



## Histological alteration in ovaries of albino mice exposed to Petroleum Refinery Bio-sludge.

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### Abstract

Sewage sludge is the solid, semisolid or liquid residue generated during treatment of domestic sewage. The occurrence of refinery sludge in considerably toxic amounts was reported by early workers in various aquatic ecosystems. It can act as an endocrine disruptor, interfering with biological functions such as reproduction, growth, development and the ability to cope up with stress. The present study investigates the endocrine activities of sludge using *in vivo* bioassays and the reproductive health of treated mice. For our study a total number of six female albino mice with body weight ranging from 15 to 20g were selected and randomly divided into two groups. Animals of treated group identically received 50mg/kg body wt. sludge for 21 days. The treated sludge leachate from Numaligarh refinery could produce adverse effects at the tested dose. The examination of the treated female albino mice shows that oocyte nuclei was degenerated, number of primary follicles were found less and the epithelial layer ruptured. Presences of atretic and degenerated corpus luteum were also noticed. In the present study, observed histological alterations in the ovary clearly establish that Bio-sludge possess adverse physiological effects. The finding of the present study confirms that it is having strong impact on reproductive system and its physiological function.

**Keywords:** Bio-sludge, albino mice, ovary

### 1. Introduction

Sewage sludge refers to the residual, semi-solid material that is produced as a by-product during sewage treatment of industrial or municipal wastewater. The majority of human populations are exposed to a variety of environmental and/or occupational toxicants. The occurrence of refinery sludge in considerably toxic amounts was reported by early workers in various aquatic ecosystems. It can act as an endocrine disruptor, interfering with biological functions such as reproduction, growth, development and the ability to cope up with stress. Female infertility is a very real medical problem and the female reproductive system is very sensitive to different harmful environmental factors<sup>1</sup> like, chemicals, industrial pollutants and food. The term "bio solids" was introduced by the wastewater treatment industry in the early 1990's and is now also used by the United States Environmental

Protection Agency (EPA)<sup>1,2,3,4</sup>, the agency responsible for federal regulations governing the application of sewage sludge to land. The occurrence of refinery sludge in considerably toxic amounts was reported by early workers in various aquatic ecosystems. It can act as an endocrine disruptor, interfering with biological functions such as reproduction, growth, development and the ability to cope up with stress. It causes significant metabolic alterations and injuries of biological system at different levels<sup>12</sup>. Pollutants found in sewage sludge can generally be divided into the following categories: inorganic contaminants (e.g., metals and trace elements); organic contaminants (e.g., polychlorinated biphenyls [PCBs] dioxins, pharmaceuticals, and surfactants); and pathogens (e.g., bacteria, viruses and parasites)<sup>1</sup>. Although they are not presently regulated, radioactive contaminants may be also present in bio solids.

The present study investigates the endocrine activities of sludge using *in vivo* bioassays and the reproductive health of treated mice.

## 2. Materials and methods

### 2.1. Study area

The study area is located at Numaligarh in the district of Golaghat (Assam). During the operation some amount of solid waste of hazardous nature is generated from the various processing units and the sludge treatment paddy fields. The major solid wastes and sludge expected to be generated during the operation of the refinery are oily sludge, Bio sludge, Chemical sludge and spent catalyst.

### 2.2. Test substance

Test substance used in biologically and chemically treated sludge was collected from the Numaligarh refinery, Golaghat district, Assam.

### 2.3. Experimental animals

Female albino mice were selected and used for the experiment. The animals were obtained from Pasteur Institute, Shillong. The animal were housed in polypropylene cages and provided with food grains and water regularly.

### 2.4. Experimental design

A total number of 6 albino mice with body weight ranging from 15 to 20g were selected and randomly divided into two groups. Animals of the treated group identically received 50mg/kg bodywt sludge. The procedure was repeated daily at 9.00 am for 21 days.

### 2.5. Anaesthesia

Diethyl ether is a volatile anaesthetic and is administered by inhalation. The mice were

anaesthetised with di-ethyl ether.

### 2.6. Administration of test compounds

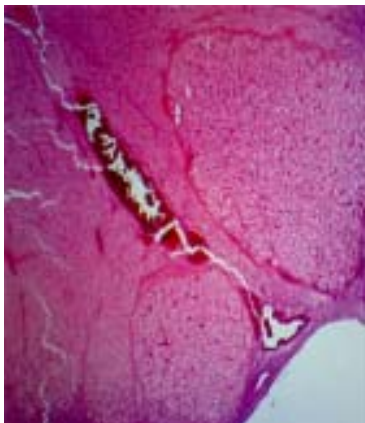
The mice received the test samples 50mg/kg body wt. sludge orally for 21 days at 24hrs interval. 1% Tween 80 was included in each sample to facilitate solubility when required. Concentration of the test samples were chosen based on the LD<sub>50</sub> values.

### 2.7 Histology

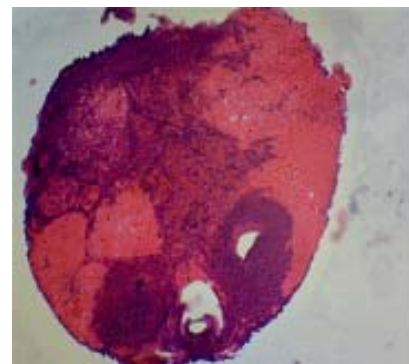
After dissecting out the ovaries of the mice were collected and fixed in 4% formaldehyde for 24 h, then washed in tap water for overnight and dehydrated in graded ethanol and embedded in paraffin wax. Sagittal sections (5µm of thickness) were cut and mounted on glass slides. Sections were deparaffinized in xylene, hydrated in ethanol and stained with hematoxylin-eosin (HE). Then the slides are mounted in DPX and studied under microscope. Changes induced by treatment of sludge on ovary tissues was photographed and analysed Labomed LX-400 Microscope.

## 3. Results

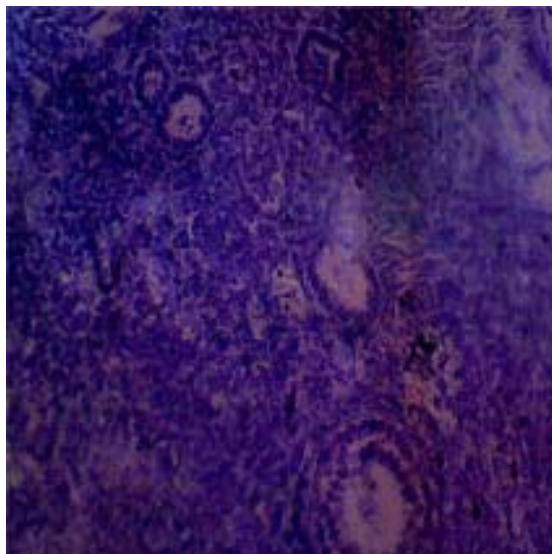
In the present investigation, the treated group at the tested dose levels could produced adverse physiological effects on ovary. The examination of the treated female albino mice shows some histoanatomical abnormalities to some extent. In ovaries of treated group, it was observed that almost all the follicles contain degenerated nucleus and the nuclei were difficult to define. The follicular epithelial layer is disrupted and the number of primary follicle was found less in number. The oocyte nuclei are degenerated; vacuolated cells are present in ovaries. Presence of atretic and degenerated corpus luteum (corpus albicans) was also evident in the treated group.



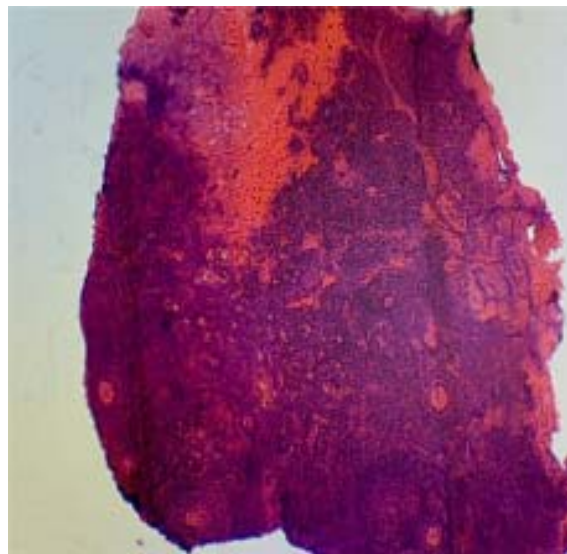
**Fig. 1:** Photomicrograph showing the cross sections of Haematoxyline and Eosin stained Ovary of control Albino mice. (x40)



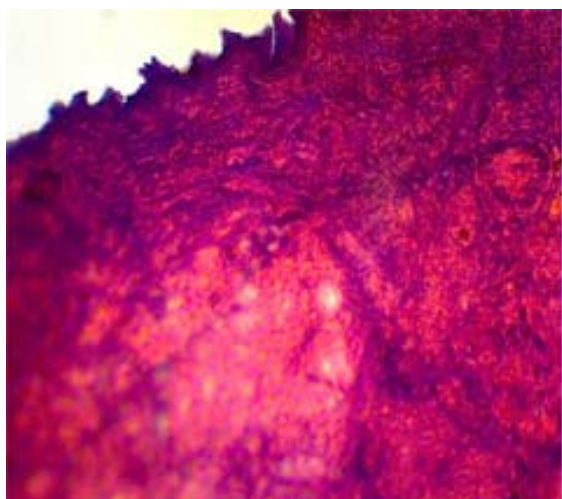
**Fig. 2:** Photomicrograph showing the cross sections of Haematoxyline and Eosin stained Ovary of Bio-sludge treated Albino mice (x10) showing ruptured epithelial layer (1); vacuolated cell (2) and atretic corpus luteum (3)



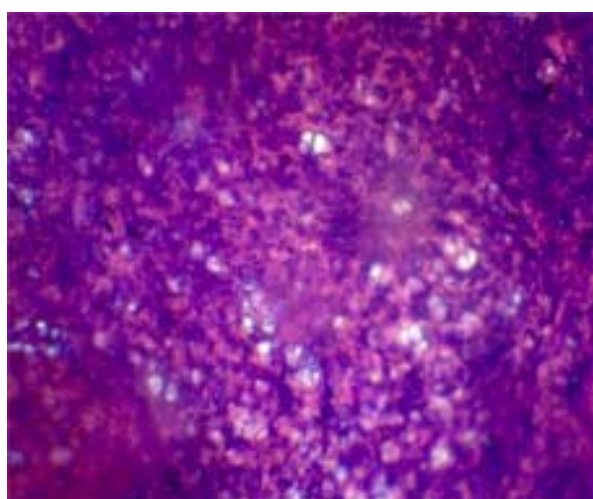
**Fig. 3:** Photomicrograph showing the cross sections of Haematoxyline and Eosin stained Ovary of Bio-sludge treated Albino mice (x40) showing less no. of follicles (1, 2- Primary follicle; 3- Secondary follicle)



**Fig. 4:** Photomicrograph showing the cross sections of Haematoxyline and Eosin stained Ovary of Bio-sludge treated Albino mice (x10) showing decrease in normal ovarian follicle (1- primordial follicle; 2, 3- primary follicle)



**Fig. 5:** Photomicrograph showing the cross sections of Haematoxyline and Eosin stained Ovary of Bio-sludge treated Albino mice (x40) with atretic corpus luteum.



**Fig. 6:** Photomicrograph showing the cross sections of Haematoxyline and Eosin stained Ovary of Bio-sludge treated Albino mice (x40) showing vacuolated cells

#### 4. Discussion and conclusion

In the present study, observed histological alterations in the ovary clearly established that Bio-sludge possess adverse physiological effects. On the 21 day of exposure of refinery sludge in female albino mice resulted in marked degenerative changes in the ovary. These changes include degenerated oocyte nuclei, less number of primary follicle, ruptured epithelial layer, degenerated corpus luteum, less number of mature follicles and presence of vacuolated cell.

Similar to these study, there are suggestions that

exposure to progesterone may reduce the number of primordial follicle<sup>10</sup>. Estrogenic action reduces follicle assembly leading to fewer primary and subsequent developing follicles<sup>9</sup>. In utero exposure to low doses of environmental pollutants disrupts fetal ovarian development in sheep<sup>11</sup>. Similar results of maternal toxicity due to sludge leachate were obtained from a study on swiss albino mice when dye wastewater untreated sludge administered during growth period of gestation<sup>7,8</sup>. Numerous studies that looked at the relationship between exposure to environmental

hazards and reproductive outcomes used residence in proximity to hazardous waste landfill sites as a surrogate for exposure. A statistically significant association between the mothers residence near hazardous waste sites and risk of having low birth weight and birth defects has been reported in some women.<sup>6,13,14</sup> Certain paternal occupations-petroleum workers, rubber worker, agricultural chemical worker have been particularly implicated as detrimental to the reproductive health of men <sup>15</sup>.The stroma appeared vacuolated<sup>5</sup>.

Hence, the present study has clearly revealed that

animals exposed to this refinery sludge may pose serious threat to human reproductive health of the females. The present investigation is a step towards evaluating the adverse effects on the reproductive aspects and it is evident by the results obtained that they could serve as baseline data for future research and studies.

#### 6. Acknowledgement

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