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# The Effect of Isobutyl Paraben an Endocrine Disrupting Chemicals on Juvenile Male Rats

**Mahesh Tanwade[1], Basawarajeshawri Indur[2], Vishwajit B Darekar[3*]**

*[1,2,3*]Department of Post Graduate Studies and Research in Zoology, Sharnbasva University, Kalabuargi-585103, Karnataka.*
**Corresponding Author: Vishwajit B Darekar*
**Email ID: vishwajeetdarekar@gmail.com, maheshtanwade1997@gmail.com*

**Abstract**

**Background:**
Isobutyl paraben (IBP) is a commonly used preservative with recognized estrogenic activity and potential endocrine-disrupting effects. Despite its widespread exposure, limited data exist on its impact during juvenile development, a critical period of male reproductive maturation.
**Methods:**
Juvenile male Wistar rats were administered IBP orally at 10, 20, and 50 mg/kg/day for 70 days, with corn oil as the vehicle control. Endpoints included clinical observations, growth performance, food intake, functional observation battery, hematological and biochemical profiling, organ weight analysis, and histopathology of reproductive and systemic organs.
**Results:**
IBP exposure resulted in dose-dependent suppression of body weight gain and food consumption, alongside delayed preputial separation. Hematological findings revealed significant reductions in leukocyte counts, while clinical chemistry demonstrated altered glucose levels, dyslipidemia, and elevations in hepatic enzyme activity. Organ weight assessments indicated significant changes in kidneys, liver, testes, and spleen. Histopathological evaluation confirmed degenerative changes in testicular tissue and epididymis, particularly at higher doses.
**Conclusion:**
Sub chronic IBP exposure during the juvenile period disrupts growth, metabolism, and male reproductive development, underscoring its endocrine-disrupting potential and raising concerns regarding long-term health risks

**Keywords**: Isobutyl paraben; Endocrine disruptors; Juvenile Wistar rats; Reproductive toxicity; Histopathology; Hematology.

## 1. INTRODUCTION:

Isobutyl paraben is a frequent preservation agent in many consumer goods since it is cheap and kills germs (Hoberman et al., 2008). But people are becoming more worried about how parabens, such as isobutyl paraben, could mess with the endocrine system. Endocrine-disrupting chemicals can mess with normal hormone function. Parabens are thought to be EDCs because they are similar in structure to estrogen and can bind to estrogen receptors (Hoberman et al., 2008) (Maske et al., 2018).

Isobutylparaben, in particular, has been a reason for concern because it is present in such small amounts. It has a longer alkyl chain and is more lipophilic than other parabens, such as methylparaben and ethylparaben. These traits may make it easier to build up in tissues and move through biological membranes. Research has indicated that IBP interacts with estrogen receptors, which could change how the endocrine system works by messing up the hormonal control of growth and reproduction. Considering these attributes, it may be deduced that isobutyl paraben, along with other parabens, may contribute to adverse health effects, particularly via its estrogenic activity (P.D. Darbre *et al.,* 2002).

People are worried about how these substances can affect human health, especially reproductive health. Recent research indicates that isobutyl paraben and other parabens may influence male fertility and sexual

conduct. In animal models, these investigations have identified alterations in mating behavior, reduced testosterone levels, and compromised spermatogenesis (Oishi, 2002; Maske *et al.,* 2018; Oishi, 2001).

The male reproductive system is very sensitive to changes in hormones, especially during important periods of development. Being around EDCs during these times can have long-term impacts that may even affect future generations (Oishi, 2001) (Oishi, 2002).

The objective of this study is to examine the impact of isobutylparaben, an endocrine-disrupting chemical, on juvenile male rats, therefore elucidating the endocrine-disruptive properties of this commonly used preservative.

## 2. MATERIALS AND METHOD

### 2.1. Ethical Approval

The Institutional Animal Ethics Committee (IAEC) gave its permission for all animal experiments. The Department of Postgraduate Studies and Research in Zoology at Sharnbasva University in Kalaburagi, Karnataka, India, gave its approval number (SUK/ZOL/IAEC/05/2024-25). The CCSEA Reg. No. 2236/PO/ReBiBt/S/23/CCSEA.

### 2.2. Test Chemical

Isobutylparaben (CAS No. 4247-02-3, with a purity of 99.38%) was sourced from Tokyo Chemical Industry (TCI) India Pvt Ltd, located at Genome Valley Road, Turkapally, Hyderabad, Telangana-500078, India, and was dissolved in corn oil (as the vehicle).

### 2.3. Animals and Husbandry

This study used Wistar rats from the National Institute of Nutrition in Hyderabad, India. Their small size, docile nature, short lifespan, ease of care (feeding, breeding, and handling), and the extensive existing research data on their biology make them suitable research subjects (Shayne Christopher, 1992). Thirty-two Juvenile male Wistar rats were housed individually in polycarbonate cages with ad libitum access to food and water. Environmental conditions were maintained at 22°C ± 3°C and 50% ± 20% relative humidity, using sterilized rice paddy husk bedding. Animals were grouped based on body weight (within ± 5 grams per group) and uniquely identified by tail markings within numbered cages (four animals per cage). The study employed specific study and group numbers for data organization.

### 2.4. Compound Preparation

IBP is a white crystalline powder that is stored at room temperature. Each day, a fresh formulation is prepared by dissolving the compound in corn oil to ensure precise dosing during administration.

### 2.5. Compound Administration

Juvenile male rats were given doses daily via oral gavage using a stainless steel, ball-tipped dosing cannula. Dosages were based on body weight (5 mL/kg) volume and calculated using the most recent body weight measurements. Juvenile male rats received doses for 70 days. Four groups of rats were studied: a control group (corn oil), a low-dose IBP group (10 mg/kg/day), a medium-dose IBP group (20 mg/kg/day), and a high-dose IBP group (50 mg/kg/day). Each group received their respective doses via oral gavage for 70 days.

### 2.6. Clinical signs

Twice a day, all rats were observed for clinical signs and mortality. Before administering the test item, a detailed clinical examination was carried out. Beginning on day 1, weekly evaluations will be carried out for all animals in the groups. During the comprehensive clinical examination, every rat exhibited various changes in its body, including alterations in skin, fur, eyes, and mucous membranes; occurrences of secretions and excretions; autonomic activities such as piloerection, lacrimation, unusual respiratory patterns, and pupil size; changes in posture and gait; responses to handling; the presence of clonic or tonic movements; and stereotypies like excessive grooming, repetitive circling, or bizarre behaviors such as self-mutilation and walking backward.

**2.6.a. Functional Observation Battery Tests:** All rats underwent a neurological examination during the final week (10th week) of their treatment period.

**2.6.b. Home Cage Observations:** The home cage was observed by all animals for posture and to check for any unusual vocalizations or convulsions.

**2.6.c. Open Field Observations:** In an open-field test, rats were put into an arena with clean absorbent paper and observed for 2 minutes. The following behavioral parameters were scored: gait, posture, mobility score, arousal level, clonic or tonic movements, stereotypic behaviors, bizarre behavior, urination, defecation, rearing, and vocalizations (IPCS, 1986).

**2.6.d. Sensory Reactivity Measurements:** The following observations were recorded and observed by rats during sensory reactivity measurements: approach response, touch response,
click response, tail-pinch response, pupil response, and aerial righting reflex. (D.E. Tupper and R.B. Wallace, 1980).

**2.6.e. Motor Activity:** The motor activity of rats was observed using an electronic animal activity measuring device from Columbus Instruments. For 45 minutes, the instrument monitored the rats individually in the activity cages. During this motor activity following parameters were measured: the stereotypic time (small movements) in seconds, the ambulatory time (large ambulatory movement) in seconds, horizontal counts, and ambulatory counts.

**2.6.f. Hindlimb Landing Foot Splay:** The landing foot splay was assessed using a similar procedure as published by P.M. Edwards and V.H. Parker (1977). The animal was marked with ink on their hindfoot heel, and then placed on a recording sheet from a height of 30 cm, repeat the procedure for thrice. The inking marks were measured in centimetres (cm). In order to conduct the statistical analysis, the mean of the three splash values was used.

**2.6.g. Grip Performance:** The grip performance of the hind and forelimbs was tested using a computerized dual gripping force measuring device (make: Orchid Scientific). Three trials were performed for each rat, i.e., three trials each for forelimbs and hindlimbs. The average of three trials for both forelimbs and hindlimbs was calculated (O. A. Meyer, H. A. Tilson, W. C. Byrd, and M. T. Riley, 1979)

**2.6.h. Indices of Sexual Maturation:** The age and body weight at preputial separation was recorded.

### 2.7. Body Weight and Food Consumption

Body weights were recorded for each individual on Day 1 of treatment (prior to dosing) and subsequently at approximately weekly intervals (7 days ±1). Food consumption was monitored weekly, and individual daily food intake was calculated by dividing the total food consumed during each interval by the number of days in that period.

### 2.8. Haematology and Biochemistry

On Day 70, clinical pathology tests were done on all of the animals in Groups 1–4 to confirm their hematological and clinical chemistry levels. Before taking blood samples, the animals were not given any food for the night. Before the animals were killed, blood samples were taken from the retro-orbital sinus using isoflurane anesthesia. Samples were taken in tubes for hematology and in heparinized tubes for clinical chemistry examination. The ADVIA 2120 hematology system was used to look at hematological parameters like hemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), platelet count (Plat), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), differential leukocyte count (DLC), reticulocyte count (Retic), and mean platelet volume (MPV). The Dimension RxL Max Clinical Chemistry System measured clinical chemistry parameters like alanine aminotransferase (ALT), albumin (Alb), alkaline phosphatase (ALP), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (Creat), gamma-glutamyl transferase (GGT), glucose (Glu), inorganic phosphate (Pi), potassium (K), sodium (Na), lactate dehydrogenase (LDH), total cholesterol (T. Chol), total protein (T. Pro), total bilirubin (T. Bil), triglycerides (Trig), globulin (Glob), albumin-to-globulin ratio (A/G), calcium (Ca), and chloride (Cl).

### 2.9. Organ Weights and Histopathology

During the terminal sacrifice, all animals from Groups 1 to 4 were euthanized via exsanguination under isoflurane anesthesia after blood collection for clinical pathology assessments. The testes, coagulating glands with seminal vesicles, epididymides, heart, kidneys, liver, pituitary glands, prostate, spleen, thymus, thyroid, and parathyroid were all weighed. The gathered tissues underwent standard paraffin embedding, were sliced into 4–5-micron sections with a rotary microtome, affixed to slides, and stained with Mayer's haematoxylin and eosin for histological examination.

### 2.10. Statistical Analysis

All data were expressed as mean ± standard deviation. Statistical analysis was performed using GraphPad Prism 10 software, utilizing two-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test to assess differences between groups. Statistical significance was defined as a *p*-value of less than 0.05.

## 3. RESULTS

### 3.1. Clinical Signs

During the study period, there were no signs of mortality, obvious toxicity, or clinical symptoms related to treatment.

### 3.2. Body weight and Food Consumption

The body weight changes in rats administered IBP (10, 20, and 50 mg/kg/day) over 70 days were different from those in rats given maize oil as a control. The groups that had IBP lost weight in a dose-dependent way, but the control group slowly gained weight, reaching roughly 230 g by the end of the experiment on Day 70. The medium- and high-dose groups showed a clear increase in attenuation, with the highest dose having the most noticeable effect. The low-dose group, on the other hand, showed very little change.

**Table 1: Body Weights of Animals Induced with IBP**

| Weeks | Vehicle Control (Corn Oil) | Low Dose (10mg/kg/day) | Medium Dose (20mg/kg/day) | High Dose (50mg/kg/day) |
|---|---|---|---|---|
| **No. of Animals** | **8** | **8** | **8** | **8** |
| **Initial** | 41.50 ± 5.210 | 46.75 ± 4.652 | 48.50 ± 7.309 | 48.50 ± 7.231 |
| **Week 1** | 60.50 ± 4.629 | 63.75 ± 8.102 | 66.50 ± 10.784 | 69.25 ± 9.677 |
| **Week 2** | 88 ± 11.212 | 97.75 ± 8.779 | 102 ± 14.89 | 103.5 ± 12.456 |
| **Week 3** | 117.375 ± 12.682 | 128 ± 12.421 | 134.75 ± 16.935 | 129 ± 14.343 |
| **Week 4** | 142.875 ± 15.615 | 166.375 ± 14.101 | 176 ± 13.18 | 164.5 ± 15.66 |
| **Week 5** | 176.375 ± 23.47 | 192.75 ± 19.912 | 203.25 ± 19.211 | 191 ± 19.683 |
| **Week 6** | 198.25 ± 23.285 | 214.75 ± 21.803 | 224.5 ± 18.815 | 210.5 ± 21.772 |
| **Week 7** | 208.125 ± 28.347 | 222.25 ± 21.579 | 228 ± 20.114 | 224 ± 23.152 |
| **Week 8** | 231.125 ± 25.136 | 242.25 ± 27.722 | 240.75 ± 19.506 | 245.25 ± 23.903 |
| **Week 9** | 244.375 ± 24.727 | 244.5 ± 22.239 | 240.75 ± 23.298 | 228.75 ± 20.401 |
| **Terminal** | 234 ± 21.804 | 232.5 ± 21.454 | 225.75 ± 21.842 | 221.125 ± 19.075** |

*Significantly different from the control according to Dunnett's test (P < 0.05); ***Highly significantly different from the control according to Dunnett's test (P < 0.001); Values are expressed as mean ± SD.*

These results show that IBP stops weight gain in a dose-dependent way, which could affect growth or metabolism over time. Food intake significantly decreased in a dose-dependent fashion in IBP-treated groups relative to the vehicle control. Medium-dose and high-dose animals exhibited a reduction in food consumption compared to the normal control group, persisting until the conclusion of the trial. These data suggest that IBP influences food intake, perhaps due to metabolic or physiological stress.

**Table 2: Food Consumption of Animals Induced with IBP**

| Organ Weeks | Vehicle Control (Corn Oil) | | Low Dose (10mg/kg/day) | | Medium Dose (20mg/kg/day) | | High Dose (50mg/kg/day) | |
|---|---|---|---|---|---|---|---|---|
| **Week** | **Mean & S.D.** | **SE** | **Mean & S.D.** | **SE** | **Mean & S.D.** | **SE** | **Mean & S.D.** | **SE** |
| **No. of Animals** | **8** | | **8** | | **8** | | **8** | |
| **Week 1** | 5.25 ± 0.44 | 0.31 | 5.28 ± 0.13 | 0.09 | 5.86 ± 0.11 | 0.08 | 5.87 ± 00 | 00 |
| **Week 2** | 9.21 ± 0.66 | 0.46 | 10.87 ± 1.50 | 1.06 | 9.69 ± 1.68 | 1.19 | 9.59 ± 0.49 | 0.34 |
| **Week 3** | 11.59 ± 0.40 | 0.28 | 10.34 ± 4.64 | 3.28 | 9.68 ± 1.50 | 1.06 | 9.12 ± 3.44 | 2.44 |
| **Week 4** | 11.58 ± 0.24 | 0.17 | 10.31 ± 2.21 | 1.56 | 8.61 ± 0.28 | 0.20 | 8.48 ± 0.02 | 0.01 |
| **Week 5** | 11.41 ± 0.22 | 0.16 | 10.42 ± 1.39 | 0.98 | 9.12 ± 0.53 | 0.37 | 7.48 ± 0.15 | 0.11 |
| **Week 6** | 11.5 ± 0.26 | 0.18 | 8.41 ± 4.19 | 2.96 | 9.93 ± 2.38 | 1.68 | 9.09 ± 1.81 | 1.28 |
| **Week 7** | 13.15 ± 1.81 | 1.28 | 13.53 ± 0.97 | 0.68 | 11.65 ± 1.76 | 1.25 | 13.93 ± 0.76 | 0.56 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Week 8** | 6.71 ± 7.29 | 5.15 | 7.78 ± 3.58 | 2.53 | 6 ± 6.18* | 4.37 | 8.82 ± 0.73 | 0.51 |
| **Week 9** | 12.07 ± 0.28 | 0.20 | 7.26 ± 3.11** | 2.20 | 9.68 ± 1.45 | 1.03 | 7.40 ± 0.44 | 0.31 |
| **Total** | 10.27 ± 1.29 | 0.91 | 9.36 ± 2.41 | 1.70 | 8.92 ± 1.77** | 1.25 | 8.87 ± 0.88** | 0.62 |

Dunnett's test showed that the groups were significantly different from each other ($P < 0.05$); ****the groups were very significantly different from each other ($P < 0.0001$); the values are given as mean ± SD.

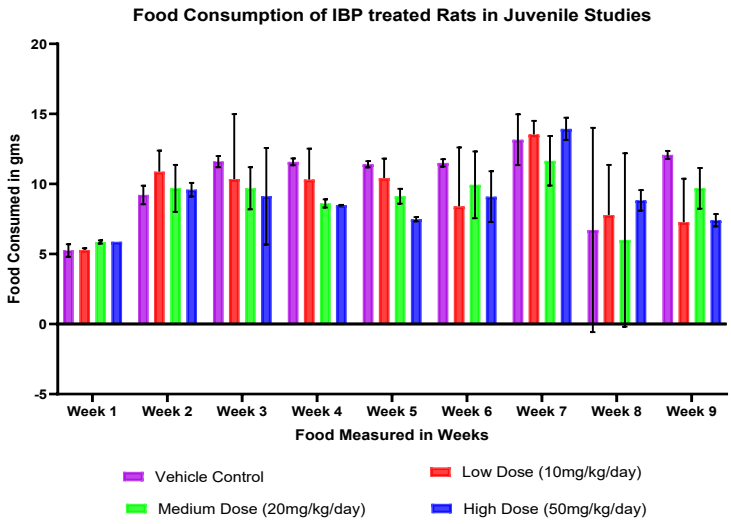



**Figure 1. Food Consumption of IBP treated rats in Juvenile Studies**

**Table 3: Clinical Examination of animals induced with IBP**

| Sl. No. | Parameters | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|---|
| 1 | Sitting and Standing Alert | 8 | 8 | 8 | 6 |
| 2 | Abnormal Vocalizations (Absent) | 8 | 8 | 8 | 7 |
| 3 | Convulsions Clonic and Tonic Movement (Absent) | 8 | 8 | 8 | 7 |
| 4 | Gait (Normal gait) | 8 | 8 | 8 | 7 |
| 5 | Mobility Score (No impairment) | 8 | 8 | 8 | 7 |
| 6 | Arousal level (Alert) | 8 | 8 | 8 | 6 |
| 7 | Clonic Movement or tonic movement (Absent) | 8 | 8 | 8 | 6 |
| 8 | Stereotype Behaviour (Absent) | 8 | 8 | 8 | 7 |
| 9 | Bizarre Behaviour (Absent) | 8 | 8 | 8 | 7 |
| 10 | Urination (Normal) | 8 | 8 | 8 | 6 |
| 11 | Defecation (Normal) | 8 | 8 | 8 | 8 |
| 12 | Rearing | 58 | 62 | 60 | 56 |
| 13 | Vocalization (Absent) | 8 | 8 | 8 | 7 |
| 14 | Sensory Reactivity (approach, response, touch response, click response, tail pinch response, pupil response, and aerial righting reflux) (Normal) | 8 | 8 | 7 | 6 |

**Home Cage, Open Fild Observations, and Sensory Reactivity Measurements**:
The doses administered did not cause any treatment-related abnormalities to be observed in both open field observations and the home cage.

**Motor Activity**:
When compared to animals treated with IBP, concurrent controls showed no discernible differences in automated motor activity evaluations.

**Hindlimb Landing Foot Splay and Grip Performance:**
In rats, the hindlimb foot splay and grip performance did not have any significant differences when compared to concurrent treatment.
**Indices of Sexual Maturation (Balano-preputial separation):**
Applying gentle pressure causes the prepuce to totally retract from the penis. In comparison to the medium and high doses, the average body weight and the day of the separation are a little higher.

**Table 4: Balano-preputial separation of animals induced with IBP**

| Parameters | Vehicle Control (Corn Oil) | Low Dose (10mg/kg/day) | Medium Dose (20mg/kg/day) | High Dose (50mg/kg/day) |
|---|---|---|---|---|
| No. of Animals | 8 | 8 | 8 | 8 |
| balano-preputial separation (body wt) | 131.1 ± 4.02 | 133.5 ± 1.11 | 133.87 ± 2.89 | 133.31 ± 1.86 |
| balano-preputial separation (in days) | 42.12 ± 0.99 | 43 ± 1.06 | 44.75 ± 1.28 | 46.12 ± 1.24 |

Data express in Mean ± SD

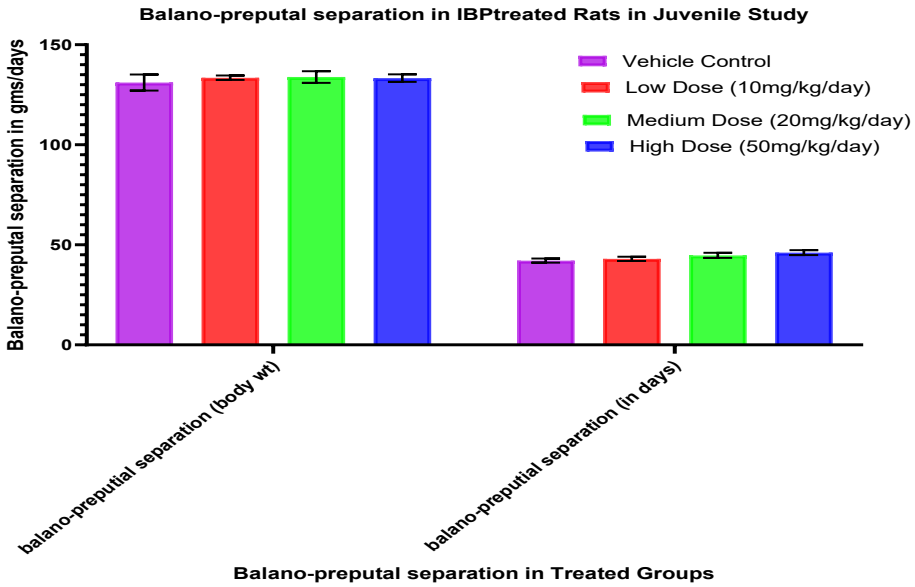



**Figure 2. Balanopreputial separation in IBP treated animals in Juvenile Studies**

**Table 5: Hind Limb & Motor Activity of animals induced with IBP**

| Parameters | Vehicle Control (Corn Oil) | Low Dose (10mg/kg/day) | Medium Dose (20mg/kg/day) | High Dose (50mg/kg/day) |
|---|---|---|---|---|
| No. of Animals | 8 | 8 | 8 | 8 |
| Hind limb foot splay (in cm) | 7.35 ± 0.90 | 8.73 ± 0.83 | 8.26 ± 0.84 | 9.38 ± 1.93 |
| Grip performance (kg) Fore limb | 1.59 ± 0.09 | 1.48 ± 0.15 | 1.40 ± 0.12 | 1.35 ± 0.14 |
| Grip performance (kg) Hind limb | 0.88 ± 0.06 | 0.80 ± 0.07 | 0.81 ± 0.06 | 0.76 ± 0.05 |
| Motor Activity Total stereotypic time (min) | 12.62 ± 0.35 | 12.72 ± 0.41 | 12.58 ± 0.46 | 12.67 ± 0.48 |
| Motor Activity Total ambulatory time (min) | 15.38 ± 0.27 | 15.4 ± 0.28 | 15.57 ± 0.43 | 15.52 ± 0.41 |
| Motor Activity Total horizontal counts | 4379.75 ± 155.46 | 4495.75 ± 165.35 | 4761.25 ± 443.09 | 4982.25 ± 305.12 |

| | | | | |
|---|---|---|---|---|
| Motor Activity Total ambulatory counts | 3126.62 ± 91.46 | 3334 ± 124.43 | 3409.75 ± 270.96 | 3781.62 213.22 |

*Data express in Mean ± SD*

### 3.3. Hematology Parameters

Using two-way ANOVA, the hematological study evaluated IBP-induced hematological alterations by contrasting treatment time and dosage with a vehicle control. Significant reduction in WBC count when compared to treated high dose. (9.65 ± 1.12 to 6.98 ± 2.98). There were no significant variations in other hematological parameters between rats treated at 10, 20, and 50 mg/kg/day and those in the control group.

**Table 6: Hematological parameters of animals induced with IBP**

| Parameters | Vehicle Control (Corn Oil) | Low Dose (10mg/kg/day) | Medium Dose (20mg/kg/day) | High Dose (50mg/kg/day) |
|---|---|---|---|---|
| No. of Animals | 8 | 8 | 8 | 8 |
| Haemoglobin (gm%) | 11.78 ± 2.10 | 13.13 ± 1.65 | 12.60 ± 0.32 | 10.58 ± 2.00 |
| Total RBC Count (millions/cumm) | 4.47 ± 0.24 | 4.51 ± 0.31 | 4.38 ± 0.29 | 4.25 ± 0.36 |
| Total WBC Count (cells/cumm) (in thousands) | 9.65 ± 1.12** | 9.85 ± 5.77 | 7.50 ± 1.54 | 6.98 ± 2.98 |
| Platelet Count (lakhs/cumm) | 1.77 ± 0.23 | 2.67 ± 1.20 | 2.35 ± 0.57 | 1.58 ± 0.66 |
| PCV/Hematocrit (%Vol) | 36.70 ± 2.43 | 39.77 ± 4.06 | 38.27 ± 1.16 | 34.47 ± 5.76 |
| MCV (fL) | 86.47 ± 1.93 | 90.52 ± 7.51 | 86.02 ± 4.05 | 72.03 ± 10.10 |
| MCH (pg) | 26.77 ± 2.26 | 28.10 ± 2.16 | 28.58 ± 0.52 | 27.23 ± 3.83* |
| MCHC (g/dL) | 33.05 ± 1.54 | 32.93 ± 1.89 | 33.53 ± 0.39 | 32.60 ± 1.98 |
| Neutrophils (%) | 48.50 ± 3.27 | 41.83 ± 21.48* | 46.83 ± 4.36 | 49.17 ± 18.18** |
| Lymphocytes (%) | 44.17 ± 3.49 | 52.00 ± 21.23 | 47.83 ± 3.06 | 45.83 ± 18.61 |
| Eosinophils (%) | 3.17 ± 0.75 | 2.00 ± 0.00 | 3.00 ± 1.26 | 2.67 ± 0.82 |
| Monocytes (%) | 3.00 ± 0.89 | 3.00 ± 0.00 | 4.00 ± 0.89 | 3.17 ± 0.75 |
| Basophils (%) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Reticulocyte Count (%) | 1.52 ± 0.25 | 1.08 ± 0.12 | 1.20 ± 0.24 | 1.37 ± 0.25 |

∗Significantly different from the control, $p \leq 0.05$.

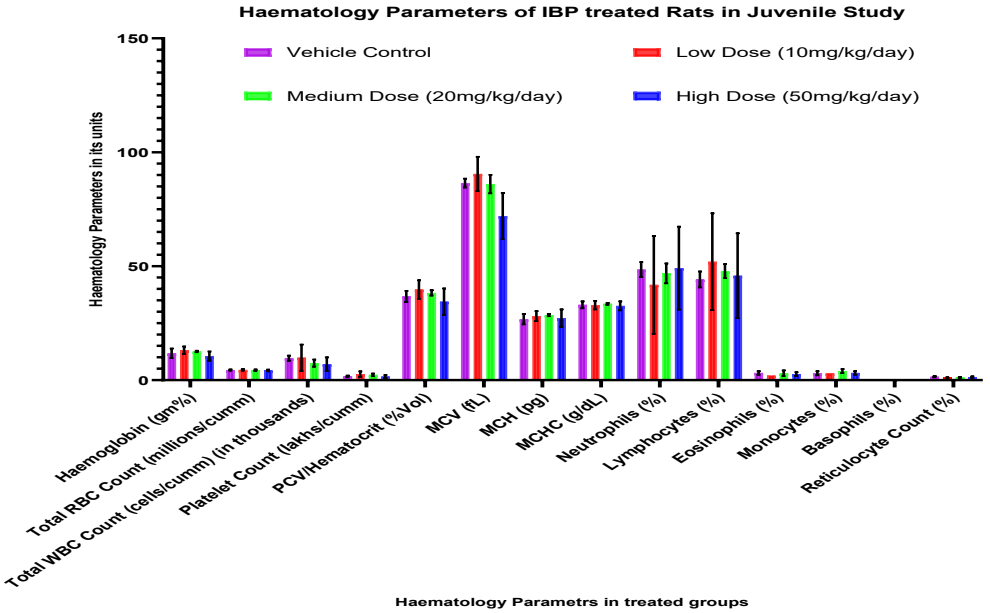



**Figure 3. Haematological Parameters of IBP treated animals in Juvenile Studies**

### 3.4. Clinical Biochemistry

Total cholesterol was significantly lower in the vehicle control group compared to all treatment doses where $p < 0.0001$. Blood urea levels in the vehicle control were substantially normal, apart from all other treatment

groups. In the high-dose treatment group, blood glucose levels are higher than in the vehicle control, low-dose, and medium-dose groups. The majority of metrics did not show any significant differences between dosing groups. These findings demonstrate IBP's significant impact on lipid metabolism, protein synthesis, and renal indicators.

**Table 7: Clinical Biochemistry parameters of animals induced with IBP**

| Parameters | Vehicle Control (Corn Oil) | Low Dose (10mg/kg/day) | Medium Dose (20mg/kg/day) | High Dose (50mg/kg/day) |
|---|---|---|---|---|
| No. of Animals | **8** | **8** | **8** | **8** |
| Blood Urea Nitrogen (BUN) (mg/dL) | 23.20 ± 1.49 | 23.67 ± 2.80 | 27.67 ± 5.92 | 26.20 ± 8.92 |
| Glucose (mg/dL) | 73.17 ± 1.83 | 72.67 ± 1.21 | 74.17 ± 1.47 | 91.33 ± 18.18* |
| Bilirubin Total (mg/dL) | 0.28 ± 0.10 | 0.45 ± 0.08 | 0.63 ± 0.16 | 0.45 ± 0.23* |
| Bilirubin Direct (mg/dL) | 0.15 ± 0.08 | 0.20 ± 0.00 | 0.27 ± 0.08 | 0.17 ± 0.08 |
| Bilirubin Indirect (mg/dL) | 0.22 ± 0.12 | 0.27 ± 0.10 | 0.37 ± 0.16 | 0.33 ± 0.20* |
| ALT (SGPT) (U/L) | 44.67 ± 2.42 | 22.17 ± 3.66 | 57.17 ± 27.09 | 75.67 ± 33.60* |
| AST (SGOT) (U/L) | 340.00 ± 16.15 | 30.33 ± 6.86 | 56.83 ± 70.64 | 290.17 ± 29.97* |
| Alkaline Phosphatase (U/L) | 154.50 ± 16.21 | 123.33 ± 30.10 | 138 ± 12.25 | 190.50 ± 74.48 |
| Gamma Glutamyl Transferase (GGT) (U/L) | 9 ± 1.26 | 33.5 ± 6.47 | 28.83 ± 8.82 | 17 ± 15.9* |
| Protein Total (g/dl) | 6.5 ± 0.21 | 6.97 ± 0.19 | 7.15 ± 0.36 | 6.88 ± 0.6 |
| Albumin (g/dl) | 3.45 ± 0.14 | 3.77 ± 0.59 | 2.3 ± 0.98 | 2.95 ± 0.61 |
| Globulin (g/dl) | 3 ± 0.4 | 3.05 ± 0.46 | 4.72 ± 1.67 | 4.12 ± 0.44 |
| Albumin/Globulin Ratio (%) | 1.33 ± 0.35 | 1.3 ± 0.46 | 4.72 ± 1.67 | 4.12 ± 0.44 |
| Cholesterol Total (mg/dL) | 73.50 ± 20.19 | 102.67 ± 1.63 | 99.83 ± 4.75 | 91.5 ± 17.01* |
| HDL Cholesterol (mg/dL) | 34.5 ± 2.43 | 36.17 ± 1.47 | 38.17 ± 5.34 | 35.5 ± 2.43 |
| Cholesterol LDL (mg/dL) | 37.33 ± 8.26 | 46.83 ± 1.94 | 39 ± 6.13 | 38 ± 13.73 |
| VLDL Cholesterol (mg/dL)013 | 21.33 ± 7.03 | 19.17 ± 0.75 | 23.17 ± 3.66 | 17.5 ± 4.14 |
| Triglycerides (mg/dL) | 97 ± 27.6 | 93.17 ± 4.36 | 108.83 ± 11.99 | 86.33 ± 16.52* |

∗Significantly different from the control, $p \leq 0.05$.

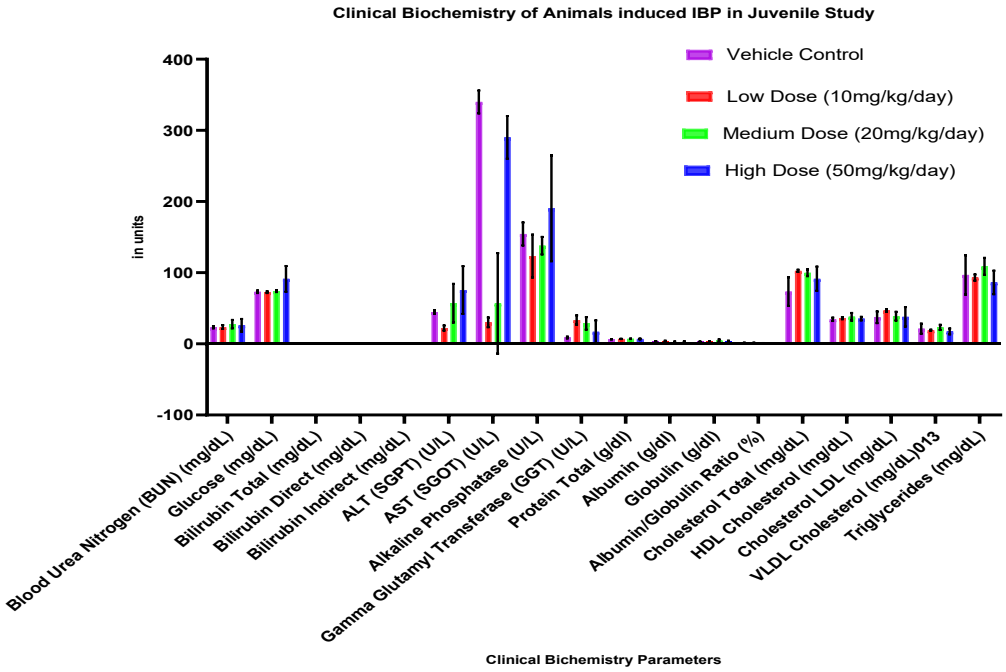



**Figure 4. Clinical Biochemistry Parameters of IBP treated animals in Juvenile Studies**

### 3.5. Organ Weight

The absolute and relative weights of the kidney, liver, testes and spleen significantly rose. The majority of the organs exhibit slight modifications in comparison to both the vehicle and treated groups.

**Table 8: Organ Weights of Animals Induced with IBP**

| Organ | Vehicle Control (Corn Oil) | Low Dose (10mg/kg/day) | Medium Dose (20mg/kg/day) | High Dose (50mg/kg/day) |
|---|---|---|---|---|
| **No. of Animals** | 8 | 8 | 8 | 8 |
| **Kidney** | 2.037 ± 0.188 | 2.347 ± 0.244 | 2.830 ± 0.455 | 2.457 ± 0.293** |
| **Liver** | 9.013 ± 1.421 | 9.414 ± 1.480 | 9.752 ± 1.712 | 8.762 ± 0.552* |
| **Adrenal** | 0.076 ± 0.043 | 0.060 ± 0.030 | 0.066 ± 0.013 | 0.061 ± 0.016* |
| **Brain** | 1.417 ± 0.182 | 1.680 ± 0.124 | 1.679 ± 0.072 | 1.708 ± 0.084** |
| **Epididymis** | 1.031 ± 0.159 | 0.973 ± 0.137 | 0.940 ± 0.101 | 0.856 ± 0.156 |
| **Heart** | 0.813 ± 0.076 | 0.861 ± 0.088 | 0.864 ± 0.054 | 0.804 ± 0.079 |
| **Seminal Vesicle** | 0.442 ± 0.139 | 0.462 ± 0.109 | 0.681 ± 0.176 | 0.295 ± 0.049 |
| **Spleen** | 0.775 ± 0.082 | 0.797 ± 0.081 | 0.892 ± 0.104 | 0.733 ± 0.072 |
| **Testes** | 2.362 ± 0.346 | 2.402 ± 0.19 | 2.399 ± 0.266 | 2.322 ± 0.13*** |
| **Thyroid** | 0.124 ± 0.033 | 0.143 ± 0.02 | 0.165 ± 0.049 | 0.135 ± 0.01** |
| **Thymus** | 0.287 ± 0.103 | 0.3 ± 0.1 | 0.251 ± 0.037 | 0.2 ± 0.03 |

Asterisks indicate statistically significant differences compared to the vehicle control:
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Dunnett's multiple comparison test).

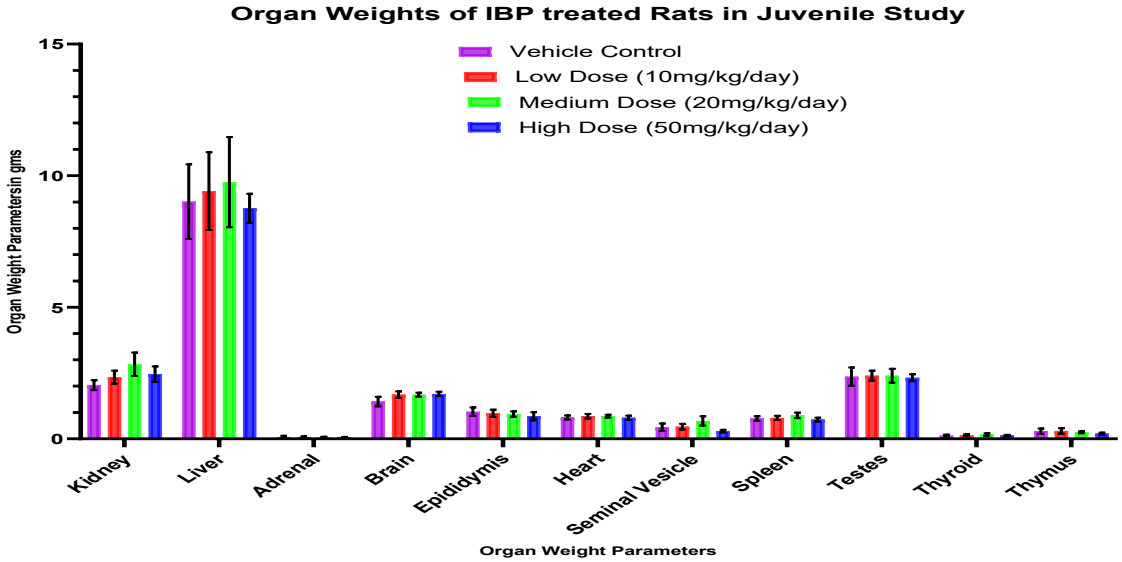



**Figure 5. Organ Weight of IBP treated animals in Juvenile Studies**

### 3.6. Histopathological Examination

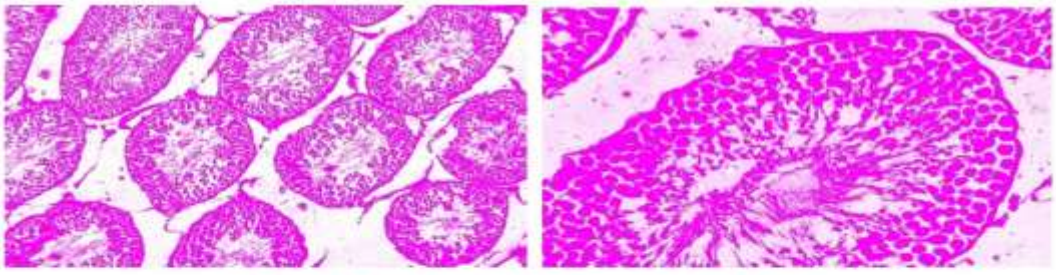
The absolute and relative weights of the kidney, liver, testes and spleen significantly rose. The majority of the organs exhibit slight modifications in comparison to both the vehicle and treated groups.

**Testis**

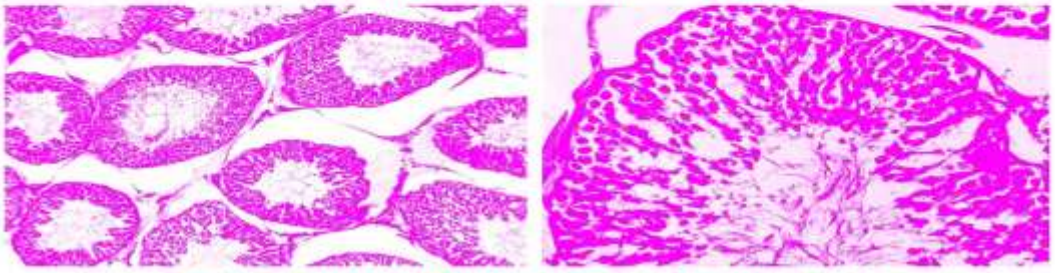
H&E sections from the normal control exhibited a well-structured testis characterized by a robust connective tissue layer known as the tunica albuginea, which encompasses pyramid-shaped lobules partitioned by septae composed of connective tissue. Each lobule of the seminiferous tubules has up to four of them, and they are lined by germinal epithelium. Sertoli cells are big, column-shaped cells that will grow until they reach the germinal epithelium. Spermatids are tiny round cells with nuclei that are very slightly coloured. Leydig cells are big and are located in groups that make testosterone.

In animals treated with IBP at 10 & 20 mg/kg/day, the tunica albuginea is a little bit reduced, and the lobules have large spaces between them. The germinal epithelium, which is slightly thinner, lines each lobule of the seminiferous tubules, which can contain up to four of them. Sertoli cells are moderate, column-shaped cells that will continue to expand until they reach the germinal epithelium. Spermatids are diminutive, circular cells with nuclei that are only faintly colored. The size of Leydig cells is diminished, and they are situated in groups that produce testosterone.

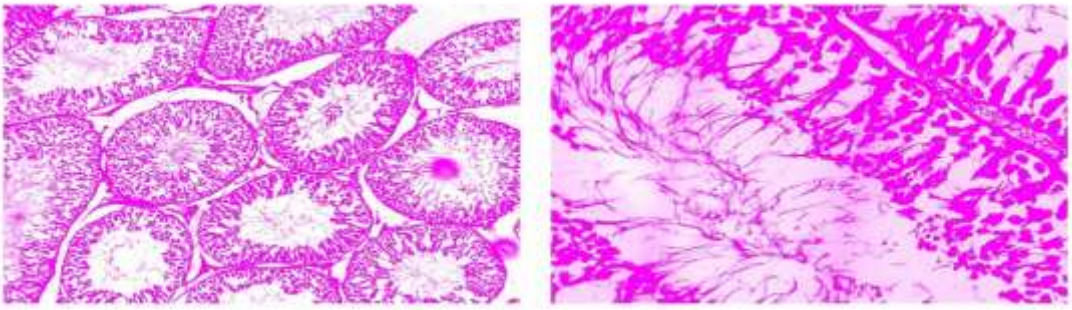
The rats administered a high dose of 50 mg/kg/day have significant alterations in the histoarchitecture of their testes. Consequently, in the outermost layer, specifically the tunica albuginea, there exists no interstice between the tissues; the tunica albuginea appears contracted. The lobules are pyramidal in shape and are delineated by septa composed of connective tissue. Seminiferous tubules comprise a maximum of three lobules, whereas Sertoli cells are diminutive relative to low doses and exhibit a columnar shape with extensions of the germinal epithelium. Spermatids are much fewer in quantity relative to all treatment groups, have irregular morphology, and possess a nucleus with a lumen.



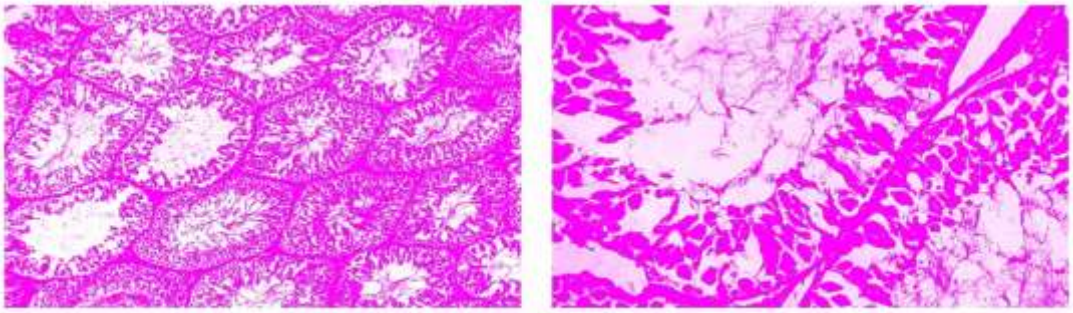
Testis: Group 1: Vehicle Control 10X & 40X



Testis: Group 2: Low Dose 10 mg/kg/day 10X & 40X



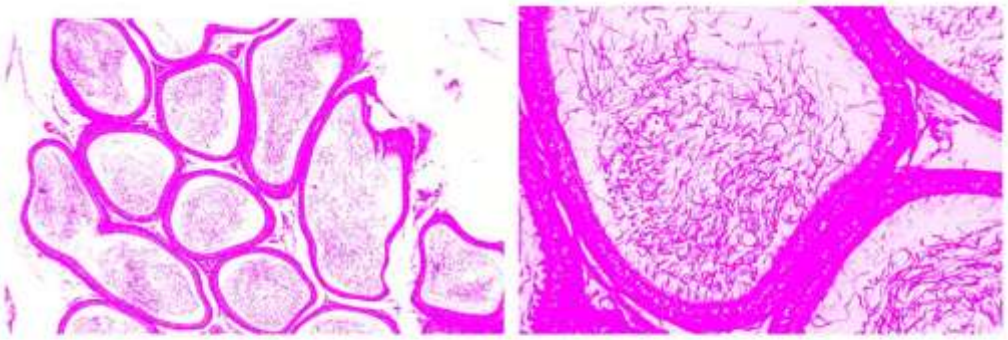
Testis: Group 3: Medium Dose 20 mg/kg/day 10X & 40X



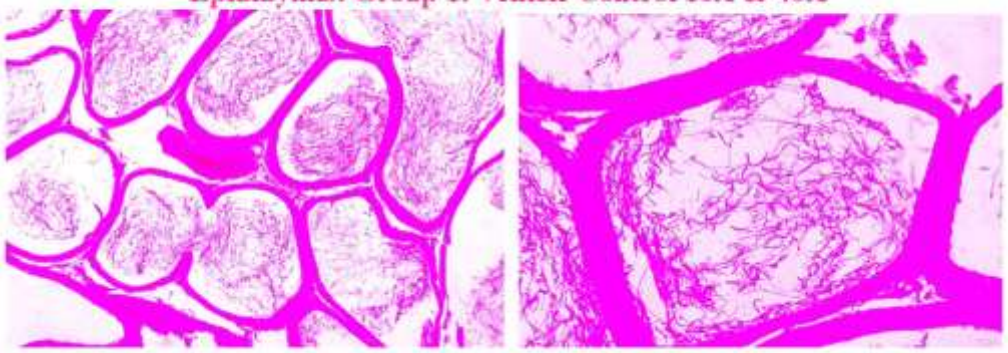
Testis: Group 4: High Dose 50 mg/kg/day 10X & 40X

**Epididymis**

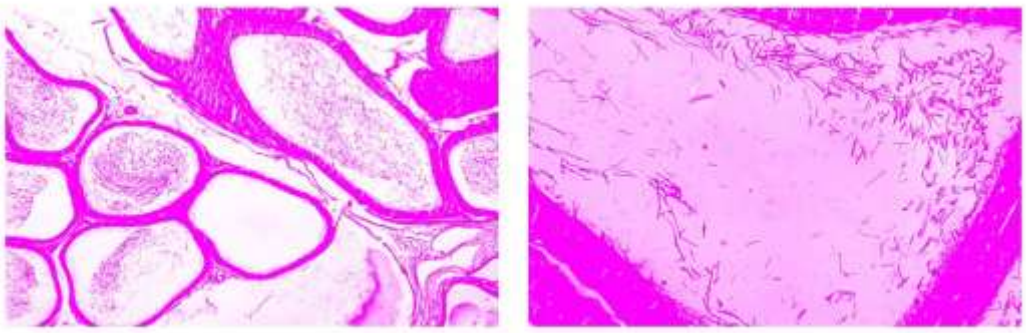
The smooth luminal surface known as pseudostratified columnar epithelium, which is rather thin and has a space between each duct, was severely affected in the medium-dose and high-dose treated animals in the epididymis, according to the H&E stains when compared to treated groups. Principal cells will not affect the stereocilia until they reach the luminal surface. There are comparatively fewer basal cells. Through the length of the ducts, the slightly thicker layer of muscle is reduced.



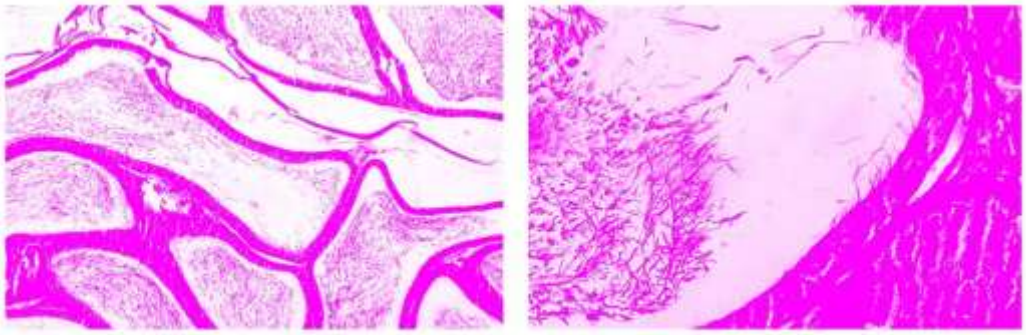
Epididymis: Group 1: Vehicle Control 10X & 40X



Epididymis : Group 2: Low Dose 10 mg/kg/day 10X & 40X



Epididymis: Group 3: Medium Dose 20 mg/kg/day 10X & 40X



Epididymis: Group 4: High Dose 50 mg/kg/day 10X & 40X

## 4. DISCUSSION

The present investigation demonstrated that sub-chronic exposure to isobutyl paraben (IBP) during the juvenile stage of male Wistar rats adversely influenced somatic growth, hematological and biochemical profiles, organ weights, and reproductive histoarchitecture. These results collectively confirm the endocrine-disrupting potential of IBP, particularly in the context of male reproductive development.

A consistent, dose-dependent reduction in body weight gain and food consumption indicates a possible interference of IBP with metabolic regulation. Similar findings have been reported in rodent models exposed to parabens, where disrupted energy homeostasis and altered lipid metabolism were linked to estrogenic and peroxisome proliferator-activated receptor (PPAR)-γ mediated pathways (Darbre et al., 2002; Riu et al., 2011). Moreover, the delayed balano-preputial separation observed in higher IBP-treated groups reflects a clear delay in the onset of puberty, a hallmark of anti-androgenic or estrogen-mimetic activity (Lee & Koo, 2007; Toppari et al., 1996).

The observed hematological suppression, especially in total leukocyte counts, implies mild immunotoxic or stress-related responses, consistent with reports showing that chronic paraben exposure may influence hematopoietic function and oxidative stress (Anjum et al., 2011). The biochemical alterations, including elevated glucose, cholesterol, and hepatic transaminases (ALT and AST), suggest metabolic disturbances and hepatic stress, corroborating earlier findings that parabens impair liver detoxification capacity and promote hepatocellular changes (Hoberman et al., 2008; Harvey et al., 2007). The hyperglycemia detected at the high dose could result from altered insulin signaling or hepatic gluconeogenesis, mechanisms proposed by LeBlanc et al. (1979) and further supported by recent endocrine disruptor models (Vandenberg et al., 2012). Significant increases in absolute and relative organ weights of the liver, kidney, and testes suggest compensatory hypertrophy or accumulation of the compound within metabolically active tissues. The alterations in testicular and epididymal histoarchitecture, including seminiferous tubular atrophy, reduced spermatid populations, and degeneration of Leydig cells, align with previous findings that parabens such as propyl- and butyl-paraben compromise spermatogenesis and androgen synthesis (Oishi, 2001; Oishi, 2002; Maske et al., 2018). The structural disorganization of seminiferous tubules observed in the current study further supports the notion that IBP exerts estrogen receptor-mediated disruption of Sertoli-Leydig cell communication and hormonal feedback mechanisms (Darbre et al., 2002; Diamanti-Kandarakis et al., 2009).

Interestingly, the absence of pronounced neurological or behavioral changes in the functional observation battery indicates that the neurotoxic potential of IBP, at the studied doses, remains minimal compared to known neurotoxicants (Atlı et al., 2016; Awogbindin et al., 2023). However, subtle metabolic stress and altered hormonal feedback could indirectly affect neural development over longer exposure periods. Collectively, these outcomes are in line with the Endocrine Society's consensus that even low-dose, long-term exposure to endocrine-disrupting chemicals (EDCs) can elicit non-monotonic dose-response effects and disrupt developmental programming (Vandenberg et al., 2012; Diamanti-Kandarakis et al., 2009). Given IBP's structural similarity to natural estrogens and its capacity to bind estrogen receptors (Darbre et al., 2002; Delfosse et al., 2015), the alterations observed in the present study strongly reinforce its categorization as an environmentally relevant endocrine disruptor capable of perturbing hormonal and reproductive homeostasis during critical developmental windows.

## 5. CONCLUSION

In conclusion, the findings of this 70-day sub-chronic study demonstrate that isobutyl paraben (IBP) exposure during the juvenile stage induces a spectrum of toxicological effects, including growth retardation, metabolic dysregulation, hematological alterations, and reproductive tissue degeneration in male Wistar rats. The consistent dose-dependent pattern highlights IBP's potential to interfere with endocrine signaling pathways particularly those regulating androgen production and testicular development.

Given IBP's widespread use in personal care and pharmaceutical products, these results raise significant concerns regarding early-life exposure and its potential long-term consequences on male reproductive health. Therefore, this study underscores the need for regulatory revaluation of paraben exposure limits and advocates for expanded mechanistic research on endocrine and reproductive toxicity across developmental stages.

## 6. REFERENCES

1. Anderson DM, Glibert PM, Burkholder JM. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries. 2002 Aug;25(4):704-26.
2. Anjum, N. A., Umar, S., Iqbal, M., & Khan, N. A. (2011). Cadmium causes oxidative stress in mung bean by affecting the antioxidant enzyme system and ascorbate–glutathione cycle metabolism. Russian Journal of Plant Physiology, 58(1), 92–99. https://doi.org/10.1134/S1021443711010022
3. Atlı, Ö., Demir-Ozkay, U., Ilgın, S., Aydın, T., Akbulut, E. N., & Şener, E. (2016). Evidence for neurotoxicity associated with amoxicillin in juvenile rats. Human & Experimental Toxicology, 35(8), 866–876. https://doi.org/10.1177/0960327115607948
4. Awogbindin, I. O., Ikeji, C. N., Adedara, I. A., & Farombi, E. O. (2023). Neurotoxicity of furan in juvenile Wistar rats involves behavioral defects, microgliosis, astrogliosis and oxidative stress. Social Science Research Network, 113934. https://doi.org/10.2139/ssrn.4340457
5. Bryan KL. Language prosody and the right hemisphere. Aphasiology. 1989 Jun 1;3(4):285-99.
6. D.E. Tupper and R.B. Wallace, "Utility of the neurologic examination in rats," Acta Neurobiologiae Experimentalis, Vol. 40, 1980.
7. Darbre, P. D., Byford, J. R., Shaw, L. E., Horton, R. A., Pope, G. S., & Sauer, M. J. (2002). Oestrogenic activity of isobutylparaben in vitro and in vivo. Journal of Applied Toxicology, 22(4), 219–226. https://doi.org/10.1002/JAT.860
8. Delfosse N. Decoding color codes by projection onto surface codes. Physical Review A Atomic, Molecular, and Optical Physics. 2014 Jan;89(1):012317.
9. Delfosse V, Maire AL, Balaguer P, Bourguet W. A structural perspective on nuclear receptors as targets of environmental compounds. Acta Pharmacologica Sinica. 2015 Jan;36(1):88-101.
10. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocrine reviews. 2009 Jun 1;30(4):293-342.
11. Gray, L.E., et al. (2001). "Endocrine screening methods and evaluations of non- receptor-mediated endocrine-disrupting chemicals." Environmental Health Perspectives, 109(Suppl 1), 77-84.
12. Harvey P.W., Everett D.J., Springall. C. J. 2007. Adrenal toxicology: a strategy for assessment of functional Toxicity to the adrenal cortex and steroidogenesis. J ApplToxicol 27:103-115.
13. Hoberman, A. M., Schreur, D., Leazer, T., Daston, G. P., Carthew, P., Re, T., Loretz, L., & Mann, P. (2008). Lack of effect of butylparaben and methylparaben on the reproductive system in male rats. In Birth Defects Research (Vol. 83, Issue 2, p. 123). Wiley. https://doi.org/10.1002/bdrb.20153
14. IPCS, "Principles and methods for the assessment for the assessment of neurotoxicity associated with exposure to chemicals," Environmental Health Criteria Document No. Vol. 60, 1986.
15. LeBlanc J, Nadeau A, Boulay M, Rousseau-Migneron S. Effects of physical training and adiposity on glucose metabolism and 125I-insulin binding. Journal of Applied Physiology. 1979 Feb 1;46(2):235-9.
16. Lee, B. M., & Koo, H. J. (2007). Hershberger assay for antiandrogenic effects of phthalates. Journal of Toxicology and Environmental Health Part A, 70(14), 1365–1370. https://doi.org/10.1080/15287390701457769
17. Maness LM, Kastin AJ, Farrell CL, Banks WA. Fate of leptin after intracerebroventricular injection into the mouse brain. Endocrinology. 1998 Nov 1;139(11):4556-62.
18. Maske, P., Dighe, V., & Vanage, G. (2018). n-butylparaben exposure during perinatal period impairs fertility of the F1 generation female rats. In Chemosphere (Vol. 213, p. 114). Elsevier BV. https://doi.org/10.1016/j.chemosphere.2018.08.130
19. Max-Stoelting P, Pfeil R, Solecki R, Ulbrich B, Grote K, Ritz V, Banasiak U, Heinrich-Hirsch B, Moeller T, Chahoud I, Hirsch-Ernst KI. Assessment strategies and decision criteria for pesticides with endocrine disrupting properties to humans. Reproductive Toxicol. 2011;31:574-84.
20. Meyer OA, Tilson HA, Byrd WC, Riley MT. A method for the routine assessment of fore-and hindlimb grip strength of rats and mice. Neurobehavioral toxicology. 1979 Jan 1;1(3):233-6.
21. Oehlmann J, Schulte-Oehlmann U, Tillmann M, Markert B. Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part I: Bisphenol A and octylphenol as xeno-estrogens. Ecotoxicology. 2000 Dec;9(6):383-97.
22. Oishi, S. (2001). Effects of butylparaben on the male reproductive system in rats. Toxicology and Industrial Health, 17(1), 31–39. https://doi.org/10.1191/0748233701TH093OA
23. Oishi, S. (2002). Effects of propyl paraben on the male reproductive system. In Food and Chemical Toxicology (Vol. 40, Issue 12, p. 1807). Elsevier BV. https://doi.org/10.1016/s0278-6915(02)00204-1

24. P.M. Edwards and V.H. Parker, “A simple, sensitive, and objective method for early assessment of acrylamide neuropathy in rats,” Toxicology and Applied Pharmacology, Vol. 40, pp. 589-591, 1977.
25. Riu A, Grimaldi M, Le Maire A, Bey G, Phillips K, Boulahtouf A, Perdu E, Zalko D, Bourguet W, Balaguer P. Peroxisome proliferator-activated receptor γ is a target for halogenated analogs of bisphenol A. Environmental health perspectives. 2011 Sep;119(9):1227-32.
26. Schettler TE. Human exposure to phthalates via consumer products. International journal of andrology. 2006 Feb;29(1):134-9.
27. T. Schug., Thaddeus, Amanda Janesick, Bruce Blumberg, et al., 2011. The Journal of steroid biochemistry and molecular biology. 127(3-5):204-215.
28. Tamura H, Tanaka K. Visual response properties of cells in the ventral and dorsal parts of the macaque inferotemporal cortex. Cerebral Cortex. 2001 May 1;11(5):384-99.
29. Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette Jr LJ, Jégou B, Jensen TK, Jouannet P, Keiding N, Leffers H. Male reproductive health and environmental xenoestrogens. Environmental health perspectives. 1996 Aug;104(suppl 4):741-803.
30. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs Jr DR, Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV, Zoeller RT. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocrine reviews. 2012 Jun 1;33(3):378-455.
31. Vijaykumar Malashetty, Raghunandan Deshpande, and Somnathreddy Patil. 2022. Seventy-Day Toxicity Study in Juvenile Sprague-Dawley Rats with Semicarbazide (SEM) from Weaning to Sexual Maturity. https://doi.org/10.1155/2022/5059761
32. Vinggaard AM, Hnida C, Larsen JC. Environmental polycyclic aromatic hydrocarbons affect androgen receptor activation in vitro. Toxicology. 2000 Apr 14;145(2-3):173-83.
33. Vos J.G. et al., 2000. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. Crit Rev Toxicollan.
34. Wetherill Y.B., Akingbemi B, Kanno J, McLachlan J.A, et al., 2007. In vitro molecular mechanisms of bisphenol A action. Reprod Toxicol. 24:178-198.
35. Willingham E, Crews D. Sex reversal effects of environmentally relevant xenobiotic concentrations on the red-eared slider turtle, a species with temperature-dependent sex determination. General and Comparative Endocrinology. 1999 Mar 1;113(3):429-35.
36. Zlatnik MG. Endocrine-disrupting chemicals and reproductive health. Journal of midwifery & women's health. 2016 Jul;61(4):442-55.