risk associatedwith the reproductive function. Finally, if the association between formaldehyde exposure and reproductive/developmental toxicity is strengthened, regulation in the workplace and the environment will be adjusted accordingly in the interest of public health.

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