

**Original Research Article**

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**ESTROUS CYCLE DISRUPTION IN RATS BY FLUORANTHENE**

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**ABSTRACT:** Polycyclic Aromatic Hydrocarbons (PAHs) are organic compounds containing prominent benzene rings in them. They are commonly found pollutants in the atmosphere. Many of them are known to be carcinogenic or teratogenic in nature. Fluoranthene (FLA) is one among the 16 priority PAHs listed by the US EPA. It is the most abundant PAH found in vehicle exhaust and often generated as a product of incomplete combustion, burning of woods and cooking at high temperatures. The toxicity of PAHs has been checked in aquatic organisms and less explored in other organisms. Humans are exposed to PAHs on daily basis, both indoors and outdoors. The toxicity assessment in rats may help to corroborate their effect in humans. Particularly the reproductive toxicity of FLA is not yet reported. This study was designed to study the effect of FLA in disrupting the regular estrous cycle (reproductive cycle) in rats. The results revealed a significant reduction in the number of normal cycles in FLA administered rats compared to that of intact. The oxidative stress imposed in the ovary and uterus by FLA administration was confirmed through histological examination. This study makes it clear that FLA has a significant influence on the reproductive organs thereby affecting the estrous cycle.

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**KEYWORDS:** PAHs, Fluoranthene, Toxicity, Pollutants and Estrous cycle.

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**1. INTRODUCTION**

Polycyclic Aromatic Hydrocarbons (PAHs) are ubiquitous organic pollutants that are commonly generated by anthropogenic activities involving combustion apart from natural sources like volcanic eruption and forest fires [1] [2] and [3]. PAHs are known to possess toxicity and they are often

carcinogenic or teratogenic in nature. The United States Environmental Protection Agency (US EPA) has listed 16 priority PAHs. Humans are exposed to various PAHs through different modes like inhalation, ingestion and dermal contact. The exposure to various toxic PAHs in day-day life leads to multiple health disorders [4]. There has been an alarming increase in the concentration of pollutants in the atmosphere and PAHs are the major parent compounds. FLA is listed as the priority PAH by the US EPA, along with 15 others. FLA is abundantly found in vehicle and diesel exhaust. It's a product generated by overheating and burning of firewood, incomplete combustion of fossil fuels etc. [5]. FLA is produced by the pyrolysis reaction at high temperatures by organic raw materials like coal and petroleum. Home heating, cooking with coal or wood is basic routes of human exposure [6]. FLA has been classified as a genotoxic agent and also enlisted as a carcinogen [7] [8]. There are very less reports related to the toxicity of FLA. FLA along with other PAHs are known to be present in aquatic systems [9] [10] [11] and [12]. The previous toxicity studies on FLA has been done mainly in aquatic organisms. Environmental pollutants are known to adversely affect the fertility in a wide range of organisms and they pose a major threat to the balance of biodiversity [13]. Most of the environmental pollutants and PAHs are known to have endocrine disrupting activity leading to hormonal imbalance ultimately leading to fertility disorders. Infertility is on the rise in the current decade compared to the previous few decades. Previous reports reveal that prolonged exposure to various environmental pollutants like PAHs cause endocrine disruption leading to infertility. Most of the PAHs have been reported as endocrine disruptors [14] and [15]. World Health Organization (WHO) reports an exponential increase in the number of infertile couples globally. Apart from genetic and other reasons environmental factors still exist to be a causal factor of infertility. Both male and female infertility has been increased mainly due to exposure to unwanted pollutants and lifestyle changes. The reproductive and developmental disorders caused by PAHs are studied in aquatic organisms [16] and [17]. The reproductive toxicity of FLA has not yet been explored in higher animals. Hence this study was designed to analyze the reproductive toxicity through the assessment of regularity in the estrous cycle of rats, which is a preliminary and important criteria for knowing the reproductive status of an individual.

## 2. MATERIALS AND METHODS

### Experimental Animals

Female adult rats (*Rattus norvegicus*) of wistar strain in the weight range 180 – 200g were procured from Indian Institute of Science, Bengaluru, Karnataka. The animals were maintained in polypropylene cages in a controlled environment with 12h light and dark cycle. The animals were given access to feed (Standard laboratory feed from Sai Durga feeds and Foods, Bengaluru) and water. Animal model used in the study was approved by the Institutional Animal Ethical Committee (IACE) and CPCSEA regulations were embraced during the study.

### **Experimental Profile**

The regularly cycling rats were randomly divided into three groups with six animals each. Group 1: Intact; Group 2: Fluoranthene administered (FLA 100mg/kg. B.W in corn oil); Group 3: Vehicle control (Corn oil). After 10 days of acclimatization, the FLA was administered for 21 days.

### **Determination of Estrous Cycle**

The stages of estrous cycle were determined by microscopic analysis of the predominant cell type in vaginal smears obtained daily from rats between 7:00 to 9:00am from a few days of purchase to the end of the experiment [18]. Those animals with 4-6 days regular cycle was included in the experiment and the irregular ones were taken out.

### **Blood Collection and Tissue Processing**

After the end of treatment duration, the rats were anesthetized with an overdose of ketamine (90-120 mg/kg B.W), and blood samples were obtained by cardiac puncture. The blood samples were centrifuged (3000 rpm for 15 minutes at 4°C and the serum was stored at -20°C until further analysis. After blood sampling, the animals were sacrificed, ovaries and uterus were excised, cleaned, weighed and stored at -80°C for analysis.

### **Histological Analysis**

Histology of ovary and uterus was studied by fixing the tissues in Bouin's fluid and adopting the routine paraffin method for light microscopic studies [19]. 3-5µm thick transverse or longitudinal paraffin sections, as may be applicable of ovary and uterus were obtained using a rotary microtome (Lieica, jena, Germany) and stained with haematoxylin and eosin, mounted in DPX (Dibutyl phthalate in xylene) mounting and observed in a Hund wetzlar microscope (Germany). Images were captured through Magnus live camera.

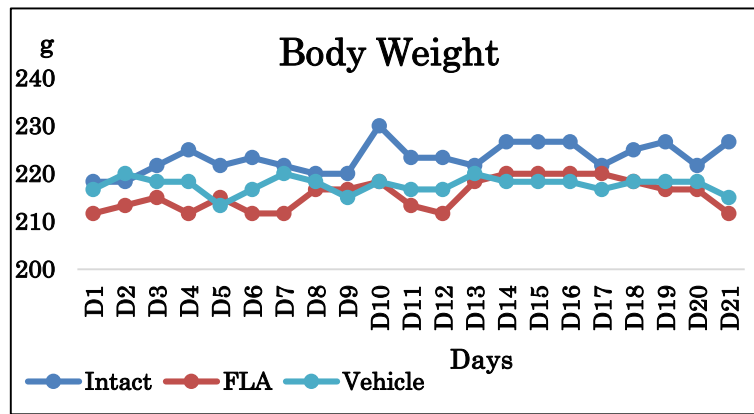
### **Statistical Analysis**

The values were expressed as mean  $\pm$  SD of six animals. The statistical significance was evaluated by one- way analysis of variance (ANOVA) using SPSS version 16 (SPSS Inc., Chicago, IL, USA) and individual comparisons were obtained by Duncan's Multiple Range Test [20]. Data were considered significant where the value of p was less than 0.05.

## **3. RESULTS AND DISCUSSION**

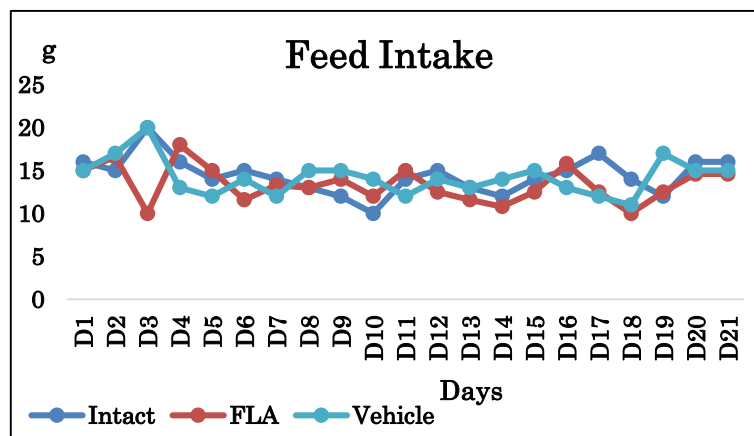
### **Body weight and feed intake**

The body weight and feed intake of the experimental animals were monitored throughout the experimental duration after one week of acclimatization.



**Figure 1:** Effect of Fluoranthene on body weight of experimental animals. Values were expressed as mean  $\pm$  SD (N=6). Bar with different alphabets are significantly different from each other and with same alphabets has insignificant changes ( $p < 0.05$ ).

The intact group animals exhibited a regular increase in their body weight. The FLA administered and other treatment groups did not show any significant increase in the body weight but exhibited moderate variations.

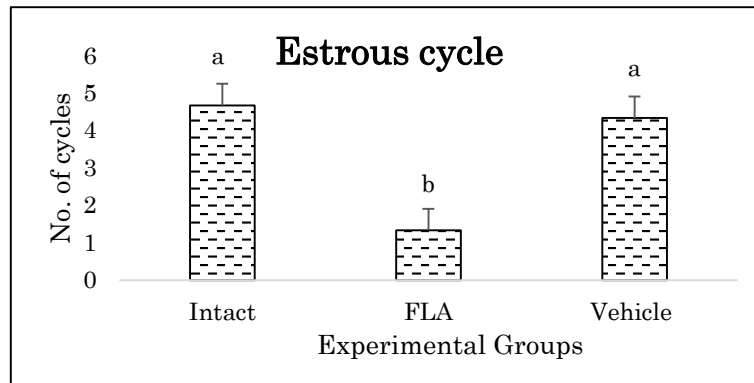


**Figure 2:** Effect of Fluoranthene on feed intake of experimental animals. Values were expressed as mean  $\pm$  SD (N=6). Bar with different alphabets are significantly different from each other and with same alphabets has insignificant changes ( $p < 0.05$ ).

The feed intake pattern did not significantly change among all the experimental groups. The feed intake of all the groups was similar at the start of the experiment and they exhibited fluctuations during the mid-treatment progress, but during the end of the experimental period all the groups exhibited similar levels of feed intake (Fig 1 and 2).

### Estrous cyclicity

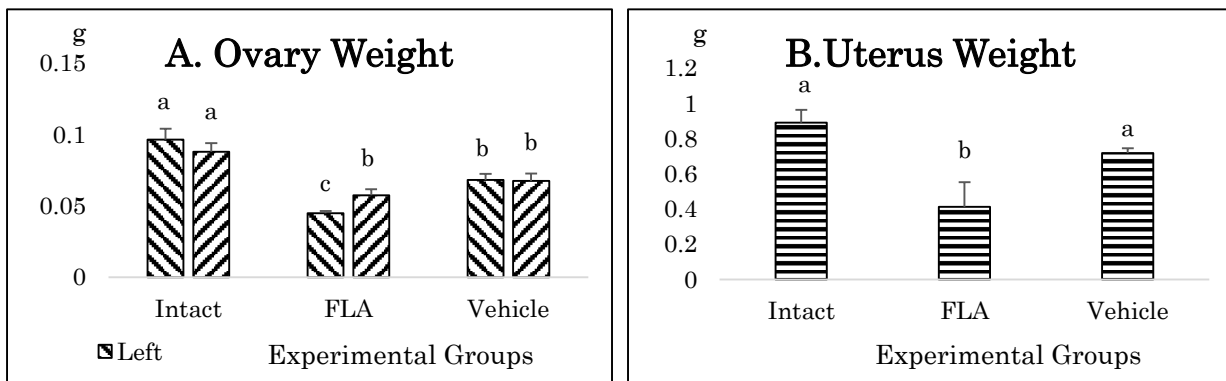
Estrous cycle is the reproductive cycle in rats. Generally, the estrous cycle in normal adult rats lasts 4-6 days comprising of Proestrus, Estrus, Metestrus and Diestrus [21]. The regular cycling indicates healthy reproductive status of an individual. If there is any alteration in the length of estrus cycle, it is an indicator of poor reproductive health.



**Figure 3:** Effect of Fluoranthene on number of regular estrous cycles of experimental animals. Values were expressed as mean  $\pm$  SD (N=6). Bar with different alphabets are significantly different from each other and with same alphabets has insignificant changes ( $p < 0.05$ ).

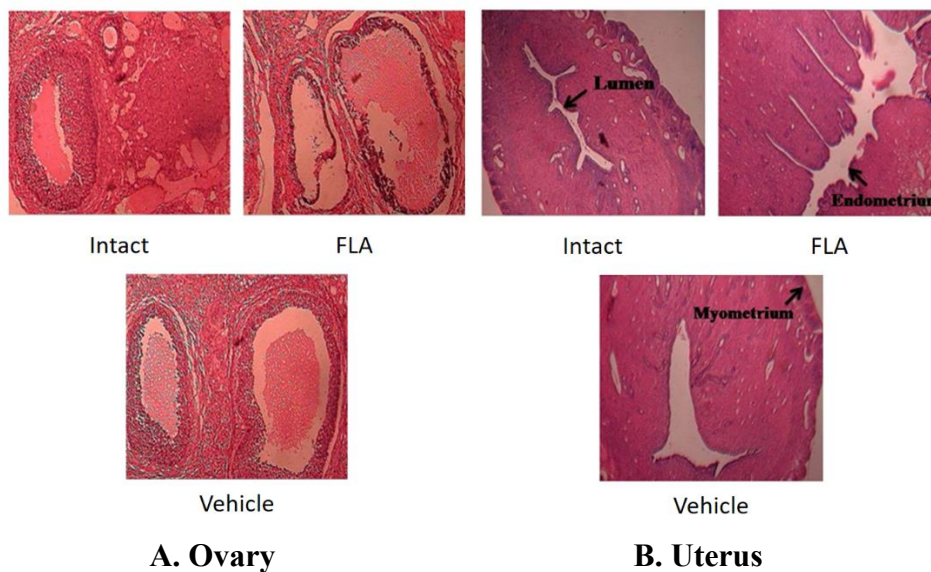
The assessment of the estrous cycle is less invasive compared to blood or tissue collection and this is a preliminary indicator of the reproductive health status of an adult individual. In this study, the FLA administered group exhibited a significant decline in the number of normal estrous cycles compared to that of intact and other vehicle groups (Fig 3). The animals exhibited prolonged diestrus stage. Earlier studies have shown that animals with impaired fertility show a reduction in the number normal estrous cycles upon exposure to pollutant compounds [22]. The FLA induced toxicity has been previously studied in various aquatic organisms [23]. FLA is known to be a potential teratogen, which has been already proven [24].

#### Ovary and Uterus weight



**Figure 4:** Effect of Fluoranthene on ovarian weight (A) and uterine weight (B) of experimental animals. Values were expressed as mean  $\pm$  SD (N=6). Bar with different alphabets are significantly different from each other and with same alphabets has insignificant changes ( $p < 0.05$ ).

The weight of reproductive organs ovary and uterus were compared across the experimental groups. The weight of ovaries in the FLA administered group significantly decreased compared to that of intact (Fig 4A). The uterus weight was not significantly different in the FLA administered group compared to intact (Fig 4B).

**Histological studies**

**Figure 5:** Effect of Fluoranthene on histology of ovary (A) and uterus (B) in experimental animals. The histological examination of ovaries showed a notable distortion in the ovarian morphology with an irregular arrangement of cells and follicles in FLA administered group. Whereas, the intact and vehicle group ovaries had well developed follicles and properly arranged cells (Fig 5). It has been reported that the administration of FLA in F344 rats did not cause any changes in the ovarian tissue morphology [25]. A similar trend was seen in the uterine tissues. The uterus of the FLA administered group had enlarged and disrupted lumen. The intact animals had normal thickness of uterine wall with a regular lumen (Fig 5).

**4. CONCLUSION**

The environment is heavily loaded with various pollutants. Particularly, the PAHs are ubiquitous and the chances of living beings getting exposed to them is pretty high. The PAHs are known to impose various adverse effects in different organisms. In this study, the effect of a PAH Fluoranthene has been studied in rats and the findings clearly indicate that the FLA has the ability to disrupt the regular reproductive cycle in rats. This may cause various reproductive disorders, thereby leading to infertility.

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**CONFLICT OF INTEREST**

The authors hereby declares there is no conflict of interest.

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