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Toxicological Evaluation of Isobutyl Paraben: A Combined Repeated Dose and Reproductive/Developmental Study in Rats

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ABSTRACT

Background: The chemical isobutyl paraben, used in the cosmetic, pharmaceutical, and food industries, has garnered attention as a potential hormonal disruptor. Its chemical structure, which includes an estrogen-like component and lipophilic properties, leads to widespread toxicities and hormonal disorders. The available toxicological data on IBP offers insufficient information regarding its long-term effects and developmental risks.

Methodology: This study analyzed the impact of oral IBP administration on the systemic health and reproductive and developmental systems of Wistar rats. Following the OECD Test Guidelines, Wistar rats received IBP orally via a corn oil vehicle.

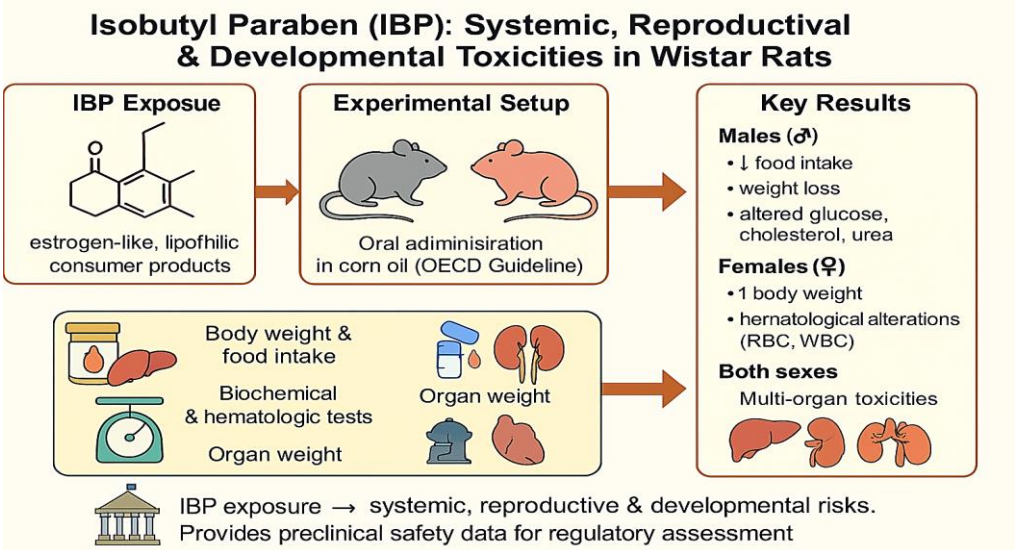
Results: Assessments of organ weights and tissue investigations revealed multiple organ toxicities in both male and female rats. Male rats given higher doses of IBP exhibited reduced food intake and weight loss, while female rats gained weight across all treatment groups. Biochemical tests on male subjects indicated that the drug dose directly influenced changes in blood glucose, cholesterol, and urea levels. Female rats showed significant alterations in both red and white blood cells, impacting their overall hematological profile. Researchers employed organ weight measurements together with histopathological results to identify specific toxic effects affecting individual organs in both male and female test subjects.

Conclusion: This study provides essential preclinical data necessary18 for regulatory agencies to assess the safety of consumer products containing IBP. The findings will aid regulatory bodies in evaluating the safety of IBP for consumer use.

Major Findings: IBP decreased food intake and weight in males, increased weight in females, and caused systemic biochemical, hematological, and organ toxicities.

***Keywords**: Isobutyl Paraben, Parabens, Repeated-dose toxicity, Reproductive Toxicity, Toxicology, Wistar Rats*

***GRAPHICAL ABSTRACT**:*



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1. INTRODUCTION

The alkyl esters of para-hydroxybenzoic acid, known as parabens, serve as preservatives in cosmetics, pharmaceutical products, and food items because they effectively protect against multiple microorganisms [1]. The personal care and hygiene industry uses isobutyl paraben (IBP) as one of its primary preservative agents [2]. Research now focuses on the endocrine disrupting properties of parabens, including IBP, because of its longer alkyl chain structure. The structural characteristics, combined with the higher lipophilicity of IBP, enable stronger estrogenic effects than ethyl paraben, while also making it more toxicologically significant [3]. The existing toxicological information about IBP is spread across multiple sources because researchers have focused on short-term exposure rather than long-term exposure and developmental toxicity [4]. Although research on other parabens exists, scientists need comprehensive data regarding IBP's effects on repeated-dose and developmental outcomes. The European Scientific Committee on Consumer Safety (SCCS), along with other regulatory agencies, advocates for sound preclinical research protocols that should analyze chronic IBP hazard exposures to human health [5].

This research project investigated the systemic, reproductive, and developmental toxicities of IBP in Wistar rats through multiple oral dose administrations [6]. The researchers conducted their study by following OECD Test Guidelines for repeated-dose toxicity and reproductive screening. The study evaluated food intake, body weight changes, blood tests, organ weight measurements, and tissue examination. Results from this study will supply essential scientific evidence that regulatory bodies need to make decisions about IBP safety in consumer products.

2. MATERIALS AND METHODS

Test Substance and Dosing

The researchers delivered IBP through oral gavage using corn oil as carrier solution. The researchers prepared test substance solutions daily to achieve dose levels of 10 mg/kg/day (Low) and 20 mg/kg/day (Medium) and 50 mg/kg/day (High). The vehicle control group received corn oil as their only substance.

Experimental Animals

The research utilized Wistar rats from both sexes that were in a healthy state. The animals spent their time under standard laboratory conditions with a 12-hour light/dark cycle along with a controlled temperature of 22 ± 3°C and relative humidity between 30–70% [7]. The rats received unlimited food pellets together with unlimited water during the entire study duration. Rats in the main study received five animals per cage but the rats in recovery groups received three animals per cage. The pre-mating phase for male rats contained four groups named Groups I to IV, which were placed in cages numbered 1 to 8, each with five rats per cage. The 14-day feeding period offered 1500 grams of food to each cage. Researchers measured total food intake per cage along with daily cage consumption and food consumption per rat daily. They also calculated mean values and standard deviation (SD) and standard error of the mean (SEM).

Experimental Design

The research followed three sequential phases, starting with pre-mating and continuing to mating, and finishing with recovery. The pre-mating period of 14 days required daily medication administration to male rats while tracking their food intake and body weight measurements. The mating phase allowed dosed males to live with females for mating purposes. The recovery phase started after treatment cessation, where researchers monitored selected male and female rats from both control Group I and high dose Group IV for 14 more days to check for reversible effects.

Body Weight Measurement

The study tracked body weight changes for all animals by measuring them at Day 0 and at set time points until study completion. Body weights of male and female animals were measured on Days 0, 7, 14, 21, 28 and Day 56 (terminal point) and Days 35, 42, 49, 56 for recovery group animals. The recovery group animal body weights received measurements on Days 35, 42, 49 and 56 to determine if treatment effects reversed during the recovery phase.

Hematological Analysis

Blood collection occurred from every animal at termination to perform a complete analysis of their blood cells. The assessment included measurements of red blood cell (RBC) count expressed in million/cumm and white blood cell (WBC) count in cells/cumm and hemoglobin concentration in gm/dL and platelet count in lakhs/cumm. The analysis of white blood

cells through differential leukocyte count measured the percentages of neutrophils, lymphocytes, eosinophils, monocytes, and basophils.

Biochemical Analysis

The researchers analyzed biochemical factors present in serum samples obtained from experimental animals. The biochemical analysis measured random blood sugar levels (mg/dL) together with total cholesterol (mg%) and total protein concentration (g%). Total bilirubin (mg%) and its direct and indirect components were among the measured substances. Blood urea levels (mg/dL) served as a renal function indicator to evaluate metabolic health.

Organ Weights and Gross Pathology

The researchers obtained vital organs from the necropsy procedure to measure absolute weights for morphological change assessment. A thorough examination of testes and thyroid glands as well as kidneys and liver and adrenal glands and spleen and heart and epididymis and thymus and brain took place. The researchers performed this procedure on vital organs from male and female animals throughout all experimental groups including the recovery group to study organ-specific effects.

Statistical Analysis

The data appeared as mean ± standard deviation (SD) and standard error of mean (SEM) depending on the situation. The analysis of data involved one-way ANOVA with post hoc tests performed to compare control versus treated groups at $p < 0.05$ significance level.

3. RESULTS AND DISCUSSION

Food Consumption

The research tracked food consumption among male rats across 14 days through four distinct treatment groups, which kept three rats each in separate cages. The researchers measured food consumption both at the cage level and the individual rat level and found a steady decrease from Group I (control) to Group IV (highest dose of isobutyl paraben). The rats in Group I consumed 15.03 grams of food daily, but Group IV rats consumed only 13.08 grams each day, showing a direct correlation between food intake and dose level (Table 1) [8]. The rats displayed decreased food consumption because systemic toxicity, together with gastrointestinal discomfort and endocrine disruption affected hypothalamic hunger regulation specifically. At higher dose levels these factors cause hypophagia and potential stress-induced anorexia [9]. Figure 1 demonstrates food consumption reduction patterns observed within every treatment group.

Table 1. Average Daily Food Consumption Per Rat Across Treatment Groups During Mating (Male & Female)

Group	Cage	Total Rats in Cage	Total Food Provided (g)	Total Consumed Food (g)	No. of Days	Food Consumed (Cage/Day)	Food Consumed (Rat/Day)	Mean	SD	SEM
I	1	3	1000	736	14	52.57143	17.52381	18.6349	1.38461	0.56527
	2	3	1000	824	14	58.85714	19.61905			
	3	3	1000	862	14	61.57143	20.52381			
	4	3	1000	776	14	55.42857	18.47619			
	5	3	1000	702	14	50.14286	16.71429			
	6	3	1000	796	14	56.85714	18.95238			
II	1	3	1000	732	14	52.28571	17.42857	17.3571	1.64061	0.66978
	2	3	1000	721	14	51.50000	17.16667			
	3	3	1000	736	14	52.57143	17.52381			
	4	3	1000	810	14	57.85714	19.28571			
	5	3	1000	605	14	43.21429	14.40476			
	6	3	1000	770	14	55.00000	18.33333			
III	1	3	1000	602	14	43.00000	14.33333	15.9563	2.89446	1.18166
	2	3	1000	522	14	37.28571	12.42857			
	3	3	1000	550	14	39.28571	13.09524			
	4	3	1000	780	14	55.71429	18.57143			
	5	3	1000	769	14	54.92857	18.30952			
	6	3	1000	786	14	56.28571	18.76190			
IV	1	3	1000	546	14	39.00000	13.00000	13.0833	1.86006	0.75959
	2	3	1000	596	14	42.57143	14.19048			
	3	3	1000	553	14	39.50000	13.16667			
	4	3	1000	650	14	46.42857	15.47619			
	5	3	1000	537	14	38.35714	12.78571			
	6	3	1000	415	14	29.64286	9.88095			

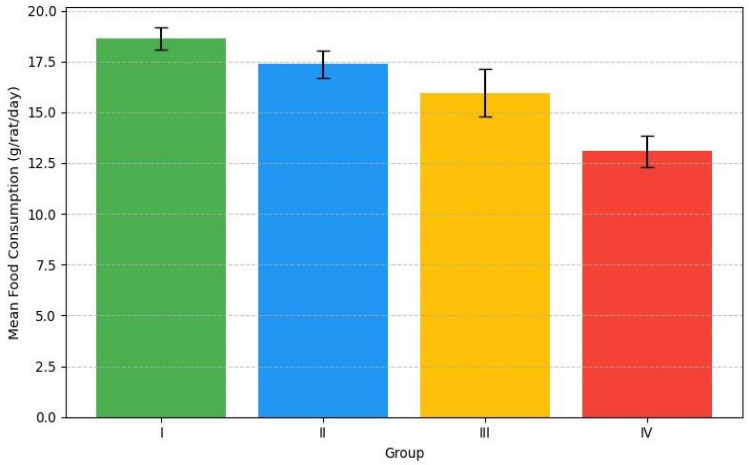

Figure 1. Dose-Dependent Decline in Food Consumption Among Male Rats Treated with Isobutyl Paraben.

Body Weight Trends in Male and Female Groups

The 28-day study with repeated doses showed that male rats subjected to isobutyl paraben presented a decrease in body weight gain in a dose-dependent manner, mainly at low and high doses, which suggested some systemic toxicity and metabolic interference in Table 2. Conversely, the body weight of female rats increased in all treated groups, probably related to sex physiology and hormones. These observations suggest sex-dependent responses to isobutyl paraben, underscoring the importance of including both sexes in toxicological studies, as well as having more studies to unravel the possible underlying mechanisms.

Table 2. Effect of Isobutyl Paraben on Weekly Body Weight Progression in Male and Female Rats During the 28-Day Repeated Dose Toxicity Study

Group	**Initial BW (g)**	**7th Day**	**14th Day**	**21st Day**	**28th Day**	**Terminal BW (g)**
Male						
Vehicle Ctrl	109.5 ± 7.40	151.0 ± 9.15	173.5 ± 10.38	213.5 ± 8.66	232.5 ± 7.75	240.0 ± 7.67
Low Dose	95.0 ± 5.68	123.0 ± 5.97	141.5 ± 6.87	186.0 ± 7.48	197.5 ± 7.04	205.0 ± 7.23
Medium Dose	111.5 ± 6.54	139.7 ± 7.31	159.0 ± 8.02	205.5 ± 8.67	221.0 ± 8.12	228.5 ± 8.30
High Dose	115.5 ± 5.98	152.5 ± 7.31	168.5 ± 6.46	203.0 ± 7.42	212.3 ± 7.05	217.0 ± 7.35
Female						
Group	**Initial BW (g)**	**7th Day**	**14th Day**	**21st Day**	**28th Day**	**Terminal BW (g)**
Vehicle Ctrl	100.83 ± 4.60	117.08 ± 5.38	140.0 ± 4.73	166.25 ± 4.97	182.5 ± 6.17	195.83 ± 4.60
Low Dose	112.92 ± 4.58	130.42 ± 4.33	152.92 ± 4.33	178.75 ± 4.73	192.5 ± 5.52	207.5 ± 4.11
Medium Dose	120.83 ± 8.52	140.42 ± 8.76	159.17 ± 7.12	182.92 ± 6.20	195.42 ± 7.00	205.08 ± 7.96
High Dose	125.83 ± 6.48	148.75 ± 7.26	172.08 ± 8.20	193.33 ± 10.12	207.92 ± 10.84	216.25 ± 10.98

The study revealed that male rats in all groups experienced progressive weight gain during the research period Figures 2 (a) and (b). The Vehicle Control group maintained the highest weight increase since the beginning of the study until its completion. The high-dose group members displayed substantially lower weight increases compared to other groups, as the statistical difference emerged starting at day 14. The Control group achieved near 300 g at the end of the study, but the high-dose group maintained a stable weight of 270 g, illustrating significant growth restrictions [10]. The weight measurements for female rats displayed parallel results to those of male rats, Figures 2 (c) and (d). The weight gain of all groups remained steady until day 42, when the High Dose group experienced a weight plateau followed by a decline. The rats in this group maintained lower body weights than the Control group throughout the experiment, as the drug dose directly impacted their growth rate [11]. The Control group maintained an average weight of 220 grams throughout the study period the high-dose group stayed close to 200 grams.

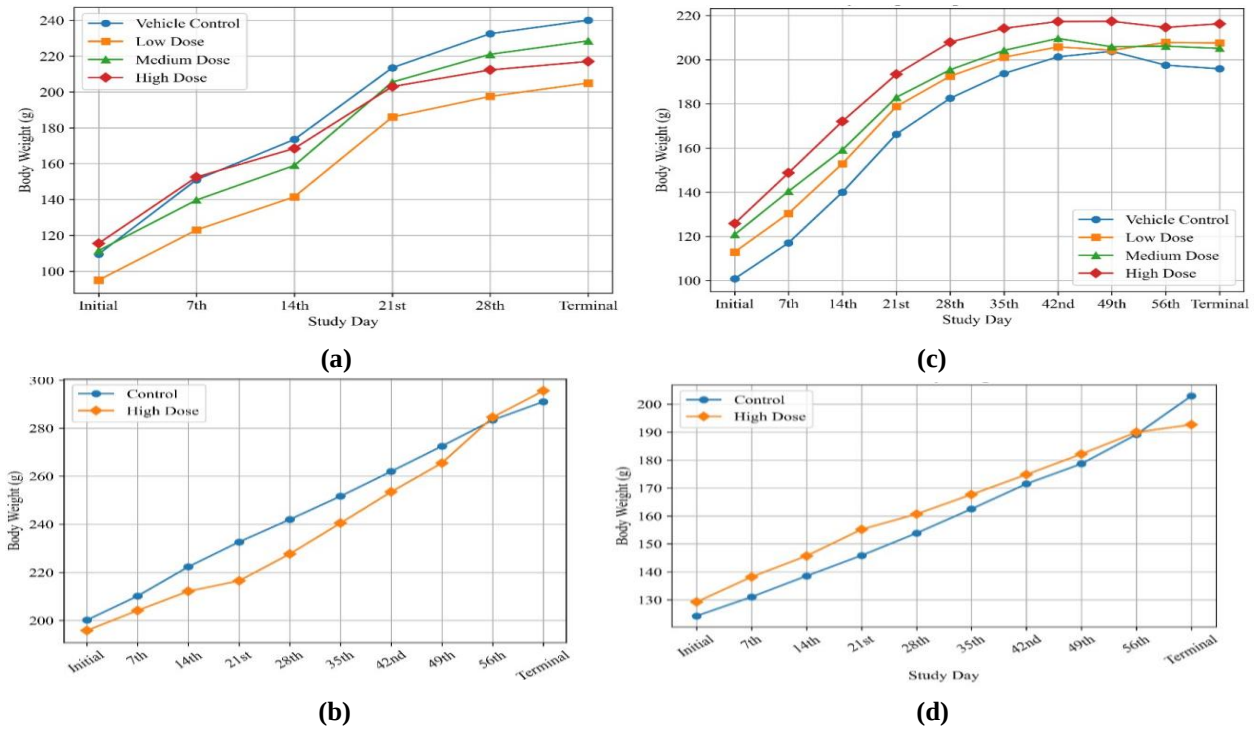

Figure 2. (a) Time-Dependent Body Weight Progression in Male Rats Across Treatment Groups, (b) Comparison of Final Body Weights in Male Rats Between Control and High Dose Groups, (c) Time-Dependent Body Weight Progression in Female Rats Across Treatment Groups, (d) Comparison of Final Body Weights in Female Rats Between Control and High Dose Groups

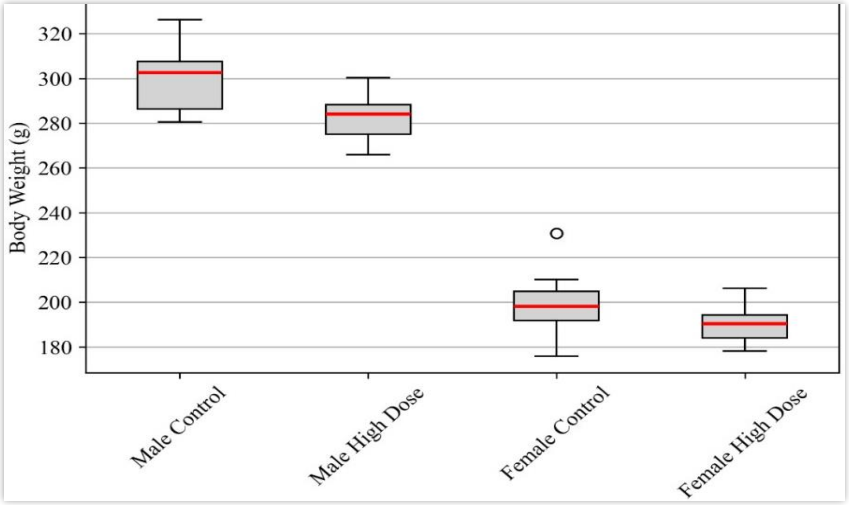

Figure 3. Boxplot of Terminal Body Weights in Male and Female Subjects under Control and High-Dose Treatment Conditions

The boxplot figure 3 illustrates terminal body weights across four groups: Male Control, Male High Dose, Female Control, and Female High Dose. The body weights of male subjects surpassed those of females throughout the control and high-dose testing groups. The study revealed a direct relationship between dosage and body weight decrease, where high-dose groups maintained lower median weights than control groups, independently for males and females [12,13]. Body weight reductions were greater among male subjects who lost approximately twenty grams, whereas female subjects lost approximately ten grams. The Female Control group had more weight variation, including one extreme value, whereas the Female High Dose group exhibited reduced weight range variability. High-dose exposure appears to induce systemic

effects that cause metabolic toxicity disturbances, leading to weight reduction [14].

Table 3. Mean Body Weight (g) ± SD in Male and Female Rats

Day	Control Group (g)	High-Dose Group (g)
Male		
Initial	200.17 ± 41.31	195.83 ± 19.34
7th Day	210.17 ± 37.58	204.17 ± 20.22
14th Day	222.33 ± 33.16	212.17 ± 20.22
21st Day	232.67 ± 30.79	216.50 ± 20.48
28th Day	242.00 ± 27.23	227.67 ± 19.02
35th Day	251.67 ± 27.40	240.50 ± 16.08
42nd Day	262.00 ± 23.87	253.50 ± 13.50
49th Day	272.50 ± 20.99	265.50 ± 12.18
56th Day	283.33 ± 21.83	284.50 ± 9.01
Terminal	291.00 ± 22.37	295.50 ± 15.49
Female		
Day	Control Group (g)	High-Dose Group (g)
Initial	124.17 ± 12.01	129.17 ± 11.14
7th Day	131.00 ± 12.30	138.17 ± 9.30
14th Day	138.50 ± 11.38	145.67 ± 8.87
21st Day	145.83 ± 12.50	155.17 ± 7.68
28th Day	153.83 ± 13.48	160.67 ± 7.39
35th Day	162.50 ± 13.46	167.67 ± 5.32
42nd Day	171.50 ± 13.97	174.83 ± 5.78
49th Day	178.67 ± 14.95	182.17 ± 6.91
56th Day	189.17 ± 21.99	190.00 ± 7.01
Terminal	203.00 ± 18.97	192.67 ± 7.37

Table 3 shows that male and female rats displayed different body weight patterns throughout the 56-day experiment when exposed to high-dose TBT (50 mg/kg/day). The male rat populations from both control and treated groups demonstrated continuous weight gain, with control rats maintaining greater mean weights during the entire research period [15]. Weight recovery in high-dose male rats exceeded control weights by Day 56 (295.5 ± 15.49 g vs. 291 ± 22.37 g), suggesting their system had adapted to the treatment. High-dose-treated female rats developed early growth suppression, which continued until the end of the 56-day experiment. The treated females maintained a body weight of 192.67 ± 7.37 g on Day 56, but control females weighed 203 ± 18.97 g, which suggests their condition was more severe and persistent[16]. The male rats regained normal weight after the treatment period, but the female rats continued to display lower weight measurements during the recovery period. The lower standard deviation observed in treated females supports the hypothesis of endocrine disruption because it indicates a stable stress anorexic response. Research results confirm results from rodent experiments to show that gender is a vital component in toxicological risk assessments.

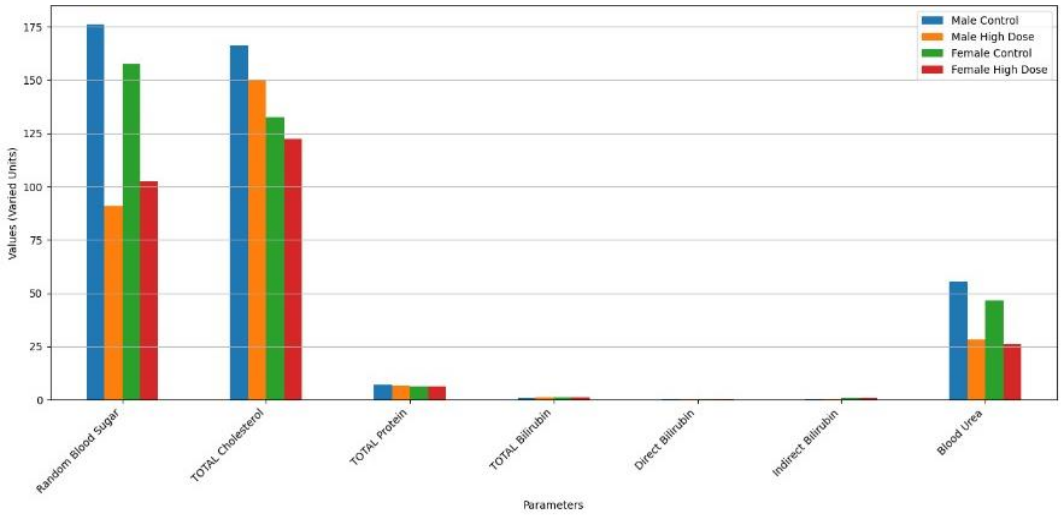

Figure 4. Biochemical Parameters in Male and Female Rats During the Recovery Phase

The biochemical evaluation of male and female rats (Figure 4) receiving high isobutyl paraben doses occurred throughout the recovery phase. Random blood sugar measurements reached their maximum levels in male control rats, followed by decreasing trends in male high-dose rats and female control rats until reaching their minimum levels in female high-dose rats [17,18]. The male control group exhibited the highest total cholesterol levels, which decreased across the other groups until reaching their lowest point in the female high-dose group. The observation data indicate that high-dose exposure to isobutyl paraben leads to hypoglycemic and hypolipidemic effects primarily within female subjects. The experimental conditions did not affect overall protein synthesis as total protein levels remained constant between all tested groups. The recovery phase showed no differences in total bilirubin, direct bilirubin and indirect bilirubin levels between groups, indicating liver function and bilirubin metabolism remained unaffected in every group. The results could indicate that exposure did not cause hepatotoxicity.

The blood urea levels decreased from male controls to female rats treated with high doses of the drug, while showing the highest levels in male controls and the lowest in female high-dose rats. The lowered blood urea levels could result from either modified nitrogen metabolism or renal function after rats stopped receiving isobutyl paraben. The rats displayed limited biochemical improvement during the recovery period, according to data that revealed metabolic patterns that depended on dosage and sex [19]. Female rats experience more pronounced effects than males due to specific metabolic differences in how their bodies handle isobutyl paraben.

Haematological Parameters Analysis

Male rats who received different doses of isobutyl paraben exhibited minimal non-linear changes in their hematological results, shown in Table 4.

Table 4. Mean Male Hematological Values across Experimental Groups

Parameter	High Dose (50 mg/kg/day)	Medium Dose (20 mg/kg/day)	Low Dose (10 mg/kg/day)	Vehicle Control (Corn Oil)	Male Recovery (50 mg/kg/day)
RBC (mill/cumm)	4.89	5.11	4.43	4.64	4.71
WBC (cells/cumm)	7650.00	6950.00	7325.00	7316.67	7283.33
Hemoglobin (gm/dl)	15.67	15.67	15.45	15.52	15.55
Platelet Count (lakh/cumm)	5.17	5.86	4.48	4.53	4.58
Neutrophils (%)	59.17	50.67	62.50	59.17	58.33
Lymphocytes (%)	35.33	47.00	34.33	37.83	37.17
Eosinophils (%)	6.33	4.00	6.33	6.33	6.33
Monocytes (%)	0.00	0.00	0.00	0.00	0.00
Basophils (%)	1.00	1.00	1.00	1.00	1.00

RBC counts experienced minimal changes between all groups, with a dose-dependent reduction followed by complete recovery during the measured period. The percentages of basophils and eosinophils stayed stable throughout testing, while no monocytes appeared, indicating no substantial inflammatory immunotoxic response. The measured blood parameters, including WBC counts and hemoglobin levels, and differential counts, remained stable across all experimental groups [20]. Observation data showed that monocytes were not found in the blood samples, and the numbers of basophils and eosinophils remained constant, suggesting that little inflammatory immunotoxic effects occurred. The blood parameter measurements show that the exposure to isobutyl paraben causes moderate dose-dependent alterations leading to mild toxicity and recovery.

Table 5: Female Hematological Parameters Across Treatment Groups

Parameter	Vehicle Control (Corn Oil)	Low Dose (10 mg/kg/day)	Medium Dose (20 mg/kg/day)	High Dose (50 mg/kg/day)	Recovery Group (50 mg/kg/day)
RBC (mill/cumm)	4.13	5.21	5.47	6.80	5.47
WBC (cells/cumm)	9900	8700	7800	4300	4300

Hemoglobin (gm/dL)	15.7	15.9	16.4	17.9	16.3
Platelet Count (lakhs/cumm)	4.16	5.81	5.46	4.97	5.46
Neutrophils (%)	58	60	45	52	45
Lymphocytes (%)	39	30	49	41	49
Eosinophils (%)	7	6	5	6	5
Monocytes (%)	0	0	0	0	0
Basophils (%)	1	1	1	1	1

The isobutyl paraben exposure showed a direct correlation with changes in blood cell parameters. The results showed that both RBC count and haemoglobin levels rose steadily across different dose levels, indicating better erythropoiesis [21]. High dose administration of the substance resulted in significant reductions of WBC counts, thus demonstrating immunosuppressive effects. High-dose treatment of isobutyl paraben resulted in a two-phase reaction where platelet numbers first decreased, then increased at lower doses. Blood cell differential counts showed changing proportions between neutrophils and lymphocytes without any modifications in eosinophil, basophil, and monocyte levels in Table 5. The recovery of RBC and platelet numbers approached normal values while WBC suppression continued after treatment, which indicated partial recovery. The study shows that isobutyl paraben induces dose-dependent modifications in blood cell counts that could disrupt immune response and enhance red blood cell production.

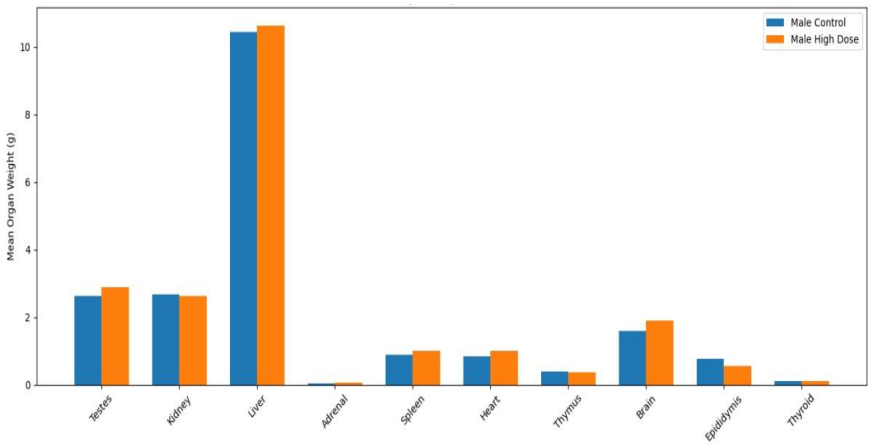

Figure 5. Comparison of Mean Organ Weights in Control and High-Dose Male Rats

Figure 5 illustrates that the high-dose group demonstrated slightly elevated weights in liver and brain tissues compared to the control group, which suggested possible tissue enlargement and brain inflammation. The study revealed minor weight changes in spleen and heart tissue alongside reduced thymus weight, which might indicate immunological suppression [22]. The weights of testes and kidneys demonstrated no substantial variations between groups. The slight increase in adrenal weight likely happened because of stress exposure. The epididymis and thyroid organs, alongside other minor structures, showed minimal alterations in this study.

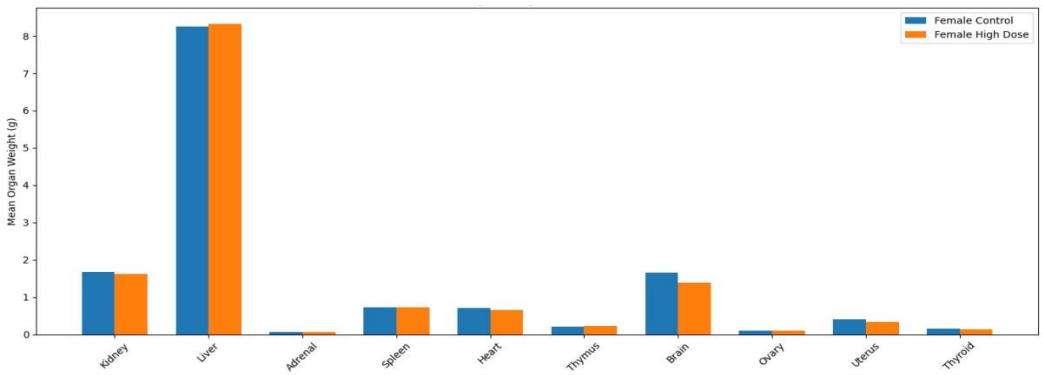

Figure 6. Organ-Specific Changes Induced by High-Dose Exposure in Female Rats

The substance's high dosage level triggered organ-specific changes in female rats that produced noticeable modifications across multiple organs in Figure 6. The liver weight increased slightly, indicating hepatic changes, while the brain weight decreased slightly, suggesting possible neurotoxic results [23]. The renal system and thymus tissue displayed minimal weight reduction, possibly indicating kidney and immune system dysfunction [24]. Weight reductions were observed in female reproductive organs, including the ovaries and uterus, which indicates potential issues with female reproductive health. The observed outcomes differ from male rat results, which suggests the substance might trigger different effects between sexes.

Study of systemic toxicity

The biochemical analysis (Figure 7) evaluated the physiological changes in six male rats per treatment group who received Low Dose (10 mg/kg/day), Medium Dose (20 mg/kg/day), and High Dose (50 mg/kg/day) of Isobutyl Paraben [25]. The assessment involved measuring Random Blood Sugar together with Total Cholesterol and Total Protein and Direct Bilirubin and Indirect Bilirubin and Serum Creatinine, and Blood Urea.

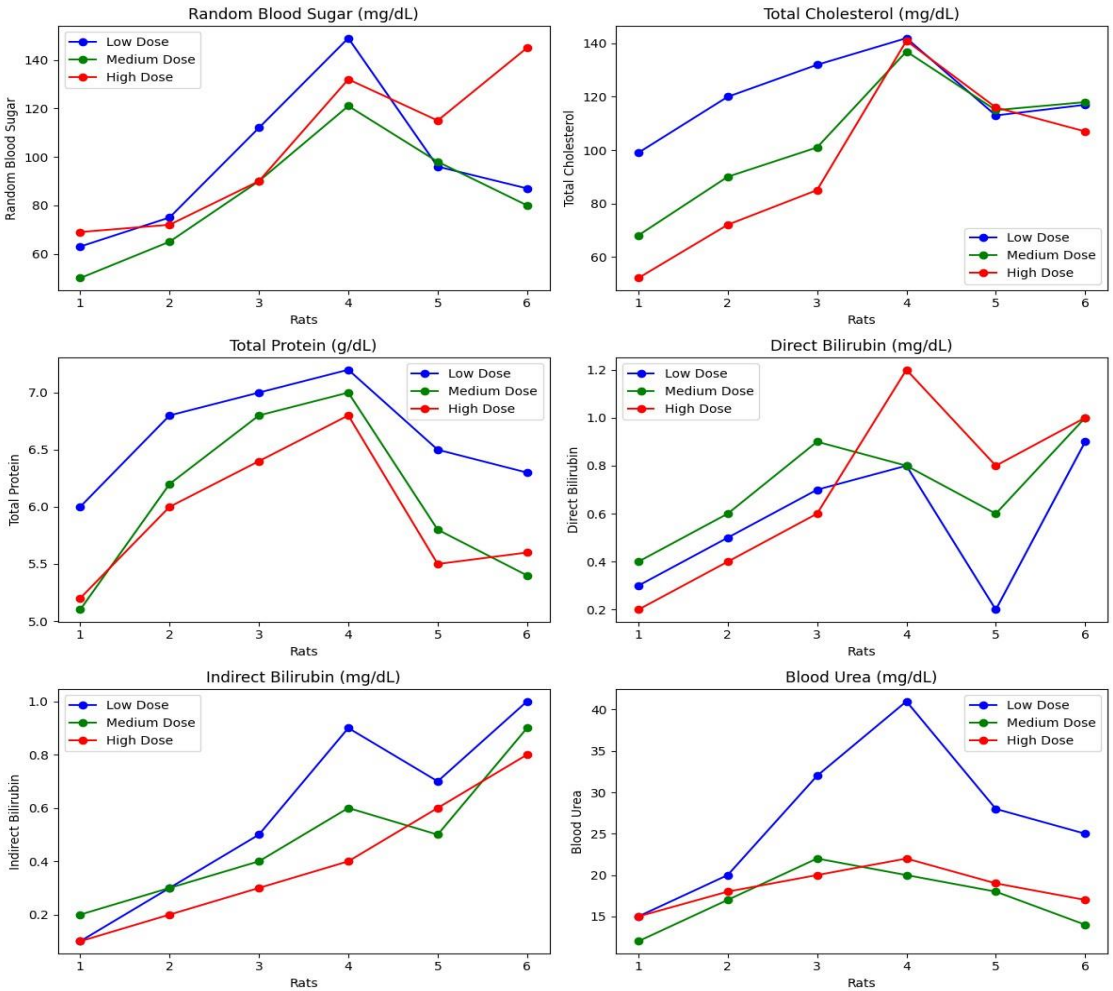

Figure 7. Individual Animal Variability in Biochemical Parameters Across Isobutyl Paraben Dose Groups in Male Rats

3.5.1 Random Blood Sugar (RBS)

Blood glucose levels decreased as the concentration of Isobutyl Paraben in the test solutions increased. The Medium Dose group demonstrated the most reduced blood glucose measurement at 50 mg/dL, indicating potential hypoglycemic effects. The High Dose group showed higher variability, while specific animals measured very low glucose levels at 69 and 72 mg/dL, which confirmed the glycemia reduction pattern at elevated exposure doses [26].

Total Cholesterol

The data showed that cholesterol levels decreased proportionally with increasing dose concentrations. Cholesterol levels in the High Dose group reached their lowest point at 52 mg/dL while exhibiting consistently lower average values than both Low and Medium Dose groups [27]. Research data shows that Isobutyl Paraben may have a suppressive impact on lipid metabolic processes.

Total Protein

The total protein levels in serum showed minimal changes across all dose groups with no established dose-response pattern. The tested doses of Isobutyl Paraben showed no noticeable effects on protein synthesis and breakdown processes.

Total, Direct, and Indirect Bilirubin

The values for direct bilirubin in all groups stayed within normal physiological ranges. The data showed no dose-dependent pattern of consistent change in direct bilirubin excretion by the liver. The study showed that indirect bilirubin values stayed stable between groups, and treatment did not produce noticeable changes. The experimental period showed that Isobutyl Paraben did not modify bilirubin metabolic activities.

Blood Urea

The Medium and High Dose groups demonstrated significantly lower blood urea levels compared to the Low Dose group. Blood urea levels decreased gradually throughout the study because Isobutyl Paraben either modified nitrogen metabolism or boosted renal clearance in a dose-dependent fashion. The results show that male rats exposed to Isobutyl Paraben develop dose-dependent biochemical changes that specifically affect blood glucose, cholesterol, and urea levels. Total protein levels, together with bilirubin and creatinine, showed no dose-related changes in the male rat subjects. The field needs to investigate biological mechanisms that affect metabolism and identify potential health threats from paraben exposure.

Histopathological Changes in Uterine Tissue

Histological examination of uterine tissue from adult rats exposed to isobutyl paraben (IBP) for 56 days revealed distinct dose-dependent alterations in uterine architecture. In the control group (Figure 8a) and the low-dose IBP group (10 mg/kg/day) (Figure 8b), the uterine lumen (UL) was lined with a continuous, uniform columnar epithelium. The lumen diameter appeared within normal limits, and the endometrial glands were regular in shape, predominantly round or oval, with no signs of structural disruption. In contrast, moderate (20 mg/kg/day) (Figure 8c) and high-dose (50 mg/kg/day) (Figure 8d) IBP exposure induced marked histopathological changes compared to controls. These included a pronounced enlargement of the uterine lumen, irregularity and distortion of the columnar epithelial lining, and a notable increase in luminal epithelial (LE) thickness with evident folding. The endometrium (E) and myometrium (M) exhibited increased thickness in these groups, accompanied by a significant rise in the number of uterine glands (UG). The observed alterations were more severe at the highest dose, indicating a clear dose-related progression in IBP-induced uterine structural changes. Such findings are consistent with reports that parabens act as a weak estrogen capable of binding to estrogen receptors and inducing abnormal endometrial proliferation [3]. Similar uterine hyperplasia and glandular remodeling after paraben exposure have also been demonstrated IBP rodent studies, supporting the view that IBP exerts estrogen receptor-mediated effects on uterine tissues [7].

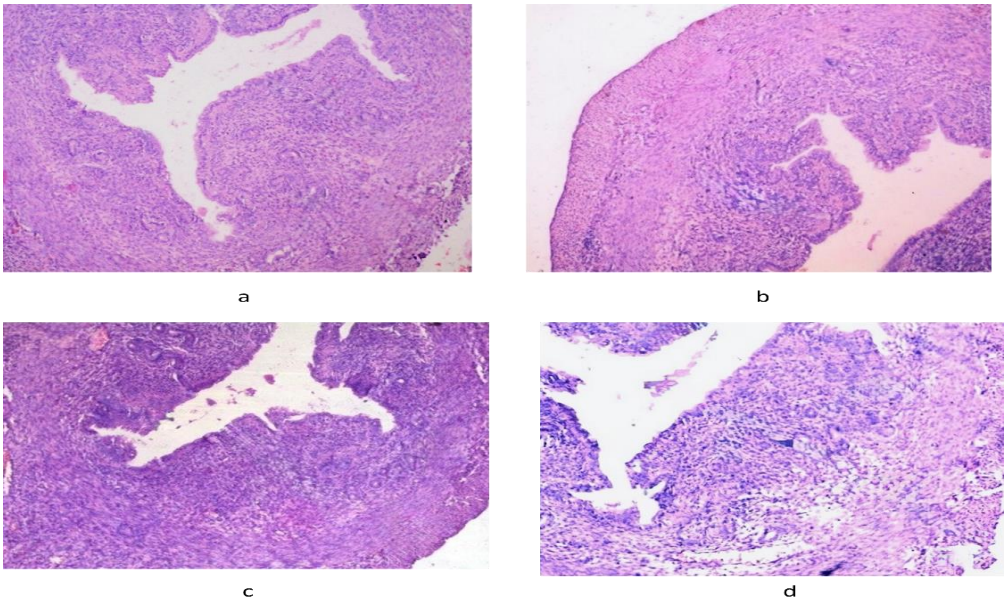

Figure 8. Dose-dependent histopathological alterations in uterine tissue of rats exposed to isobutyl paraben (IBP) for 56 days, showing (a) normal architecture in the control group, (b) intact epithelial lining at low dose 10 mg/kg/day, (c) enlarged lumen and epithelial irregularities at medium dose 20 mg/kg/day, (d) pronounced epithelial folding with glandular hyperplasia at high dose 50 mg/kg/day

4. CONCLUSION

A study evaluates how multiple oral exposures to IBP influence Wistar rats throughout their systemic development and reproductive growth. The research found that IBP doses resulted in proportional changes in food consumption, body weight measurements, and blood indicators, demonstrating metabolic imbalances, particularly in male rats. Further studies indicate that IBP testing should focus on consumer products that result in prolonged user contact with the substance. The complete impact of IBP exposure on human health necessitates extensive research to establish.

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