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Effect on ovarian histoarchitecture in white albino mice following exposure to xenoestrogen butylparaben

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Abstract: Environmental chemicals with estrogenic properties which are commonly known as 'Xenoestrogen' are found in wide variety of substances to which human is widely exposed. Human particularly females are mostly exposed to xenoestrogen Butylparaben (BuPben) through use of pharmaceuticals and cosmetics where the chemical is used as a preservative. In the present study exposure to BuPben on ovarian histology including change in follicles, corpus luteum and histology was studied. Adult mice were exposed to 10 mg/kg bw dose, 50 mg/kg bw and 100 mg/kg bw dose dose of BuPben for 7 and 21 days through subcutaneous route of administration. In this study 0.001 mg/Kg body weight 17β estradiol was used as positive control and Olive oil and olive oil + ethanol (1:10) was used as control and vehicle control respectively. Exposure of mice to BuPben showed adverse affects on ovary with significant decrease in primordial follicle, primary follicle, secondary follicle and graffian follicle and increase in number of atretic follicle. Number of corpus luteum is also found to show significant decrease along adverse affects on ovarian histology. In summary these results clearly indicate adverse affect of BuPben on female reproduction.
Keywords: Xenoestrogen; Butylparaben; estrogen; progesterone; follicle; corpus luteum.

Introduction:

Butylparaben (BuPben) retards microbial growth in in some foods, cosmetics, and drug formulations[1, 2]. In 2003, BuPben was approved for used as a flavour additive in food by the U.S. Food and Agriculture Organization and the World Health Organization[3].

BuPben have been of concern to the scientific community for its ability to bind with estrogen receptors [4]. Presence of BuPben was reported in breast cancer tissue at a level of 2.3 ng/g tissue [5]. Studies showed that BuPben exerts reproductive, developmental as well as terratogenic toxicity in experimental animals [6,7]. The biological and toxicological effects of parabens on reproductive, cardiovascular, skeletal, and gastrointestinal system have also been reported[8].

Experimental

The albino mice Mus musculus of C3H/HE strain was selected for the present study which were fed with commercially available animal diet, vitamins and mineral supplement (purchased from Agrivet Farm Care Division, Glaxo Smithkline, Chennai, India). Body weight and clinical sign were recorded on daily basis throughout the period of experiments. Permission from Animal Ethical committee was taken before conducting the experiments on the animal model. All ethical norms were strictly followed during the experiments (Animal

ethical clearance number: 902/AC/05/CPCSEA). All chemicals used in the present study were obtained from Sigma (Sigma- Aldrich Corporation. The stock solution of BuPben was prepared 2.5 g of BuPben was dissolved in 20 ml 100 % ethanol.

17β estradiol was prepared by dissolving in analytical grade ethanol. The selected dose was 0.001 mg/Kg body weight. Olive oil was used as control. A vehicle control group was also used for all the experimental purposes which were ethanol and olive oil (1:10).

Experimental design:

Weighing of tissues

The complete ovary was collected and was trimmed of fat and weighed on a standard electronic balance. The relative ovarian weight to body weight were calculated from the following formula-

$$\text{Relative ovary weight} = \frac{\text{Ovary weight}}{\text{Body weight}} \times 100$$

Histological preparation of samples

The tissues were cut into 5-6 µm sections using a microtome (Ernst Leitz Wetzlar GMBH, Germany).The sections were stained with haematoxylin and eosin (H&E). dehydrated Slides were then observed under microscope (Leitz, Ortholux II, Germany) for histological changes in the tissues.

At the start of each experiment mice were divided into six groups - olive oil (control), olive oil-ethanol(1:10), 17β estradiol (positive control), 10 mg/Kg body weight, 50 mg/Kg body weight and 100 mg/Kg body weight of BuPben.

Study and classification of ovarian follicles

Morphological classification of follicles:

At an average, about 200 serial sections were obtained in each ovary after cutting at the thickness of 6 µm. For each ovary, every 12th and 20th section was examined for counting smaller (primordial, primary and secondary) and larger (graffian and atretic) follicles respectively to obtain an overall view of the follicular populations per ovary [9, 10, 11].

Quantification of corpus luteum in adult mice

The number of corpus luteum was counted in both the ovaries considering three sections per ovary [12].

Result

Effect of BuPben on ovarian weight in adult mice

The single ovarian weight of mice was found to decrease in treated groups when compared to both control and vehicle control groups following short term exposure of 7 and 21 days. Following 7 days BuPben exposure 100 mg/Kg bw BuPben and estradiol treated group showed significantly lower ($p < 0.05$) single ovarian weight than both control and vehicle control groups. 10 mg/Kg bw and 50 mg/Kg bw dose of BuPben showed statistically insignificant decrease in ovarian weight

Following 21 days BuPben exposure BuPben dose of 50 mg/Kg bw and 100 mg/Kg bw recorded decrease in single ovarian weight which was statistically significant at $p < 0.05$ and $p < 0.01$ compared to control groups. Estradiol treated group showed single ovarian weight of 52.74 ± 1.68 mg which was significant at $p < 0.01$ compared to control groups.

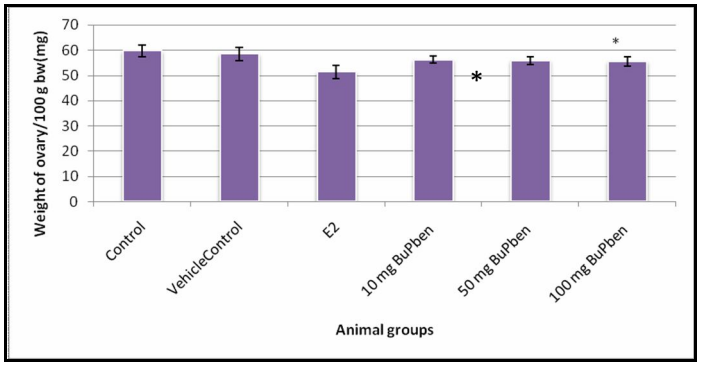


Fig 1: Effects of 7 days exposure of BuPben on ovarian weight (mg/100 g bw) in adult mice. Data are expressed as Mean ± SEM. (n=5/group). Asterisks denote significant relationship with control groups (*p<0.05)

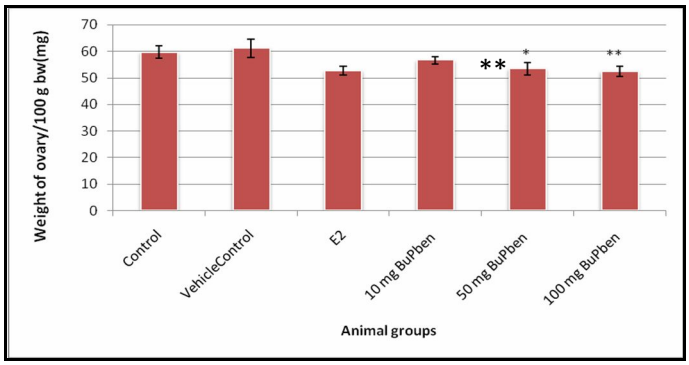


Fig 2: Effects of 21 days exposure of BuPben on ovarian weight (mg/100 g bw) in adult mice. Data are expressed as Mean ± SEM. (n=5/group). Asterisks denote significant relationship with control groups (*p<0.05) (p<0.01)**

Effect of BuPben on ovarian follicular population in adult mice

Exposure to BuPben dose of 100 mg/Kg bw for 7 days showed significant ($p<0.05$) decrease in primary follicle compared to both control and vehicle control group. However there is no significant change in number of primordial follicle, primary follicle, secondary follicle, graffian follicle and atretic follicle in BuPben dose of 10 mg/Kg bw and 50 mg/Kg bw.

In estradiol treated group significant ($p<0.05$) decrease in number of primordial follicle, primary follicle, secondary follicles were observed compared to the control groups.

When mice were exposed to BuPben dose of 100 mg/Kg bw for 21 days significant decrease in primordial follicles ($p<0.05$), primary follicles ($p<0.05$), secondary follicles ($p<0.01$) and graffian follicles ($p<0.05$) were observed when compared to both control and vehicle control group. However no significant change in number of primordial follicles, primary follicles, secondary follicles and graffian follicles were recorded in 10 mg /Kg bw BuPben and 50 mg /Kg bw BuPben when compared with both control and vehicle control group.

Estradiol treated group similar to BuPben dose of 100 mg/Kg bw showed significant decrease in primordial follicles ($p<0.01$), primary follicles ($p<0.01$), secondary follicles ($p<0.01$) and graffian follicles ($p<0.05$) were observed when compared to both control and vehicle control group.

Increase in number of atretic follicles was observed in both 100 mg/Kg bw dose of BuPben and estradiol treated group which was significant ($p<0.05$) when compared to both control and vehicle control group.

Table 1: Effects of 7 days exposure of BuPben on ovarian follicular population in adult mice. Data are expressed as Mean ± SEM. (n=5/group). Asterisks denote significant relationship with control groups (*p<0.05)

Ovarian follicles → / Animal groups ↓	Primordial Follicle	Primary follicle	Secondary follicle	Graffian follicle	Atretic follicle
Control	739.54 ± 12.6	172.32 ± 6.8	98.12 ± 3.2	8.13 ± 1.23	13.82 ± 2.26
Vehicle Control	744.36 ± 13.32	169.29 ± 5.6	96.23 ± 2.93	8.11 ± 1.34	12.46 ± 1.92
Estradiol	711.46 ± 10.6 *	156.38 ± 9.6 *	92.27 ± 1.63 *	6.34 ± 1.86	14.63 ± 3.86
10 mg BuPben	740.38 ± 13.4	176.37 ± 8.4	97.49 ± 1.46	7.61 ± 1.26	12.54 ± 2.24
50	741.42 ± 12.3	168.25 ± 7.6	97.38 ± 2.61	7.26 ± 1.35	12.14 ± 3.42
100	728.30 ± 11.6	158.22 ± 9.4 *	95.96 ± 2.12	6.54 ± 1.41	13.68 ± 2.98

Table 2: Effects of 21 days exposure of BuPben on ovarian follicular population in adult mice. Data are expressed as Mean ± SEM. (n=5/group). Asterisks denote significant relationship with control groups (*p<0.05) (p<0.01)**

Ovarian follicles → / Animal groups ↓	Primordial Follicle	Primary follicle	Secondary follicle	Graffian follicle	Atretic follicle
Control	752.61 ± 13.61	186.35 ± 5.45	122.15 ± 4.23	13.59 ± 1.15	14.19 ± 1.31
Vehicle Control	749.26 ± 12.33	179.43 ± 9.28	118.30 ± 3.35	12.61 ± 1.22	15.16 ± 1.12
Estradiol	543.41 ± 11.63 **	142.87 ± 4.38 **	95.10 ± 2.15 **	8.11 ± 0.83*	19.4 ± 2.11*
10 mg BuPben	754.26 ± 12.28	183.48 ± 6.91	123.57 ± 3.11	12.32 ± 1.13	14.27 ± 2.18
50	723.32 ± 8.92	172.51 ± 9.13	116.73 ± 3.21	11.21 ± 1.72	15.04 ± 2.33
100	711.28 ± 9.34*	163.27 ± 5.11*	92.45 ± 1.83**	7.65 ± 1.61*	17.12 ± 1.46*

Effect of exposure of BuPben on corpus luteum in adult mice

Exposure to BuPben for 7 days did not showed any significant change in number of corpus luteum at any dose when compared with both control and vehicle control group.

Exposure of adult mice to BuPben dose of 100 mg/Kg bw showed significant decrease ($p<0.05$) in corpus luteum compared to control and vehicle control group. Estradiol treated group also showed a significant decrease ($p<0.01$) in number of corpus luteum. Decrease in number of corpus luteum was also found in 10 mg/Kg bw and 50 mg/Kg bw dose of BuPben but the decrease was not significant when compared to control and vehicle control group.

Table 3: Effects of exposure of BuPben for 7 days on number of corpus luteum in adult mice. Data are expressed as Mean ± SEM. (n=5/group).

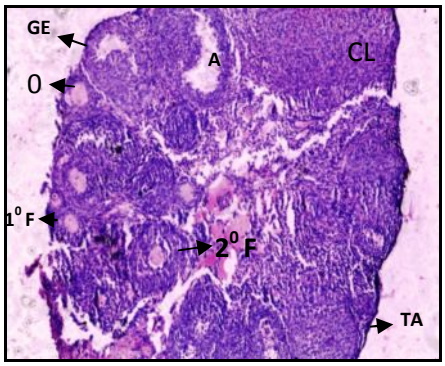
Animal groups	No. of mice with corpus luteum (%)	No. of corpus luteum
Control	5 (100 %)	13.4 ± 0.8
Vehicle Control	5 (100 %)	12.2 ± 0.2
0.001mg/ Kg bw Estradiol	5 (100 %)	8.3 ± 0.7
10 mg BuPben	5 (100 %)	14.6 ± 0.5
50 mg BuPben	5 (100 %)	13.4 ± 0.6
100 mg BuPben	5 (100 %)	13.2 ± 0.7

Table 4: Effects of exposure of BuPben for 21 days on number of corpus luteum in adult mice. Data are expressed as Mean ± SEM. (n=5/group). Asterisks denote significant relationship with control groups (*p<0.05) (p<0.01)**

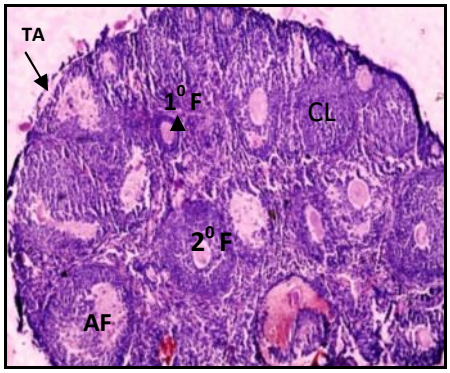
Animal groups	No. of mice with corpus luteum (%)	No. of corpus luteum
Control	5 (100 %)	16.1 ± 0.4
Vehicle Control	5 (100 %)	14.5 ± 0.3
0.001 mg/ Kg bw Estradiol	3 (60 %)	6.8 ± 0.1**
10 mg BuPben	5 (100 %)	14.3 ± 0.2
50 mg BuPben	5 (100 %)	13.2 ± 0.3
100 mg BuPben	4 (80 %)	10.4 ± 0.4*

4.2.9. Effect of exposure of BuPben on ovarian histology in adult mice

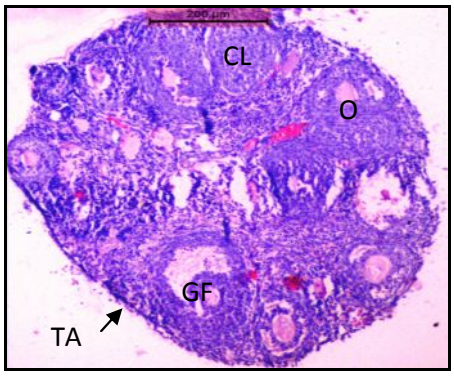
However unlike control groups and similar to estradiol treated group, 50 mg/Kg bw BuPben and 100 mg/Kg bw BuPben showed significant decrease in follicular population and increase in number of atretic follicles and polycystic follicle (PF). Significant decrease in number of corpus luteum was also observed in the treated groups. The basement membrane (BM) in the treated groups was found to be disorganized.



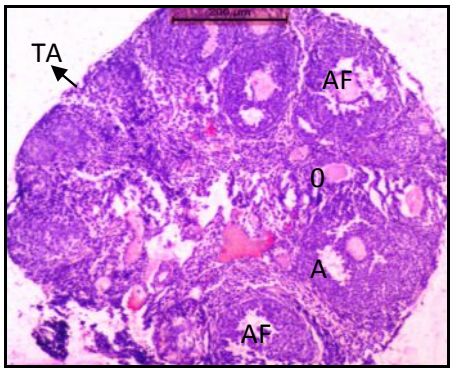
Control



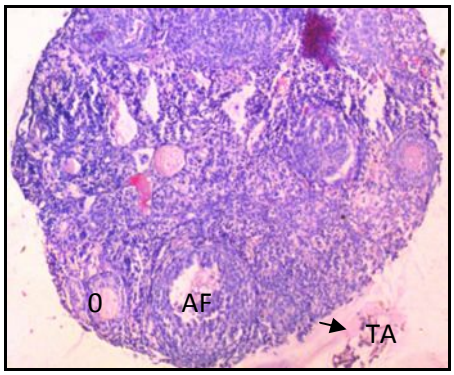
Vehicle Control



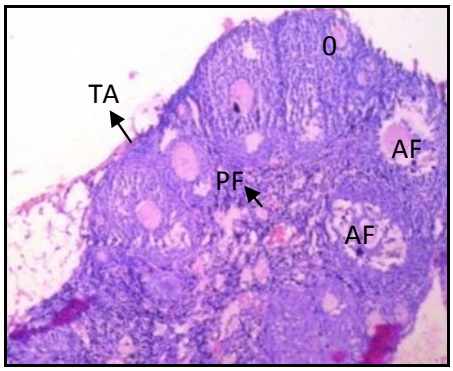
10 mg BuPben



50 mg BuPben



100 mg BuPben



Estradiol

Plate1: Effect of BuPben on histo architecture of ovary of adult female mice. Control groups and 10 mg/Kg bw BuPben showed normal follicles and corpus luteum. However estradiol treated group, 50 mg/Kg bw BuPben and 100 mg/Kg bw BuPben showed decrease in healthy follicle and corpus luteum. Abbreviations: 1 ^{0} F - Primary follicle; 2 ^{0} F - Secondary follicle; CL -Corpus luteum; GE - Germinal epithelium; TA - Tunica albuginea; PF - Polycystic follicle; BM - Basement membrane.

Discussion

Wistar rats exposed to 0, 5, 15 or 50 µg/kg bw/day of ethinyl estradiol during gestation and lactation showed significant reduction in ovarian weight [13].

Alternation in folliculogenesis similar to the present study was reported involving other xenoestrogens [14, 15, 16]. Exposure of female Sprague–Dawley rat orally to 62.5, 250 and 1000 mg/kg bw/day dose of BuPben from postnatal day 21–40 showed significant decrease in number of corpus luetum in all dose of BuPben [17].Similar adverse effects on ovarian histology was reported in many studies following exposure to different environmental estrogen [18, 19, 20].

As BuPben is also reported to bind to estrogen receptor it may interfere with secretion of GnRH resulting in decrease in ovarian weight, adverse effect on ovarian folliculogenesis and ovarian histology as reported in the present study.

Conclusion

BuPben is of major concern to the scientific community for its wide spread use in formulation which are daily used by man. It can be understood from the study that exposure to BuPben exerts adverse effects on female reproductive system. Decrease in follicle can lead to decrease in reproductive capacity with decrease in ovulation. The effects on ovary can even lead to permanent sterility. Another concern with the chemical exposure is the fact that this chemical can enter to human through wide variety of sources including dermal, oral and inhalation. Thus more studies should be conducted on the low dose but long term exposure to the chemical.

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