Original Research Article

Evaluation of Low-Dose Effects and Non-Monotonous Dose Response of Butyl p-hydroxybenzoate, or Butylparaben on Female Wistar Rats

Abstract

Butylparaben (BP) is an extensively used synthetic preservative that has been testified as an endocrine disruptor and speculated to be toxic for the body all together. The present study evaluated the possible noxious impact of BP at minimum doses, i.e., 1, 5, and 10 mg/kg BW/day, on female Wistar rats following consecutive subcutaneous exposure for 7 and 21 days. The results of the genotoxic study exhibited that BP at a dose of 10 mg/kg BW/day significantly ($p < 0.05$) induced micronucleated polychromatic erythrocytes (MNPCEs) formation in bone marrow of rats, both in short (7.3 ± 0.87 MNPCEs/1000 PCEs) and long-term exposures (6.6 ± 0.33 MNPCEs/1000 PCEs), which was in line with the results obtained for 17β-estradiol (E2)-treated reference control rats. Levels of hepatic toxicity marker enzymes, i.e., aspartate transaminase (AST) and alanine transaminase (ALT), were not altered substantially in any of the study durations, but the alkaline phosphatase (ALP) level increased significantly ($p < 0.05$) at all BP doses, including E2. Variations in the serum HDL as well as cholesterol levels were found to be non-monotonous with respect to the applied BP doses. However, contrasting specifics were observed for triglycerides in terms of the study durations. Conclusively, the current study established that BP, even at low doses, could evidently disturb the circulating lipid content, hepatic enzyme levels, and even cause genotoxicity, and also pointed toward its non-monotonous dose response.

Keywords: Butylparaben, preservative, endocrine disruptor, genotoxicity.

1. Introduction:

Since the term "endocrine disruptor" was introduced in 1991, there has been an ever increasing scientific and public interest regarding the issue of exposure to endocrine disrupting chemicals (EDCs) and possible adverse health consequences in humans and other living organisms [1, 2]. Well-documented scientific reports and discoveries strongly indicated the reproductive and developmental processes as vulnerable targets of EDCs, and the growing body of evidence has now highlighted many other targeted physiological pathways [3]. For instance, a ubiquitous synthetic chemical called butylparaben (BP) has been in wide applications as preservatives since the mid-1920s and is anticipated to be a thyroid as well as gonadal disruptor [4, 5]. Although this butyl ester of p-hydroxybenzoic acid (PHBA) has a short half-life and is hence reported as non-hazardous at the systemic level, many studies have detected the presence of BP in biological samples like serum, urine, breast milk, fatty tissue, amniotic fluid, seminal plasma, and even in breast tumor samples [6, 7, 8, 9, 10, 11, 12]. Several studies have shown that butylparaben causes oxidative stress [13,14].

A number of studies established interference of BP with the functions of sex steroid hormones, which chiefly regulate reproductive development, sex differentiation, puberty, early embryonic development, etc. [15, 16, 17, 18, 19, 20]. BP has been found to induce breast cancer progression, gestational diabetes, obesity, etc. [11, 21, 22]. Our previous study revealed BP as a thyroid disruptor [23]. As thyroid hormones (THs) regulate most of the body's metabolic activities, including lipid metabolism, any disruption of these hormones may have a substantial impact on the serum lipid profile of an individual. In view of the facts that BP has estrogenicity, widespread applications, efficient endocrine disrupting properties, and bio-accumulative

potential, it has been speculated that BP may hold immense potential to disrupt the functioning of virtually the entire body. The present study was aimed evaluating the genotoxic effects and alterations in circulating HDL, cholesterol and triglycerides levels as well as AST, ALT and ALP levels of female Wistar rats following exposure to BP at very low doses.

2. Materials and method:

2.1. Animal

Healthy adult female Wistar rats (approximately 9 weeks of age with BW, 130 ± 10 g) were taken from the Dept. of Pharmacology, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam. All the animals were kept in the Animal House Facility of the Dept. of Zoology, Gauhati University, for a period of 15 days to acclimate them to the new laboratory environment. Standard laboratory conditions like humidity of 40- 70%, temperature of 20-26ºC, and 12 hrs light:12 hrs dark photoperiod were constantly maintained. Animals were fed with a pelleted diet (soya-free) and clean drinking water *ad libitum*.

2.2. Selection of the doses of the test chemical

The three doses of BP used in the current study were selected on the basis of the existing literature and the subsequent results of a pilot study performed in our laboratory. A recent report published by the National Institute for Public Health and the Environment (RIVM) mentioned that in rodents, the oral LD_{50} value of BP is greater than 5000 mg/kg BW, intraperitoneal BP treatment has an LD_{50} value of 230 mg/kg BW and rabbit dermal exposure has an LD_{50} more than 2000 mg/kg BW [24]. In the present study, a comparatively low dose of BP, 1mg/kg BW/day was considered which was below the NOEL value i.e. 2 mg/kg BW/day of BP [25]. This value reported by Fisher et al. has been considered by the SCCS of EU as the present conservative NOEL value for BP [26]. Butylparaben at a concentration of 10 mg/kg BW/day was taken as a high BP dose in the present study. This particular value has been reported as the lowest observed adverse effect level (LOAEL) value of BP in a number of earlier reports [24, 27, 28]. To determine the dose response relationship, 5 mg/kg BW/day was taken as a medium dose of BP in the current study. On the basis of the conversion coefficient between rats and humans [29], the corresponding doses of 1, 5 and 10 mg/kg BW/day of BP were calculated for humans and found to be 0.16, 0.81 and 1.62 mg/kg BW/day of BP respectively. These values were below the daily level of PBs exposure in humans and lower than the recommended BP level in various products [26, 30].

2.3. Administration of doses

The present study includes five groups (n = 12); a control group (represented as C), three BP treatment groups, viz., 1 mg/kg BW/day of BP, 5 mg/kg BW/day of BP, and 10 mg/kg BW/day of BP, which were shown as BP1, BP5, and BP10, respectively, and a positive control group, i.e., 0.01 mg/kg BW/day of E2 (shown as E2). Doses were injected subcutaneously in a volume of 100µL aliquot daily under the scruff region. After 7 and 21 days of treatment, animals were euthanized under mild anaesthesia and all surgical procedures were followed.

2.4. Estimation of serum lipids

The present study looked into the possible imbalance or alteration of serum lipids owing to BP exposure in rats. To fulfill the aim, quantifications of serum triglyceride, HDL, and cholesterol levels were performed using a Semi Auto Biochemistry Analyzer (Erba Chem 5X, TRANSASIA®, ERBA Diagnostics Mannheim GmbH, Mallaustrasse, Germany). For each biochemical assay, specific kits from ERBA (ERBA Diagnostics Mannheim GmbH, Mallaustrasse., Germany) were used following the manufacturer's instructions.

2.5. Assays for toxicity marker enzymes

Toxicity markers of the liver, i.e., AST, ALT, and ALP levels, were measured in a Semi Auto Biochemistry Analyzer (Erba Chem 5X, TRANSASIA®, ERBA Diagnostics Mannheim

GmbH, Mallaustrasse, Germany). All the assay kits were from ERBA Diagnostics, and the procedures followed were in accordance with the manufacturer's specifications.

2.6. Micronuclei assay for genotoxicity study

The genotoxic potency of butylparaben was evaluated through a Micronuclei assay following the procedure of Schmid [31]. Femora bone of rats was removed, and using a 1 mL syringe, the bone marrow was aspirated and suspended in FBS. The suspension was centrifuged at 1,000 rpm for 5 min, the supernatant was discarded, and using a Pasteur pipette, the cells in the sediment were transferred to glass slides to prepare the smears. Slides were first stained for 3 min in undiluted May-Grunwald's stain and then in diluted May-Grunwald's stain (1:1 in distilled water) for 2 min. In Giemsa (diluted with phosphate buffer in a ratio of 1:6), the slides were stained for 10 min and subsequently rinsed in distilled water. After blot dried with filter paper, the slides were cleared in xylene for 5 min and then mounted in DPX to examine under a microscope with high magnifications (100x). 1000 polychromatic erythrocytes (PCEs)/smear were screened for examining the presence of MNPCEs.

3. Results

3.1. Micronuclei induction in PCEs of bone marrow after butylparaben exposure

Table 1 shows the frequency of micro-nucleated PCEs (MNPCEs) in the respective groups. Photomicrographs showing the occurrence of MNPCEs after 7 and 21 days of treatment periods are displayed in Fig.1a-e and Fig.2a-e respectively. Results revealed that BP induced the generation of micronuclei in a dose-dependent manner. However, a significant effect was observed only at the selected maximum dose (10 mg/kg BW/day). At a low dose of BP (1 mg/kg BW/day), there was a slight elevation in the number of MNPCEs after 7 and 21 days of exposure, but statistical significance was not found against the control group ($p > 0.05$) (Fig. 1b and 2b). BP5 caused further rise of micronuclei; 5 ± 0.57 MNPCEs/1000 PCEs and 6 ± 0 MNPCEs/1000 PCEs after 7 and 21 days of treatment respectively, as depicted in Table 1 and Fig.1c&2c. Yet again, the effect was not significant ($p > 0.05$) since the control animals showed 3.3 ± 0.33 MNPCEs/1000 PCEs following 7 days of vehicle treatment and 3.6 ± 0.66 MNPCEs/1000 PCEs after 21 days of dosing (Table 1). BP at 10 mg/kg BW/day caused a significant ($p > 0.05$) rise in micronuclei production both in short (7.3 ± 0.87 MNPCEs/1000 PCEs) and long-term exposures (6.6 ± 0.33 MNPCEs/1000 PCEs), as shown in Fig.1d and 2d. Therefore, only BP10 generated a substantial genotoxic effect in the rats. With reference to the time-course study, the observed genotoxic effects were almost alike. Comparatively, the highest occurrence of MNPCEs was observed in the E2 group, Fig.1e and 2e. Short-term treatment of E2 resulted in the formation of 9.3 ± 0.33 MNPCEs/1000 PCEs, and in long-term administration, there were 10 ± 0.57 MNPCEs/1000 PCEs, which were significantly different than the control, ($p < 0.05$).

Table 1: Micronuclei induction in polychromatic erythrocytes (PCEs) of bone marrow in rats exposed to different doses of butylparaben (BP) for 7 and 21 days.

Animal groups	Mean MNPCEs / 1000 PCEs ± SEM	
	7 days treatment	21 days treatment
Control (olive oil)	3.3 ± 0.33	3.6 ± 0.66
BP1 (1mg/kg BW/day)	3.6 ± 0.33	4 ± 0.57
BP5 (5mg/kg BW/day)	5 ± 0.57	6 ± 0
BP10 (10mg/kg BW/day)	7.3 ± 0.87*	6.6 ± 0.33*
E2 (17β-estradiol, 0.01mg/kg BW/day)	9.3 ± 0.33*	10 ± 0.57*

All the values are shown as mean ± SEM (triplicate measurement). * $p < 0.05$, compared to control. MNPCES - micro-nucleated polychromatic erythrocytes.

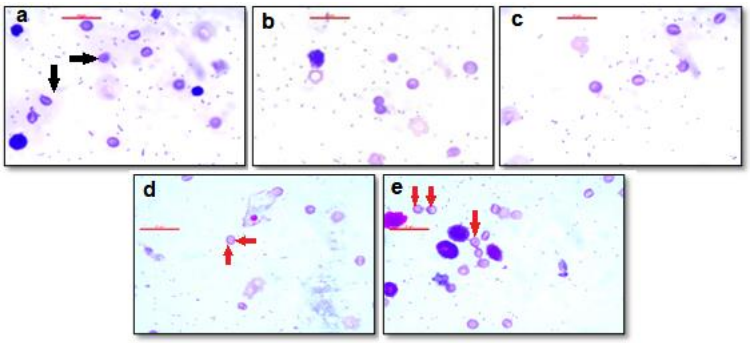

Fig.1 Micronuclei formation in polychromatic erythrocytes (PCEs) of the bone marrow of rats after butylparaben exposure for 7 days. [a] Control (olive oil), [b] 1 mg/kg BW/day of BP, [c] 5 mg/kg BW/day of BP, [d] 10 mg/kg BW/day of BP, and [e] 0.01 mg/kg BW/day of 17β-Estradiol (E2). Black arrows show polychromatic erythrocytes (PCEs), and the red arrows indicate the presence of micro-nucleated PCEs (MNPCEs) (magnification 100x).

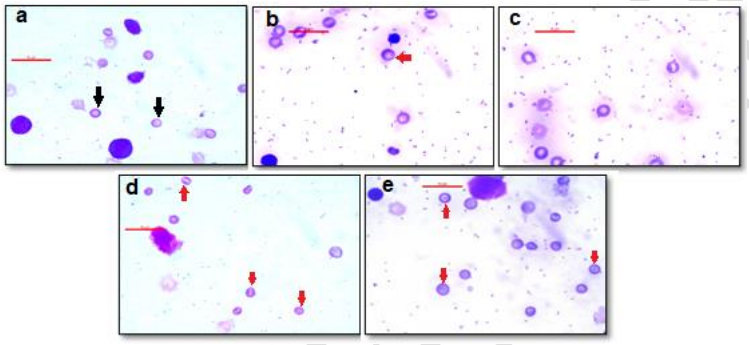

Fig.2 Micronuclei formation in polychromatic erythrocytes (PCEs) of the bone marrow of Wistar rats after butylparaben exposure for 21 days. [a] Control (olive oil), [b] 1 mg/kg BW/day of BP, [c] 5 mg/kg BW/day of BP, [d] 10 mg/kg BW/day of BP, and [e] 0.01 mg/kg BW/day of 17β-Estradiol (E2). Black arrows show polychromatic erythrocytes (PCEs) and red arrows indicate the presence of micro-nucleated PCEs (MNPCEs) (magnification 100x).

3.2. Effects of butylparaben on serum lipids

Subsequent to both short and long-term exposures, there were significant effects of the three selected doses of BP on HDL-cholesterol, total cholesterol as well as triglycerides content in the treated rats as shown in Figs 3, 4, and 5, respectively. After 7 days, BP1, BP5, and E2 doses exhibited a significant fall ($p < 0.05$) in serum HDL-cholesterol levels compared to control rats (Fig. 3a). In contrast, animals administered with BP at 10 mg/kg BW/day showed no significant changes, thus reflected a non-monotonous dose effect on the same. After 21 days of exposure, the BP doses (1, 5, and 10 mg/kg BW/day) reduced HDL-cholesterol similar to the E2 treatment (Fig. 3b). However, the HDL-cholesterol level in BP5- treated and control animals displayed no significant variations. In the short-term study, the total cholesterol level estimated in the animals was found to be effectively altered by BP as well as E2, except for the 10 mg/kg BW/day of BP. As demonstrated in Fig. 4a, BP1 and BP5 depressed the serum cholesterol content significantly (47 ± 0.09 mg/dL at BP1 and 43 ± 1 mg/dL at BP5, $p < 0.05$), while a very minimal effect was apparent at the high BP dose (54 ± 0.39 mg/dL) compared to control (61.08 ± 0.78 mg/dL). There was also a significant drop in cholesterol level in the E2 group against control ($p < 0.05$). In the exact manner, 21 days of exposure period resulted in a marked downfall of serum cholesterol level, where significant effects ($p < 0.05$) were particularly

shown by BP1, BP5, and E2 (Fig. 4b). But no obvious effect was observed following BP10 exposure ($p > 0.05$).

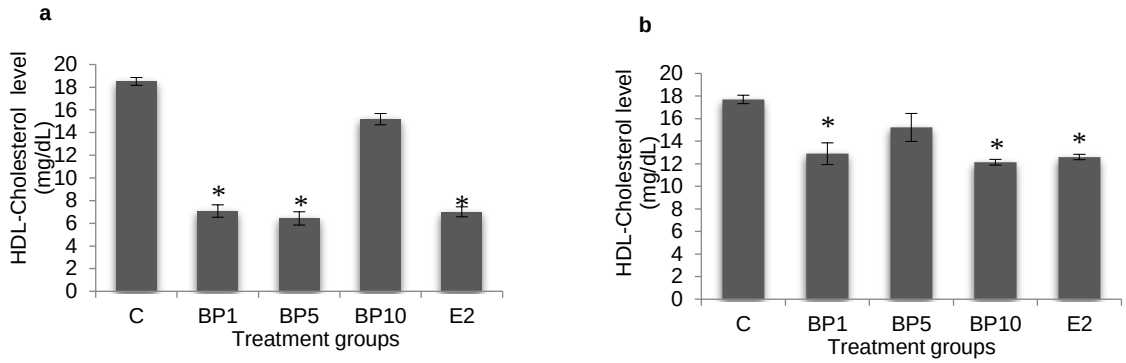

Fig.3 Changes in serum level of HDL-cholesterol in Wistar rats following exposure to butylparaben (BP) at doses of 1, 5, and 10 mg/kg BW/day (represented as BP1, BP5, and BP10, respectively) for 7 days [a] and 21 days [b]. The control rats were subjected to the vehicle (olive oil) only and 17β-estradiol (E2) was administered as a positive control dose (0.01 mg/kg BW/day). The values are expressed as mean ± SEM (n = 12). One-way ANOVA and Tukey test were performed to determine the variation against the control value. *$p < 0.05$, significantly different than the control.

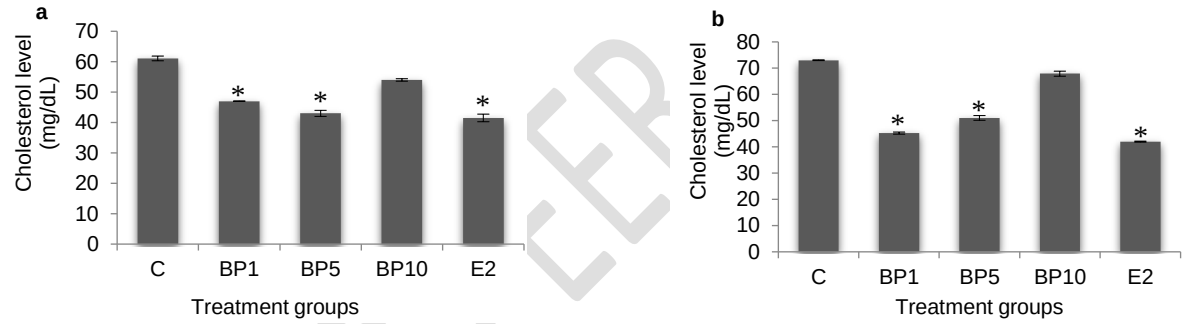

Fig. 4 Changes in serum level of cholesterol in Wistar rats following exposure to butylparaben (BP) at doses of 1, 5, and 10 mg/kg BW/day (represented as BP1, BP5, and BP10, respectively) for 7 days [a] and 21 days [b]. The control rats were subjected to the vehicle (olive oil) only, and 17β-estradiol (E2) was administered as a positive control dose (0.01 mg/kg BW/day). The values are expressed as mean ± SEM (n = 12). One-way ANOVA and Tukey test were performed to determine the variation against the control value. * $p < 0.05$, significantly different than the control.

Serum triglycerides content was determined, and the findings are shown in Figs. 5a & 5b. Subsequent to short-term treatment, BP at all concentrations showed a significant reduction of triglycerides in the animals compared to the control (142 ± 1.9 mg/dL). The estimated values for BP1, BP5, and BP10 were found to be 53.48 ± 1.4 mg/dL, 77.3 ± 0.58 mg/dL, and 85.9 ± 0.16 mg/dL, respectively (Fig. 5a). Similar was the case for E2 treatment, a significant fall ($p < 0.05$) in serum triglyceride level after 7 days of treatment (80 ± 1.24mg/dL). On the contrary, in 21 days of assessment, there was an opposite effect observed in serum triglycerides (Fig. 5b). Unlike the 7 days findings, no significant difference in serum level of triglycerides was found among the animals in BP1, BP10, E2, and control groups (157.4 ± 2.7 at BP1, 121 ± 1.76 at BP10, 130 ± 1.2 at E2 and 139 ± 4.3 mg/dL at control, $p > 0.05$). Only 5 mg/kg BW/day of BP was effective in causing a significant ascend of triglycerides in animals under 21 days of study (240 ± 0.86 mg/dL).

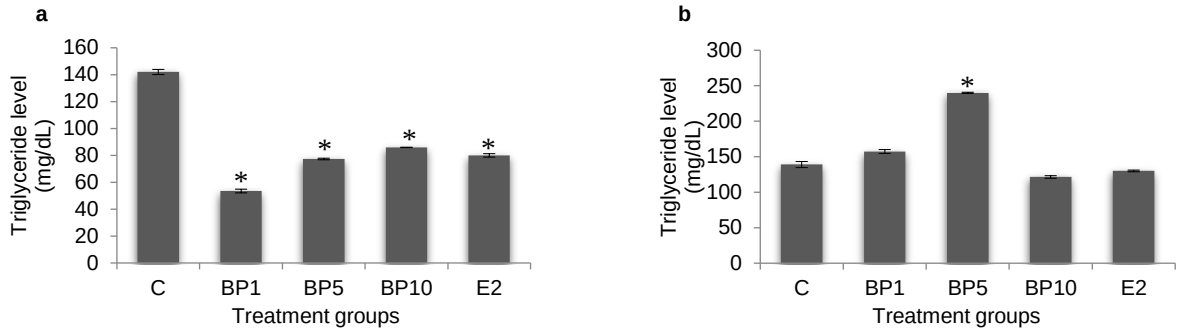

Fig. 5 Changes in serum level of triglycerides in Wistar rats following exposure to butylparaben (BP) at doses of 1, 5, and 10 mg/kg BW/day (represented as BP1, BP5, and BP10, respectively) for 7 days [a] and 21 days [b]. The control rats were subjected to the vehicle (olive oil) only, and 17β-estradiol (E2) was administered as a positive control dose (0.01 mg/kg BW/day). The values are expressed as mean ± SEM (n = 12). One-way ANOVA and Tukey test were performed to determine the variation against the control value. * $p < 0.05$, significantly different than control.

3.3. Effect of butylparaben on serum hepatic enzymes

The toxic impact of BP on the experimental animals was also assessed by evaluating serum levels of three hepatic marker enzymes, i.e., ASL, ALT, and ALP. In both the experimental durations, the animals treated with the three selected BP doses showed no significant changes in their serum AST level compared to the control animals (presented in Table 2). Similarly, the effect of E2 on AST level was not significantly different than control ($p > 0.05$). Serum ALT level was also unaffected by the BP and E2 treatments following both 7 and 21 days of exposures (Table 3). As presented in Table 4, dosing of BP for 7 days significantly altered the serum ALP level in the treated animals. Compared to the ALP level of control rats (130.6 ± 0.88 IU/1), the treated groups showed significant elevation of the enzyme level at 1, 5, and 10 mg/kg BW/day of BP (187.6 ± 0.83 in BP1; 181.5 ± 0.25 in BP5; and 171.1 ± 1.14 IU/1 in BP10; $p < 0.05$). A significant rise of serum ALP was noted in the E2exposed rats (189.00 ± 1.1 IU/1). Similarly, 21 days of dosing resulted in a significant elevation ($p < 0.05$) of the ALP level at the entire concentration range of BP tested (Table 4). At BP1, BP5, and BP10, the level of ALP was found to be 189.46 ± 0.74, 192 ± 1.45, and 194.6 ± 1.8 IU/1, respectively, whereas in the control animals it was 131.6 ± 1.76 IU/1. E2 showed a significant effect on ALP after administered consecutively for 21 days and the level of its increase was detected at 200± 2 IU/1 (Table 4).

Table 2: Serum aspartate transaminase (AST) level in rats after exposure to butylparaben (BP) for 7 and 21 days

Animal groups	AST (IU/1)	
	7 days treatment	21 days treatment
Control (olive oil)	32. 33 ± 0.33	32 ± 0.881
BP1 (1mg/kg BW/day)	30.6 ± 0.5	31.6 ± 0.33
BP5 (5mg/kg BW/day)	31. 30 ± 0.881	32.6 ± 0.33
BP10 (10mg/kg BW/day)	32. 3 ± 0.33	33.6 ± 0.33
17β-estradiol (E2) (0.01mg/kg BW/day)	31.05 ± 0.57	32.93 ± 0.57

All values are presented as mean ± SEM. No significant changes were observed ($p > 0.05$).

Table 3: Serum alanine transaminase (ALT) level in rats after exposure to butylparaben (BP) for 7 and 21 days

Animal groups	ALT (U/mL)	
	7 days treatment	21 days treatment
Control (olive oil)	35. 3 ± 0.88	36 ± 0.57
BP1 (1mg/kg BW/day)	34. 3 ± 1.76	36.6 ± 0.88
BP5 (5mg/kg BW/day)	35. 3 ± 0.57	34.66 ± 0.33
BP10 (10mg/kg BW/day)	34 ± 0.57	33.6 ± 0.66
17β-estradiol (E2) (0.01mg/kg BW/day)	35.6 ± 0.55	35.55 ± 0.57

All the values are presented as mean ± SEM. No significant changes were observed ($p > 0.05$).

Table 4: Serum alkaline phosphatase (ALP) in rats after exposure to butylparaben (BP) for 7 and 21 days

Animal groups	ALP (IU/1)	
	7 days treatment	21 days treatment
Control (olive oil)	130.6 ± 0.88	131.6 ± 1.76
BP1 (1mg/kg BW/day)	187.6 ± 0.83*	189.46 ± 0.74*
BP5 (5mg/kg BW/day)	181.5 ± 0.25*	192 ± 1.45*
BP10 (10mg/kg BW/day)	171.1 ± 1.14*	194.6 ± 1.8*
17β-estradiol (E2) (0.01mg/kg BW/day)	189.00 ± 1.1*	200 ± 2*

All values are presented as mean ± SEM.* $p < 0.05$, compared to control.

4. Discussion

A number of EDCs present in our environment have been documented as genotoxins, the chemicals or agents that induce DNA or chromosomal damage in a cell. Genetic toxicity in germ cells may result in heritable consequences, and if it occurs in somatic cells, it may induce mutations and later initiate malignant transformations. Therefore, we investigated potential genotoxic consequences due to butylparaben exposure by assessing the presence of micronuclei in the bone marrow erythrocytes of the treated rats. In our study, the induction of micronuclei in the treated animals provided suggestive evidence for the genotoxic effects of BP. In the short-term exposure, BP at 1 and 5 mg/kg BW/day enhanced micronuclei formation in the bone marrow PCEs of the rats, but the frequency of MNPCEs was not significantly different from the control animals. At 10 mg/kg BW/day, micronuclei formation was found to be greater compared to other doses of BP, and the frequency of MNPCEs was significantly elevated. Similarly, treatment with BP for 21 days induced genotoxicity in a dose-responsive manner. BP1 and BP5 induced micronuclei generation without marked variations compared to control rats, whereas the dose with a high BP concentration, 10 mg/kg BW/day, produced significant genetic toxicity and thereby generated more MNPCEs in the respective animals. E2 exposure at 0.01 mg/kg BW/day for 7 and 21 days caused further increase of MNPCEs occurrence. On the whole, it is obvious from the present study that BP at the dose of 10 mg/kg BW/day has the ability to cause considerable chromosomal breakage or separation that leads to micronuclei formations. However, the effects were independent of the exposure durations considered in the present study. The observed genetic effects in the current study were in agreement with the previously reported toxic assessments of different PBs. For example, in an *in vitro* approach, Güzel Bayülken and Ayaz Tüylü (2018) examined cytotoxic and genotoxic properties of BP, PP, iso-BP, and iso-PP at concentration of 100, 50, 25, and 10 µg/mL for 24 and 48 hrs [32]. In that study, BP induced micronuclei in the human peripheral lymphocytes after exposure for

24 and 48 hours were determined in a concentration dependent manner [32]. The study also reported that BP exhibits the potential to produce chromosomal aberrations [32]. Indeed, the ability of BP and PP to produce DNA damage, chromosomal aberrations, and sister-chromatid exchanges has been demonstrated earlier [33]. The genotoxic effects of E2 and other xenoestrogens have been well documented [34, 35, 36].

Any dysbalance of circulating THs in the body has great inferences on the circulating lipid content and the findings of the present study are somewhat the same. Animals subjected to BP for both 7 and 21 days exhibited significant changes in their serum lipid profiles. Short-term exposure to BP resulted in a reduction of both the serum HDL-cholesterol and cholesterol levels in the experimental rats. The level of triglyceride was considerably depressed after 7 days of BP administration. The long-term effects of BP demonstrated a significant reduction in serum HDL-cholesterol with low cholesterol levels. Conversely, there was a profound rise in serum triglycerides in the animals receiving 5 mg/kg BW/day of BP for 21 days, while others showed no considerable changes. It is well established that the synthesis, mobilization, and degradation of lipids are controlled by THs, and any dysbalance of these hormones like in the case of hypo- or hyperthyroidism, perturbs lipid metabolism. THs depletion raises the levels of plasma cholesterol, phospholipids, triglycerides, etc., whereas THs excess causes reverse effects [37]. THs induce cholesterol biosynthesis in the liver by stimulating the activity of the 3-hydroxy-3methylglutaryl-coenzymeA (HMG-CoA) reductase that catalyzes the first step of cholesterol biosynthesis. A crucial step in cholesterol biosynthesis is the uptake of LDL into hepatocytes through LDL receptor and it has been well established that T3 up-regulates LDL receptor gene expression in hepatic cells [38]. T3 induces the LDL receptor expression by directly binding to TREs. Furthermore, T3 controls the sterol regulatory element-binding protein-2 (SREBP-2), a transcription factor that positively regulates LDL receptor expression [39]. The effects mediated by THs on cholesterol levels occur via hepatic THRβ. Also, there has been evidence that T3 lowered levels of HDL-cholesterol and triglycerides. Such an association was also found in the present study. In the current report, a reduction in HDL-cholesterol might have resulted due to the high level of circulating T3, as supported by our previous research data [23]. As supported by our previously published study, Gogoi and Kalita [23], high T3 levels in E2treated animals may have caused a reduction in HDL-cholesterol [23]. Hence, the present study provides evidence that BP could indirectly alter lipid levels in the body by altering THs balance. It has been reported that THs mediate their effects on lipid metabolism through binding to the THRβ present in the liver cells [37]. It has become apparent in our earlier study that BP can alter gene expression of *Thrβ1* in liver tissue at all the applied doses [40]. Up-regulation of hepatic *Thrβ1* after both 7 and 21 days of BP exposure might have resulted in more access to T3 mediated genomic effects on lipid metabolism, as supported by our earlier study [23, 40]. Similarly, alterations detected for triglyceride level in the current study could possibly be due to the imbalance of the T3 level in the serum, as evident in the former study [23]. High T3 concentration might have lowered triglycerides in rats after 7 days of BP exposure. However, the effects observed after 21 days of dosing with BP were somewhat different. Here, our study did not find any changes in triglyceride levels in the BP treated rats. Such variations in plasma triglycerides have also been evident in hyperthyroid patients, where they can be in normal, lowered, or slightly elevated levels [37].

In this study, the general toxicity of BP was evaluated by estimating serum levels of three hepatic toxicity marker enzymes, i.e., ALT, AST, and ALP. But none of the BP doses elicited the levels of AST and ALT in the animals. Conversely, animals exhibited a considerably high serum ALP level after 7 and 21 days of BP exposure. It has been well documented in the former studies that following intestinal or dermal absorption, PBs undergo rapid hepatic metabolism then eliminated via urine, thus having nominal systemic exposure with a very short half-life, i.e., about 24 hours. Earlier reports have illustrated that BP has no

systemic effects in rats after oral, topical, or subcutaneous administrations [41]. In the study of Aubert *et al.* (2012), after oral and subcutaneous intake, more than 70% of the BP metabolite was excreted in urine within the first 24 hrs, whereas removal through faeces was less than 4% and content retained in the tissue and carcasses was about 2% [41]. In our study, the high ALP level in the serum owing to BP exposure could not get a precise explanation. But there has been evidence that sometimes deformities or dysbalance of bone functions or physiology may give rise to high serum ALP. Since it is well characterised in that BP disrupted thyroid functions and meanwhile remarkably elevated ALP content in serum, it can be expected that there may exist some kind of possible disrupting effect of BP on the calcitonin secreting parafollicular cells in the thyroid gland that may later affect bone functions. Recent reports also documented the association of THs imbalance with alterations of ALP. Since bone is a major target of the estrogen hormone, environmental chemicals with estrogenic potency may alter bone or bone marrow functions by directly acting on it [42]. Earlier, different PB esters had shown their potential negative impact on bone formation, which added new insight into PBs toxicity to the growing body of literature [43]. As a xenoestrogen, there are possibilities that BP may alter bone functions and ultimately reflect its effect on ALP levels. However, further studies are needed to establish the targets at mechanistic, cellular, and molecular levels.

Conclusion:

The present study showed that butylparaben has noxious health impacts, and even a minimal dose, i.e., 10 mg/kg BW/day, is sufficient to cause genotoxicity. BP not only alters circulating lipid content but also elevates serum ALP level following exposure for 7 and 21 days with low doses. Hence, further minimization of the current acceptable level of butylparaben as well as its restricted usage in different products are of utmost necessity.

Ethical approval

All the measures related to animal care and handling were conducted in accordance with the guidelines as recommended by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). Prior to initiating the present research work, all the experimental protocols of animal use were approved by Institutional Animal Ethics Committee (IAEC) with the reference number- IAEC/Per/2018-19/PP-IAEC/2018-19/47.

Disclaimer (Artificial intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

References

[1] Cuvillier-Hot, V., & Lenoir, A. (2020). Invertebrates facing environmental contamination by endocrine disruptors: Novel evidences and recent insights. *Molecular and cellular endocrinology,* 504, 110712, https://doi.org/10.1016/j.mce.2020.110712

[2] Yang, C., Song, G., & Lim, W. (2020). Effects of endocrine disrupting chemicals in pigs. *Environmental pollution*, 263(Pt B), 114505, https://doi.org/10.1016/j.envpol.2020.114505

[3] Lauretta, R., Sansone, A., Sansone, M., Romanelli, F., & Appetecchia, M. (2019). Endocrine Disrupting Chemicals: Effects on Endocrine Glands. *Frontiers in endocrinology*, 10, 178, https://doi.org/10.3389/fendo.2019.00178

[4] Nowak, K., Ratajczak–Wrona, W., Górska, M., & Jabłońska, E. (2018). Parabens and their effects on the endocrine system. *Molecular and cellular endocrinology*, 474, 238-251, https://doi.org/10.1016/j.mce.2018.03.014

[5] Thambirajah, A.A., Wade, M.G., Verreault, J., et al (2022). Disruption by stealth - Interference of endocrine disrupting chemicals on hormonal crosstalk with thyroid axis function in humans and other animals, *Environ. Res*. 203, 111906. https://doi.org/10.1016/j.envres.2021.111906

[6] Darbre, P. D., & Harvey, P. W. (2008). Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks. *Journal of applied toxicology*, 28(5), 561–578, https://doi.org/10.1002/ jat.1358

[7] Calafat, A. M., Ye, X., Wong, L. Y., Bishop, A. M., & Needham, L. L. (2010). Urinary concentrations of four parabens in the US population: NHANES 2005–2006. *Environmental health perspectives*, 118(5), 679-685, https://doi.org/10.1289/ehp.0901560

[8] Frederiksen, H., Jørgensen, N., & Andersson, A. M. (2011). Parabens in urine, serum and seminal plasma from healthy Danish men determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). *Journal of exposure science & environmental epidemiology*, 21(3), 262-271, https://doi.org/10.1038/jes.2010.6

[9] Barr, L., Metaxas, G., Harbach, C. A., Savoy, L. A., & Darbre, P. D. (2012). Measurement of paraben concentrations in human breast tissue at serial locations across the breast from axilla to sternum. *Journal of applied toxicology*, 32(3), 219-232, https://doi.org/10.1002/jat.1786

[10] Philippat, C., Wolff, M. S., Calafat, A. M., Ye, X., Bausell, R., Meadows, M., et al. (2013). Prenatal exposure to environmental phenols: concentrations in amniotic fluid and variability in urinary concentrations during pregnancy. *Environmental health perspectives*, 121(10), 1225–1231, https://doi.org/10.1289/ehp.1206335

[11] Khanna, S., Dash, P. R., & Darbre, P. D. (2014). Exposure to parabens at the concentration of maximal proliferative response increases migratory and invasive activity of human breast cancer cells in vitro. *Journal of applied toxicology*, 34(9), 1051–1059, Ahttps://doi.org/10.1002/jat.3003

[12] Moorchilot, V. S., Arun, P., Aravind, U. K., & Aravindakumar, C. T. (2024). Human exposure to methyl and butyl parabens and their transformation products in settled dust collected from urban, semi-urban, rural, and tribal settlements in a tropical environment. *Environmental Research*, *242*, 117805. https://doi.org/10.1016/j.envres.2023.117805

[13] Yin, Y., Xie, Z., Sun, X., Wu, X., Zhang, J., Shi, H., Ding, L., & Hong, M. (2023). Effect of Butyl Paraben on Oxidative Stress in the Liver of *Mauremys sinensis*. *Toxics*, *11*(11), 915. https://doi.org/10.3390/toxics11110915

[14] Kim, J.H., Moon, N., Heo, S.J., Jeong, Y.W., Kang, D.R. (2024). Repeated measurements and mixture effects of urinary bisphenols, parabens, polycyclic aromatic hydrocarbons, and other chemicals on biomarkers of oxidative stress in pre- and postpartum women. *Environmental Pollution*. 342, 123057 https://doi.org/10.1016/j.envpol.2023.123057.

[15] Vo, T. T., Yoo, Y. M., Choi, K. C., & Jeung, E. B. (2010). Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model. *Reproductive toxicology*, 29(3), 306-316, https://doi.org/10.1016/j.reprotox.2010.01.013.

[16] Alam, M. S., Ohsako, S., Kanai, Y., & Kurohmaru, M. (2014). Single administration of butylparaben induces spermatogenic cell apoptosis in prepubertal rats. *Acta Histochem,* 116(3), 474-480, https://doi.org/10.1016/j.acthis.2013.10.006

[17] Zhang, J., Li, Y., Gupta, A. A., Nam, K., & Andersson, P. L. (2016) b. Identification and Molecular Interaction Studies of Thyroid Hormone Receptor Disruptors among Household Dust Contaminants. *Chemical research in toxicology*, 29(8), 1345-1354, https://doi.org/10.1021/acs.chemrestox.6b00171

[18] Boberg, J., Axelstad, M., Svingen, T., Mandrup, K., Christiansen, S., Vinggaard, A.M., & Hass, U. (2016). Multiple endocrine disrupting effects in rats perinatally exposed to butylparaben. *Toxicological sciences*, 152(1), 244–256, https://doi.org/10.1093/toxsci/kfw079

[19] Riad, M. A., Abd-Rabo, M. M., Abd El Aziz, S. A., El Behairy, A. M., & Badawy, M. M. (2018). Reproductive toxic impact of sub chronic treatment with combined butylparaben and triclosan in weanling male rats. *Journal of biochemical and molecular toxicology*, 32(3), e22037, https://doi.org/10.1002/jbt.22037

[20] Schreiber, E., Garcia, T., Sharma, R. P., Torrente, M., Domingo, J. L., & Gómeza, M. (2019). Oxidative stress in testes of rats exposed to n-butylparaben. *Food and chemical toxicology,* 131, 110573, https://doi: 10.1016/j.fct.2019.110573

[21] Kolatorova, L., Sramkova, M., Vitku, J., Lischkova, O., Starka, L., & Duskova, M. (2018). Parabens and their relation to obesity. *Physiological research*, 67(Suppl 3), S465-S472, https://doi.org/10.33549/physiolres.934004

[22] Li, Y., Xu, S., Li, Y., Zhang, B., Huo, W., Zhu, Y., et al. (2019). Association between urinary parabens and gestational diabetes mellitus across prepregnancy body mass index categories. *Environmental Research*, 170, 151–159, https://doi.org/10.1016/j.envres.2018.12.028

[23] Gogoi, P., Kalita, J.C., 2020. Effects of butylparaben exposure on thyroid peroxidase (TPO) and type 1 iodothyronine deiodinase (D1) in female Wistar rats. *Toxicology* 443, 152562. https://doi.org/10.1016/j.tox.2020.152562.

[24] RIVM Report, 2018-0161. Review on butylparaben: exposure, toxicity and risk assessment. National Institute for Public Health and the Environment, doi: 10.21945/RIVM-2018-0161

[25] Fisher, J. S., Turner, K. J., Brown, D., & Sharpe, R. M. (1999). Effect of neonatal exposure to estrogenic compounds on development of the excurrent ducts of the rat testis through puberty to adulthood. *Environmental health perspectives*, 107(5), 397–405, https://doi.org/10.1289/ehp.99107397

[26] SCCS (Scientific Committee on Consumer Safety) (2013). Opinion on Paraben. Updated Request for a Scientific Opinion on Propyl- and Butylparaben. SCCS/1514/13, doi:10.2772/66369

[27] 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

[28] Boberg, J., Taxvig, C., Christiansen, S., & Hass, U. (2010). Possible endocrine disrupting effects of parabens and their metabolites. *Reproductive toxicology*, 30(2), 301–312, https://doi.org/10.1016/j.reprotox.2010.03.011

[29] Reagan-Shaw, S., Nihal, M., & Ahmad, N. (2008). Dose translation from animal to human studies revisited. *FASEB Journal*, 22(3), 659-661, https://doi.org/10.1096/fj.07-9574LSF

[30] Soni, M. G., Carabin, I. G., & Burdock, G. A. (2005). Safety assessment of esters of phydroxybenzoic acid (parabens). *Food and chemical toxicology*, 43(7), 985–1015, https://doi.org/10.1016/j.fct.2005.01.020.

[31] Schmid W. (1975). The micronucleus test. *Mutation Research*, 31(1), 9-15, https://doi.org/10.1016/0165-1161(75)90058-8

[32] Güzel Bayülken, D., & Ayaz Tüylü, B. (2019). In vitro genotoxic and cytotoxic effects of some paraben esters on human peripheral lymphocytes. *Drug and chemical toxicology*, 42(4), 386-393, https://doi.org/10.1080/01480545.2018.1457049

[33] Tayama, S., Nakagawa, Y., & Tayama, K. (2008). Genotoxic Effects of environmental estrogen-like compounds in CHO-K1 cells. *Mutation research,* 649(1-2), 114-125, https://doi.org/10.1016/j.mrgentox.2007.08.006

[34] Liehr J. G. (2000). Is estradiol a genotoxic mutagenic carcinogen?.*Endocrine reviews*, 21(1), 40-54, https://doi.org/10.1210/edrv.21.1.0386

[35] Caldwell J. C. (2012). DEHP: genotoxicity and potential carcinogenic mechanisms-a review. *Mutation research*, 751(2), 82-157, https://doi.org/10.1016/j.mrrev.2012.03.001

[36] Lee, S., Liu, X., Takeda, S., & Choi, K. (2013). Genotoxic potentials and related mechanisms of bisphenol A and other bisphenol compounds: a comparison study employing chicken DT40 cells. *Chemosphere,* 93(2), 434-440, https://doi.org/10.1016/j.chemospere.2013.05.029

[37] Pramfalk, C., Pedrelli, M., & Parini, P. (2011). Role of thyroid receptor β in lipid metabolism. *Biochimica et biophysica acta*, 1812(8), 929-937, https://doi.org/10.1016/j.bbadis.2010.12.019

[38] Rizos, C. V., Elisaf, M. S., & Liberopoulos, E. N. (2011). Effects of thyroid dysfunction on lipid profile. *The open cardiovascular medicine journal*, 5, 76-84, https://doi.org/10.2174/1874192401105010076

[39] Duntas, L. H., & Brenta, G. (2018). A renewed focus on the association between thyroid hormones and lipid metabolism. *Frontiers in endocrinology*, 9, 511, https://doi.org/10.3389/fendo.2018.00511

[40] Gogoi P, Kalita JC., (2019). Interaction of butylparaben with thyroid hormone receptors (TRα1 and TRβ1) and level of its mRNA transcript in rat liver. *Journal of Bioresources*, 6(2), 71-79.

[41] Aubert, N., Ameller, T., & Legrand, J. J. (2012). Systemic exposure to parabens: pharmacokinetics, tissue distribution, excretion balance and plasma metabolites of [14C]-methyl-, propyl- and butylparaben in rats after oral, topical or subcutaneous administration. *Food and chemical toxicology*, 50(3-4), 445–454, https://doi.org/10.1016/j.fct.2011.12.045

[42] Agas, D., Lacava, G., & Sabbieti, M. G. (2018). Bone and bone marrow disruption by endocrine-active substances. *Journal of cellular physiology*, 234(1), 192-213, https://doi.org/10.1002/jcp.26837

[43] Hu, P., Kennedy, R. C., Chen, X., Zhang, J., Shen, C. L., Chen, J., & Zhao, L. (2016). Differential effects on adiposity and serum marker of bone formation by post-weaning exposure to methylparaben and butylparaben. *Environmental science and pollution research international*, 23(21), 21957-21968, https://doi.org/10.1007/s11356-016-7452-0