

## 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 ~~and 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 ( $7.3 \pm 0.87$  MNPCEs/1000 PCEs) and long-term exposures ( $6.6 \pm 0.33$  MNPCEs/1000 PCEs) which was in line with the results obtained for 17 $\beta$ -estradiol (E2) treated reference control rats. The 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, micronucleated polychromatic erythrocytes.

### 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 human 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 evidences 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 mid-1920s and anticipated as thyroid as well as gonadal disruptor [4, 5]. Although this butyl ester of p-hydroxybenzoic acid (PHBA) has a short half-life and hence reported as non-hazardous at systemic level, many studies detected presence of BP in biological samples like serum, urine, breast milk, fatty tissue, amniotic fluid, seminal plasma, and even in breast tumour 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 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 substantial impact on 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

functioning of virtually the entire body. The present study was aimed ~~to evaluate~~ 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,  $130 \pm 10$ 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 ~~acclimatize~~ acclimate them ~~with~~ to the new laboratory environment. Standard laboratory conditions like humidity of 40-70%, temperature of 20-26°C and 12 hrs light:12 hrs dark photoperiod ~~was~~ ~~were~~ constantly maintained. Animals were fed with 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<sub>50</sub> value of BP is greater than 5000mg/kg BW, intraperitoneal BP treatment has ~~an~~ LD<sub>50</sub> value of 230mg/kg BW, ~~while in~~ and rabbit dermal exposure has ~~an~~ LD<sub>50</sub> more than 2000mg/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. 2mg/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 10mg/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, 5mg/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 10mg/kg BW/day of BP were calculated for humans and ~~were~~ found to be 0.16, 0.81 and 1.62mg/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. 1mg/kg BW/day of BP, 5mg/kg BW/day of BP and 10mg/kg BW/day of BP which were shown as BP1, BP5 and BP10 respectively, and a positive control group i.e. 0.01mg/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 fulfil the aim, quantification of serum triglyceride, HDL and cholesterol levels were performed using a Semi Auto Biochemistry Analyzer (Erba Chem 5X, TRANSASIA<sup>®</sup>, ERBA Diagnostics Mannheim GmbH, Mallaustrasse, Germany). For each biochemical assay, specific kits from ERBA (ERBA Diagnostics Mannheim GmbH, Mallaustrasse., Germany) were used following manufacturer's instructions.

### **2.5. Assays for toxicity marker enzymes**

Toxicity markers of liver i.e., AST, ALT and ALP levels were measured in a Semi Auto Biochemistry Analyzer (Erba Chem 5X, TRANSASIA<sup>®</sup>, 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

Genotoxic potency of butylparaben was evaluated through Micronuclei assay following procedure of Schmid (1975) [31]. Femora bone of rat was removed and using a 1mL syringe the bone marrow was aspirated and suspended in FBS. The suspension was centrifuged at 1,000 rpm for 5 min, 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 microscope with high magnifications (100x). 1000 polychromatic erythrocytes (PCEs)/smear were screened for examining presence of MNPCEs.

## 3. Results

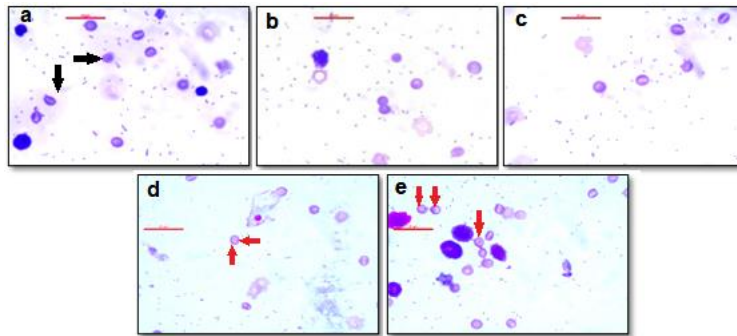
### 3.1. Micronuclei induction in PCEs of bone marrow after butylparaben exposure

Table 1 shows the frequency of micro-nucleated PCEs (MNPCEs) in respective groups. Photomicrographs showing 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 generation of micronuclei in a dose-dependent manner. However, significant effect was observed only at the selected maximum dose (10 mg/kg BW/day). At low dose of BP (1 mg/kg BW/day) there was slight elevation in 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;  $5 \pm 0.57$  MNPCEs/1000 PCEs and  $6 \pm 0$  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  $3.3 \pm 0.33$  MNPCEs/1000 PCEs following 7 days of vehicle treatment and  $3.6 \pm 0.66$  MNPCEs/1000 PCEs after 21 days of dosing (Table 1). BP at 10 mg/kg BW/day caused significant ( $p > 0.05$ ) rise of micronuclei production both in short ( $7.3 \pm 0.87$  MNPCEs/1000 PCEs) and long-term exposures ( $6.6 \pm 0.33$  MNPCEs/1000 PCEs) as shown in fig.1d and 2d. Therefore, only BP10 generated 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 E2 group, fig.1e and 2e. Short-term treatment of E2 resulted in formation of  $9.3 \pm 0.33$  MNPCEs/1000 PCEs and in long-term administration there was found  $10 \pm 0.57$  MNPCEs/1000 PCEs, which were significantly different than control,  $p < 0.05$ .

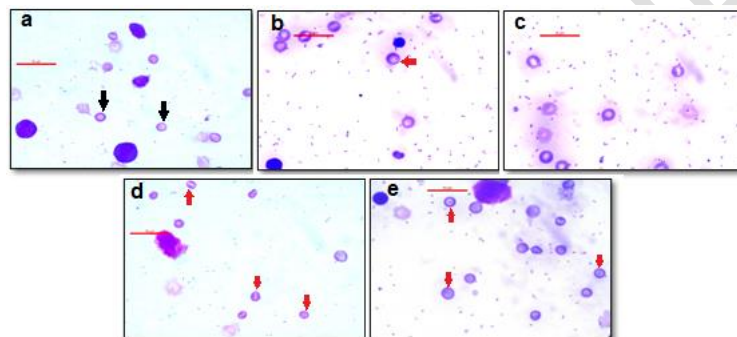
**Table 1:** Micronuclei induction in polychromatic erythrocytes (PCEs) of bone marrow in rats exposed to different doses of butylparaben (BP) for 7 and 21 days.

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

all the values are shown as mean  $\pm$  sem (triplicate measurement). \*  $p < 0.05$ , compared to control. mnpces - micro-nucleated polychromatic erythrocytes.



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

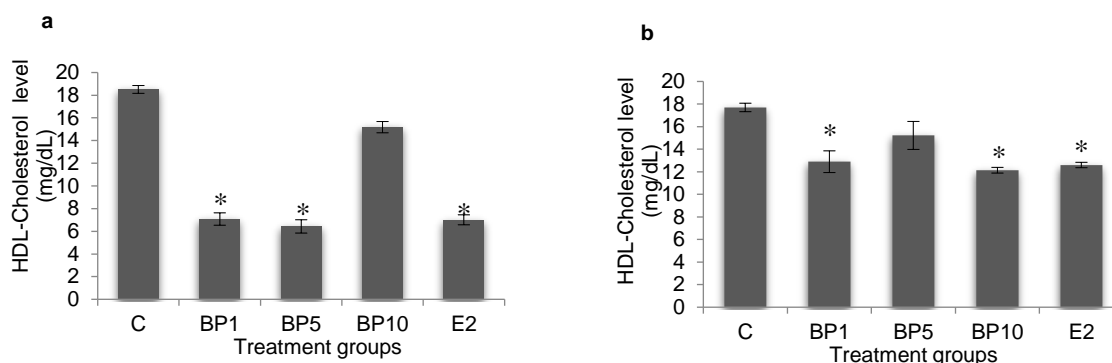


**Fig.2** Micronuclei formation in polychromatic erythrocytes (PCEs) of bone marrow of Wistar rats after butylparaben exposure for 21 days. [a] Control (Olive oil), [b] 1mg/kg BW/day of BP, [c] 5mg/kg BW/day of BP, [d] 10mg/kg BW/day of BP and [e] 0.01mg/kg BW/day of 17 $\beta$ -Estradiol (E2). Black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

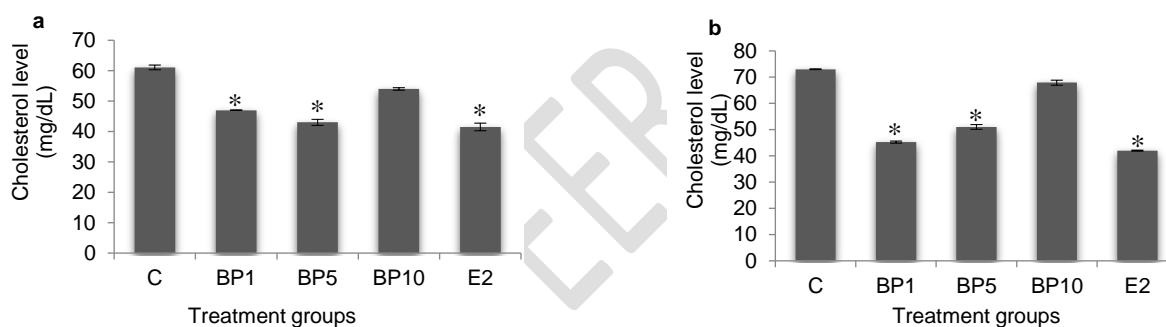
### 3.2. Effects of butylparaben on serum lipids

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

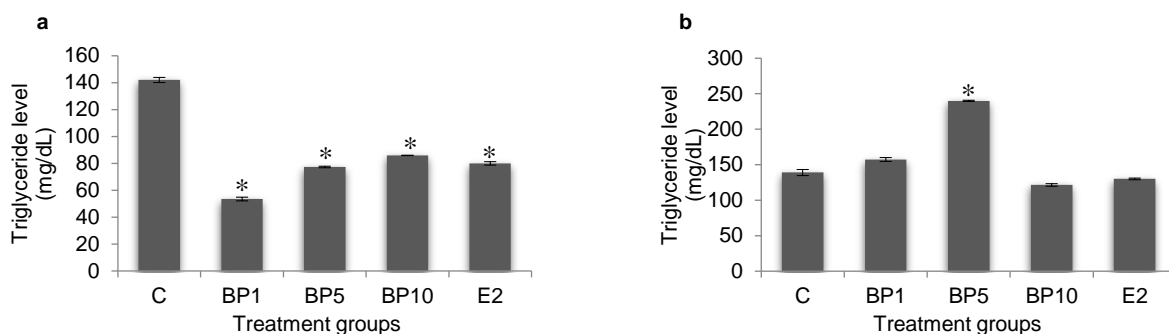


**Fig.3** Changes in serum level of HDL-cholesterol in Wistar rats following exposure to butylparaben (BP) at doses of 1, 5 and 10mg/kg BW/day (represented as BP1, BP5 and BP10 respectively) for 7 days [a] and 21 days [b]. The control rats were subjected to the vehicle (olive oil) only and  $17\beta$ -estradiol (E2) was administered as positive control dose (0.01mg/kg BW/day). The values are expressed as mean  $\pm$  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.



**Fig. 4** Changes in serum level of cholesterol in Wistar rats following exposure to butylparaben (BP) at doses of 1, 5 and 10mg/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  $17\beta$ -estradiol (E2) was administered as positive control dose (0.01mg/kg BW/day). The values are expressed as mean  $\pm$  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.

Serum triglycerides content was determined and the findings are shown in Fig 5a & 5b. Subsequent to short-term treatment, BP at all the concentrations showed significant reduction of triglycerides in the animals compared to the control ( $142 \pm 1.9$ mg/dL). The estimated values for BP1, BP5 and BP10 were found to be  $53.48 \pm 1.4$ mg/dL,  $77.3 \pm 0.58$ mg/dL and  $85.9 \pm 0.16$ 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 treatment ( $80 \pm 1.24$ mg/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 of BP1, BP10, E2 and control groups ( $157.4 \pm 2.7$  at BP1,  $121 \pm 1.76$  at BP10,  $130 \pm 1.2$  at E2 and  $139 \pm 4.3$ mg/dL at control,  $p > 0.05$ ). Only 5mg/kg BW/day of BP was effective in causing significant ascend of triglycerides in animals under 21 days study ( $240 \pm 0.86$ mg/dL).



**Fig. 5** Changes in serum level of triglycerides in Wistar rats following exposure to butylparaben (BP) at doses of 1, 5 and 10mg/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 17 $\beta$ -estradiol (E2) was administered as positive control dose (0.01mg/kg BW/day). The values are expressed as mean  $\pm$  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

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 the Table 2). Similarly, 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 ( $130.6 \pm 0.88$  IU/1), the treated groups showed significant elevation of the enzyme level at 1, 5 and 10mg/kg BW/day of BP ( $187.6 \pm 0.83$  in BP1;  $181.5 \pm 0.25$  in BP5 and  $171.1 \pm 1.14$  IU/1 in BP10;  $p < 0.05$ ). Significant rise of serum ALP was noted in the E2 exposed rats ( $189.00 \pm 1.1$  IU/1). Similarly, 21 days of dosing resulted in significant elevation ( $p < 0.05$ ) of the ALP level at the entire concentration range of BP tested (Table 4). At BP1, BP5 and BP10, level of ALP was found to be  $189.46 \pm 0.74$ ,  $192 \pm 1.45$  and  $194.6 \pm 1.8$  IU/1 respectively, whereas in the control animals it was  $131.6 \pm 1.76$  IU/1. E2 showed significant effect on ALP after administered consecutively for 21 days and level of its increase was detected as  $200 \pm 2$  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 $\pm$ 0.33	32 $\pm$ 0.881
BP1 (1mg/kg BW/day)	30.6 $\pm$ 0.5	31.6 $\pm$ 0.33
BP5 (5mg/kg BW/day)	31.30 $\pm$ 0.881	32.6 $\pm$ 0.33
BP10 (10mg/kg BW/day)	32.3 $\pm$ 0.33	33.6 $\pm$ 0.33
17 $\beta$ -estradiol (E2) (0.01mg/kg BW/day)	31.05 $\pm$ 0.57	32.93 $\pm$ 0.57

all values are presented as mean  $\pm$  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/l)	
	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 may 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 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 more higher 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 than compare to control rats, whereas the dose with high BP concentration, 10 mg/kg BW/day produced significant genetic toxicity and thereby generated more numbers of 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 10mg/kg BW/day has the ability to cause considerable chromosomal breakage or separation that leads to micronuclei formations. However, the effects were independent irrespective 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 the cytotoxic and genotoxic properties of BP, PP, iso-BP and iso-PP at ~~one~~ 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 ~~hrs~~ hours were determined in a ~~one-~~ concentration dependent manner [32]. The study also reported that BP exhibits the potential to produce chromosomal aberrations [32]. Indeed, 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 depicted somewhat the same. Animals subjected to BP for both 7 and 21 days exhibited significant changes in their serum lipid profiles. Short-term exposure of to BP resulted in 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 of the in serum HDL-cholesterol with low cholesterol levels. Conversely, there was a profound rise of in serum triglycerides in the animals receiving 5mg/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, perturb 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 catalyses the first step of cholesterol biosynthesis. A crucial step of 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 THR $\beta$ . 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 of in HDL-cholesterol might have resulted due to high level of circulating T3 as supported by our previous research data [23]. As supported by our previously published study, Gogoi and Kalita, (2020),[?] high T3 levels in E2 treated animals possibly may have caused a reduction of in HDL-cholesterol [23] Hence, the present study provides evidences 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 THR $\beta$  present in the liver cells [37]. It has become apparent in our earlier study that BP can alter gene expression of *Thr $\beta$ 1* in liver tissue at all the applied doses [40]. Up-regulation of hepatic *Thr $\beta$ 1* after both 7 and 21 days of BP exposure might have resulted in more access of to T3 mediated genomic effects on lipid metabolism as supported by our earlier study [23, 40]. Similarly, alterations detected for triglycerides 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 triglycerides levels in the BP treated rats. Such variations in plasma triglycerides have also been evident in hyperthyroid patients where they ~~it~~ 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 and then eliminated via urine, thus have 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 estrogen hormone, environmental chemicals with estrogenic potency may alter bone or bone marrow functions by directly acting on the same it [42]. Earlier, different PB esters had shown their potential negative impact on bone formation which added new insight of into PBs toxicity in to the growing body of literature [43]. As a xenoestrogen, there are possibilities that BP may alter the bone functions and ultimately reflects 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., 10mg/kg BW/day is sufficient to cause genotoxicity. BP not only alters circulating lipid content but also elevate 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.

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