Impact of Benzo (a) pyrene on thyroid glands and their associated hormones of male Mice *Mus musculus*

ABSTRACT

Benzo[a]pyrene is the main representative of polycyclic aromatic hydrocarbons, and has been repeatedly found in the air, surface water, soil, and sediments. Human exposure to Benzo[a]pyrene B[a]P is therefore common.Benzo (a) pyrane(BaP) which is mainly presented in newspaper ink and inhibits the function of the thyroid and other endocrine glands of the human system, due to several disorders that occur in the human body.Healthy mature male mice *Mus musculus* having weight of 125± 5 gm were used for the experiments. The effects of a Benzo(a) pyrene (0.65mg/25g body weight/twice in a week) during experimental periods of 60 and 90 days in male mice *Mus musculus* showed significant difference in body weights compared to control.We assessed thehormonalestimation of thyroid treated with Benzo (a) pyrene by using an appropriate ELISA assay kit and histopathological changes were observed under a light microscope (magnifications 100X) in the thyroid tissue of malemice*Mus musculus* exposed to BaPafter 60 and 90 days. During the study, we observed that in treated mice thyroid hormone concertation was affected the structure of the thyroid follicles was disturbed and epithelial cells were necrotic compared with the control mice which indicates that the BaP may lead to changes in the thyroid morphology as well as secreted hormone concentration in exposed treated male mice.

Keywords:*Benzo (a) pyrane; Mus musculus;hormonal, histopathological;thyroid follicles*

1. INTRODUCTION

“Polycyclic aromatic hydrocarbons (PAHs) such as Benzo (a) pyrene (BaP) are one of the most widespread persistent organic pollutants in terrestrial and marine environments” [1,2]. “Benzo (a) pyraneis a crystalline, aromatic hydrocarbon consisting of five fused benzene rings found in coal tar with the formula $C_{20}H_{12}$ in the nineteenth century”[1]. “The main sources of BaP in food are from pollution materials in the environment or produced by the pyrolysis of amino acids, carbohydrates, and fatty acids”[2]. “It is primarily found in automobile emissions, cigarette smoke, coal tar, charcoal-broiled foods and newspaper ink”[3]. “For their lipophilic and hydrophobic characteristics, polycyclic aromatic

hydrocarbon (PAH) finally accumulates in the food chain. Dietary exposure accounts for more than 90% of the total exposure to PAHs in the general population in various countries"[4]. "Nowadays, BaP is the first pollution indicator of PAHs in food designated by the scientific community"[5].

"BaP is the first pollution indicator of PAHs in food. Therefore, foodborne BaP contaminants are a primary source of BaP uptake by humans.After entering the body, except for a small part of BaP excreted in the feces in its original form, most of the BaP accumulated in the gastrointestinal tract, epididymal fat, lung, liver, brain, and kidney"[6]. "BaP is highly lipophilic and can be easily absorbed into cells through the plasma membrane and can be metabolized into dozens of metabolites through AhR and aromatic hydrocarbon metabolizing enzymes, such as 1, 2-dihydroxy-1, 2-dihydrobenzopyrene, benzopyrene diketone, and Benzo[A]Pyrene Diol Epoxide (BPDE)".[7,8,9] "BaPmay directly interfere with thyroid gland function and then change the its structure, leading to the interruption of hormone synthesis, thus reducing thyroid hormone (TH) circulation and tissue level"[10]. "Some studies suggested that BaP could affect TH synthesis by inhibiting thyroid peroxidase (TPO) activity" [11].

"BaP is well known for its carcinogenic activity early in 1930, and numerous studies since the 1970s have documented links between BaP intake and cancers"[1,12,13]. "The most representative PAHs is BaP, which can be absorbed through the skin, respiratory, and digestive tract and has become a public health concern due to its carcinogenic, teratogenic, and mutagenic effects" [14,12].

"In addition, collective evidence from these studies revealed that the endocrine toxicity of foodbornecontaminants like food material wrapped by newspaper ink is mainly attributed to PAHs, especially BaP". [28]In this study, oral exposure to BaP in male mice*Mus musculus*was modeled by evaluating TSH, T3 and T4 hormone concentrations in blood serum and histopathology to check the effect of thyroid tissue and glands.

2. Materialsand methods

2.1 Animal handling and ethical approval:

For this study adult male *Mus musculus*mice were used.The healthy mature male albino male mice *Mus musculus* having weight of 125± 5 gm were used for the experiments. Animals were kept at an ambient room temperature of 26 ± 2°C with a relative humidity of 75%, under a controlled 12hour light/dark cycle. The mice were reared on a standard diet and tap water. Mice were kept in the Animal House of the Endocrinology Laboratory, Bioscience

Department, Barkatullah University, Bhopal, M.P. Animal care and handling were performed according to guidelines issued by the Committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delh, India.

2.2 Chemical and Dose preparation

Current study chemical Benzo (a) pyrane a polycyclic aromatic hydrocarbon was used. This chemical was purchased from Sigma-Aldrich(St. Louis, MO, USA). and the dose was prepared and given orally alternately (twice times) in a week.

2.3 Dose of Chemical

Benzo (a) pyrene 0.65mg/25g body weightdissolved in 0.2 ml coin oilwas orally administeredtwo timesin a week for 90 dayswith and routine slandered diet.

2.4 Experimental design:

Male *Mus musculus* mice were divided into two groups of 10 each. The first controlled group was kept for a time period of 60 and 90 day's duration, received a balanced diet and water ablitium with proper photoperiodic intervals of 12 hrs (Light: Dark) duration. While, second group was treated with Benzo (a) pyrene with corn oil administrated orally two times in a week for the duration of the 60 and 90 days.

2.5 Animal Exposure

First, male mice*Mus musculus* were adapted to the experimental conditions for 7 days. The mice were then randomly divided into two experimental groups, control(0.2ml corn oil) and B[*a*]P (0.65mg/25g body weight, dissolved in 0.2ml corn oil coin oil). The mice in each group were exposed to the substance by gavage two times in week for 90 days. The mice were sacrificed 24 h after the last exposure and the thyroid gland was taken.

2.6 Body Weight:

Body weight of all the experimental male mice*Mus musculus* were taken initially at zero days and at the end of the each different interval after the ie60 and 90 days and all the values were expressed in grams. The body weight gain by the mice was calculated by the formula:

Body weight gain= Final body weight – Initial body weight

Percentage weight gain=

Weight

$$\frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

2.7 Biochemical parameters:

Blood samples were collected in a separate serum tube by orbital puncture just immediately before the sacrifice of the animal by cervical dislocation. After the collection of the blood, it was kept undisturbed for 15-30 min., then undergo for centrifugation at 10,000 rpm for 10 minutes in a cooling centrifuge and serum was separated, which was then immediately aspirated out for hormonal estimation of stored at -20 ^{0}C to -70 ^{0}C for hormonal estimations. Repeated freeze and thaw cycle will be avoided because it will destroy many serum components. Hormonal estimation of thyroid treated with Benzo (a) pyrene was done by using an appropriate ELISA assay kit (ELK0554, ELK0451, ELK2284), ELK Biotechnology, Co., Ltd. USA.

2.8 Histopathological Examination

"After 60 and 90 days the mice were sacrificed, the thyroid gland was quickly removed, and washed with precooling phosphate-buffered saline, and their color and morphological changes were observed. After being fixed in 4% paraformaldehyde, the tissues were embedded in paraffin, and cut into 3 μm sections. The tissues were stained with Hematoxylin and eosin (H&E), and observed under a light microscope (magnifications 100X)".[28]

2.9 Statistical analysis:

Results are expressed as the mean and standard error of the mean of different groups. The inter-group variation was analyzed by using a one-way analysis of variance (ANOVA) followed by Tukey's test [15]. All P values were statistically analyzed. The $P<0.05$ was considered statistically significant. In some dependent variable difference ($P<0.001$) was considered a high significance association.

3. RESULTS

3.1 Impact of Benzo[a] pyrene on mice body weight

Our experiment on the duration of 90 days gave us the favorable and substantial differences between both the groups of experimental studies, where the second group was treated with Benzo (a) pyrene with corn oil for the duration of the 90 dayswas given, to explore the impact of Benzo (a) pyran. The highly significant body weight gain of mice treated with Benzo (a) pyran group as compared to the body weight gain of control groups. The results are

summarized in Table 1 and Fig. 1.The treated group showedhighly significant differences in the body weightof mice at duration of 60 days (24.05±0.28) and 90 days(27.88± 0.27) as compared to the control group.

3.2 Impact of Benzo[a]pyrene on thyroid gland hormones

A significant effect was found in the secretion of thyroid gland hormones like $T_{3,}$ T_4 and TSH in treated groups exposed to Benzo (a) pyrene(0.65mg/25g body weight/twice in a week) compared with control groups at 60 and 90 days. The Benzo (a) pyran-treated mice showed a mark elevation in the Triiodothyronine (T3) level (ng/ml) in the blood serum after 60 and 90 days when comparedto the control group. The serum T3 level significantly ($P \leq 0.05$) increasedin 90 days, however, a more significant ($P \leq 0.01$) increased level was noticed in the duration of 60days in the treated group as compared with the control (Table2 and Fig.2).

The thyroxine (T4) level was elevated with a significant difference ($p<0.001$) in the duration of 60 and 90 days after being exposed to Benzo (a) pyran as compared with the control group (Table 3 and Fig. 3).

The effect of a Benzo(a) pyrene is a more significant difference ($p<0.01$) during experimental periods 60 and 90 days as compared to control mice of TSH hormone(Table 4 and Fig. 4).Due to the impact of Benzo (a) pyrene on the thyroid gland, which resultedin anincrease in the concentration of the T3,T4 & TSH secretion and inhibits its signaling and function.

3.3 Histopathology and Histological changes in thyroid gland exposed by Benzo(a) pyrene

The transverse section of the control thyroid gland of male mice *Mus musculus* up to 60 and 90 days showed normal follicular cells with colloid in the lumen (Fig. 5), However, the thyroid gland of mice treated with Benzo(a) pyrene for 60 days showed enlarged follicles with more vacuolization, however, the number of follicles has become reduced (Fig. 5). However, the thyroid gland of mice up to 90 days exhibited more enlarged epithelial cells, lumen were filled with a large amount of collides (Fig.5)

4. DISCUSSION

“Some published experimental studies on animal or cell culture systems report evidence of thyroid-specific effects from certain polycyclic aromatic compounds”[16,17,18]. “Toxicity studies in rodents, particularly rats, form the standard basis for risk assessment of many

substances. However, when considering the relevance of rodent data on the thyroid gland, it is well known that the rodent thyroid, both in terms of its physiology and histology, differs substantially from the primate thyroid, and the management of the thyroid hormones themselves is radically different, resulting in a much more rapid thyroid hormone turnover rate"[19]. "The binding of thyroid hormones to thyroid-binding globulin (TBG) results in their protection from degradation and elimination"[20].

The present study indicated that the toxicological effect of B[a]P (polycyclic aromatic hydrocarbons) increased the body weight of experimental mice. Similar but other studies reported a decrease in the body weight of mice exposed by polyaromatic hydrocarbons and para-nonphenol [21,22].

Some published experimental studies on animal or cell culture systems report evidence of thyroid- and other organs-specific effects from certain polycyclic aromatic compounds [16,17,18]."The most representative poly aromatic hydrocarbons is B[a]P, which can be absorbed through the skin, respiratory, and digestive tract and has become a public health concern due to its carcinogenic, teratogenic, and mutagenic effects"[14,12]."In recent years, some studies have demonstrated that EDCs (including B[a]P and BDE-47) affect thyroid structure and hormone secretion"[23,24].

"The morphology of rat thyroid cells treated with 5 mg/kg of B[a]P and 0.5 mg/kg of BDE-47 changed. Microscopically, in the B[a]P group, it was found that a part of the thyroid follicle structure was destroyed, the follicular epithelial cells were necrotic and shed into the follicular cavity, the glial cells accumulated in the individual follicular cavity, and the color was darker"[25].

"The thyroid tissue structure and secretion changed significantly after Liza Abu was exposed to B[a]P for 2 weeks"[24]. "B[a]P exposure significantly decreased the circulatory concentrations of T3 and T4 and increased the circulatory concentrations of thyroid-stimulating hormone in the treated rats and fish"[24,26]."Another study suggested that the primary toxic effects of short-term exposure to B[a]P in zebrafish was compromised hypothalamic-pituitary function, which would then hamper the adequate functional maturation of thyroid follicles and TH synthesis" [27]

During thestudy we found that the body weight of mice, hormone concentration, and histopathological changes inthe thyroid gland found significant results in male mice *Mus musculus*exposed by Benzo(a) pyrene(dose of 0.65mg/25g body weight/twice in a week) with under observation of the periods of 60 and 90 days.

This study supported other research studies and showed highly significant results in experimental animals, However, we suggest here that we avoid or minimize the polyaromatic hydrocarbons like Benzo(a) pyrene in our food diet and cure our health. In the further, future more toxicology studies are needed to its prolonged inhalation impact on human health.

5. CONCLUSION

Benzo(a) pyrene is a polycyclic aromatic hydrocarbon, that causes toxic effects in the thyroid, adrenal gland, and testes on male mice *Mus musculus*. The effects of a Benzo(a) pyrene (0.65mg/25g body weight/twice in a week) during experimental periods of 60 and 90 days in male mice *Mus musculus* showed significant difference in body weights compared to control. It may also show that both may be directly affected on the target cell or through positive, negative thyroid hypophysis gonadal axis. The Benzo(a) pyrene-induced and decreased a remarkable effect on the levels of triiodothyronine (T3), and tetraiodothyronine (T4) and TSH hormone of the thyroid gland. During the histopathological study of the thyroid gland the thyroid epithelial and colloid were more effective as compared to the control in 90 days exposure by Benzo(a) pyrene. However, we suggest here that we avoid or minimize the polyaromatic hydrocarbons like Benzo(a) pyrene in our food diet and cure our health.

Ethical Approval

The present study is a part of a research plan approved by the university/Institutional Animal Ethical Committee (IAEC) with certificate number: 1885/GOI/S/16/CPCSEA/IAEC/BU/28.

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FIGURES LEGENDS:

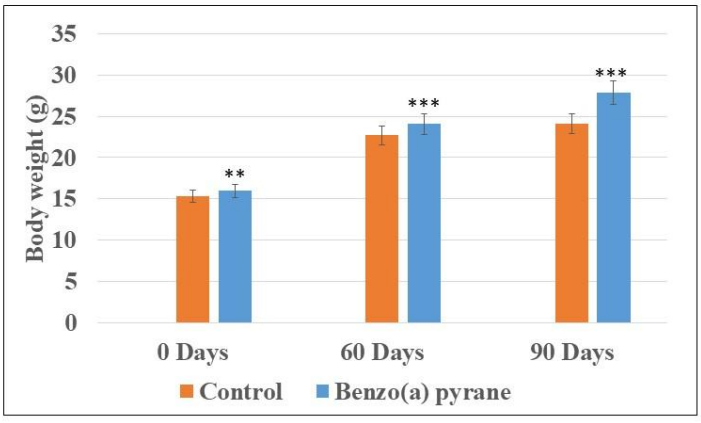

Fig. 1

Fig. 1: Effect of Benzo(a) pyrene of Body Weight (g) of male mice *Mus musculus* after 0 60 and 90 days as compared to control. Data represent Mean ± SEM values, ** ($p<0.01$) and *** ($p<0.001$) compared to control by one way ANOVA.

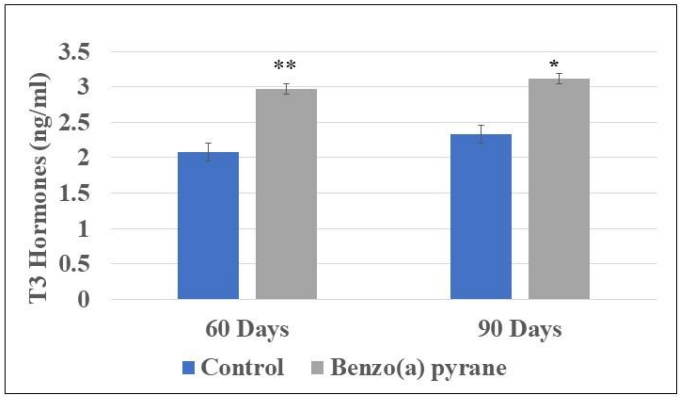

Fig. 2

Fig. 2. Effect of Benzo (a) pyrene of serum T3 Hormones (ng/ml) level of male mice *Mus musculus* after 60 and 90 days as compared to control. Data represent Mean ± SEM values, * ($p<0.05$) and** ($p<0.01$) compared to control by one way ANOVA.

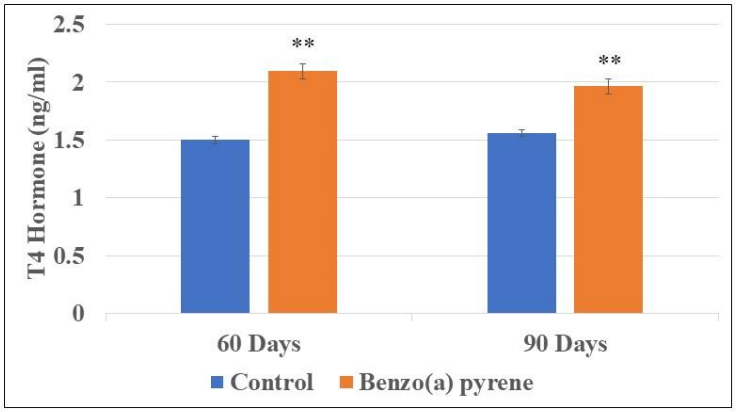

Fig. 3

Fig. 3. Effect of Benzo (a) pyrene of serum T4 hormones (ng/ml) level of male mice *Mus musculus* after 60 and 90 days as compared to control. Data represent Mean ± SEM values ** ($p<0.01$) to control by one way ANOVA.

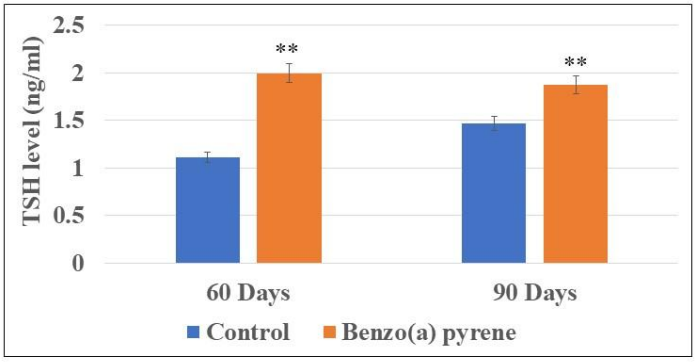

Fig. 4

Fig. 4. Effect of Benzo(a) pyrene and ink of serum TSH level (ng/ml) level of male mice *Mus musculus* after 60 and 90 days as compared to control. Data represent Mean ± SEM values, ** ($p<0.01$) compared to control by one way ANOVA.

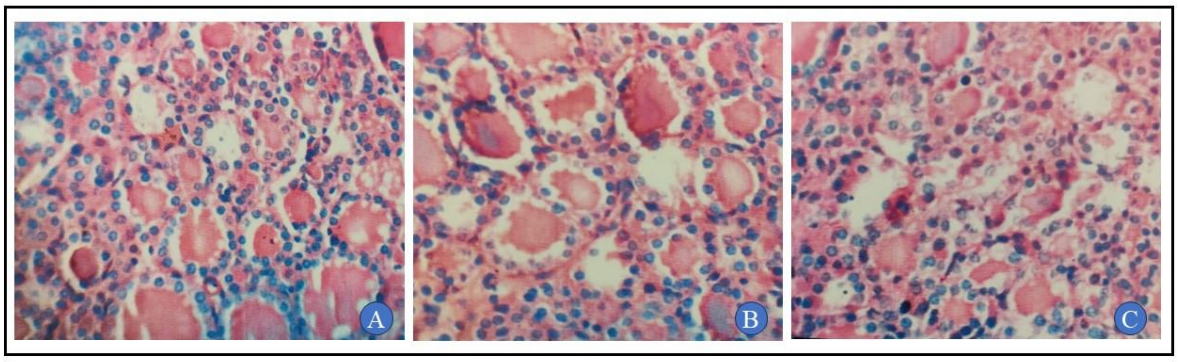

Fig. 5

Fig. 5. Microphotograph of Thyroid gland transverse section (H & E 100X). A . Control thyroid gland showing normal follicular cells with colloid in lumen B. Thyroid gland treated with Benzo(a) pyrene for 60 days showing enlarged follicles with more vacuolization, however, the number of follicles has become reduced. C. Thyroid gland treated with Benzo(a) pyrene for 90 days showing epithelial cells become enlarged, the lumens areformed.

Table 1. Effect of Benzo (a) pyrene of Body Weight (g) of male mice *Mus musculus* after 0, 60 and 90 days as compared to control.

Group	**Body Weight (g) in different durations**		
	0 Days	**60 Days**	**90 Days**
Control	15.30± 0.15	22.73± 0.08	24.10±0.21
Benzo(a) pyrane	15.96±0.07**	24.05±0.28***	27.88± 0.27***

All values are expressed in Mean±SEM

** More significance difference ($p<0.01$) compared to control by one way ANOVA.

*** High significance difference ($p<0.001$) compared to control by one way ANOVA.

Table 2. Effect of Benzo (a) pyrene of T3 Hormones (ng/ml) level of male mice *Mus musculus* after 60 and 90 days as compared to control.

Group	**Duration parameter (Mean±SEM)**	
	60 Days	**90 Days**
Control	2.08±0.12	2.33± 0.21

Benzo(a) pyrane	2.96±0.16**	3.11±0.21*

All values are expressed in Mean±SEM

* Significance difference ($p<0.05$) compared to control by one way ANOVA.

** More significance difference ($p<0.01$) compared to control by one way ANOVA.

Table 3. Effect of Benzo(a) pyrene of T4 hormone (ng/ml) level of male mice *Mus musculus* after 60 and 90 days as compared to control

Group	**Duration parameter (Mean±SEM)**	
	60 Days	**90 Days**
Control	1.51±0.01	1.56± 0.01
Benzo(a) pyrene	2.09±0.01***	1.96±0.02***

All values are expressed in Mean±SEM

*** High significance difference ($p<0.001$) compared to control by one way ANOVA.

Table 4. Effect of Benzo(a) pyrene of Serum TSH level (ng/ml) level of male mice *Mus musculus* after 60 and 90 days as compared to control.

Group	**Duration parameter (Mean±SEM)**	
	60 Days	**90 Days**
Control	1.113 ±0.017	1.465 ± 0.02
Benzo(a) pyrene	1.993 ±0.024**	1.868 ±0.021**

All values are expressed in Mean±SEM

** More significance difference ($p<0.01$) compared to control by one way ANOVA.