Research Article

ALTERATION IN UTERINE HISTOARCHITECTURE AND UTERINE PROTEIN FOLLOWING BUTYLPARABEN EXPOSURE IN ADULT MICE

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ABSTRACT

Environmental chemicals with estrogenic properties are widely found in products widely used by human. Butylparaben (BuPben), a chemical with estrogenic activities is found to have adverse effects on the female reproductive tract. Adult mice were exposed to 10 mg, 50 mg and 100mg dose of BuPben for 7 and 21 days. In the study 0.001 mg/Kg body weight 17 β estradiol was used as positive control and Olive oil and olive oil + ethanol (1:10) was used as control and vehicle control respectively. Subcutaneous exposure to 50 mg/kg body weight (bw) and 100 mg/kg bw of BuPben is found to cause significant increase in uterine weight when compared to control and vehicle control group in adult mice. In this study 50mg and 100 mg dose of BuPben, similar to 17 β estradiol (E2) (used as positive control) showed increase in thickness of myometrium and endometrium with adverse effects on uterine histology. Increase in number of uterine glands in also reported by all the doses of BuPben including 10 mg, 50 mg and 100mg when compared to control groups, along with significant decrease in concentration of serum estradiol and serum progesterone. Increase in uterine protein was also found in the study following BuPben exposure. Thus BuPben exposure adversely affects uterus in mice. This adverse effect of the chemical describes estrogenic activity of the chemical which may lead to reproductive failure in human and animals which gets exposed to the chemical.

Keywords: Estradiol, Mice, Uterus, Uterine protein, Uterine glands.

INTRODUCTION

Parabens are widely used as preservative in pharmaceuticals and personal care products as an antimicrobial agent¹. It is found to have high antimicrobial efficiency and is a very low cost preservative. Among the parabens, methylparaben, propylparaben, butylparaben (BuPben) and ethylparaben are very widely used².

Parabens is of concern due to their presence in human breast tumors³. BuPben is used as an antimicrobial agent in wide variety of human used products like cosmetics, food stuffs and in medicines. In 1981, Food and Drug Administration (FDA) have reported the used of BuPben in personal care products up to 5%. Among the products surveyed in the study baby cosmetics, personal hygiene products, bath products were reported². BuPben is reported to possess estrogenic property because of its structural similarity with endogenous estrogen produced in the human body. Routledge et al., 1998, have reported uterotrophic effects of BuPben at dose 400 to 1200 mg/kg body weight following exposure to the chemical for three days in ovariectomized and immature rats⁴. Oishi, 2001 have reported decrease in sperm count and serum testosterone in male rats⁵.

The present study aims to study the effect of BuPben on uterus of adult albino mice.

MATERIALS AND METHODS

Experimental animal

Swiss albino mice were selected for the present study. The mice were fed with commercially available animal diet (Agrivet Farm Care Division, Glaxo Smithkline, Chennai, India). All the experiments were approved by the institutional animal ethical

committee (Animal ethical clearance number: 902/AC/05/CPCSEA).

Chemicals

All chemicals used in the present study were obtained from Sigma (Sigma- Aldrich Corporation. 2.5 g of BuPben in 20 ml 100 % ethanol was used as stock solution. The selected dose for 17 β estradiol was 0.001 mg/Kg body weight which was prepared in analytical grade ethanol. Olive oil was used as control and vehicle control group was ethanol and olive oil (1:10). Animals were exposed to the test compound through subcutaneous route of administration at 24hour interval. The dosing volume for the test compounds was 5 ml/kg body weight⁶.

Weighing of tissues

The complete uterus was collected without the loss of any luminal fluid and was trimmed of fat and weighed on a standard electronic balance.

The relative uterine wet weight to body weight were calculated from the following formula:

Relative uterine wet weight = Uterine weight / Body weight x 100

Histological preparation of samples

The tissues were cut into 5 μ m sections using a microtome (Ernst Leitz Wetzlar GmbH, Germany). The sections were stained with haematoxylin and eosin. 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 (n $\geq$ 5)- olive oil (control), olive oil- ethanol (1:10), 17 β estradiol (positive control) respectively and 10 mg/Kg body

weight, 50 mg/Kg body weight, 100 mg/Kg body weight of BuPben respectively.

Morphometric analysis of mice uterus in adult cyclic mice

For morphometric analysis of mice uteri, the method followed by Lemini et al., 2004 and Markey et al., 2005 were used^{7,8}. A distance of 50 μ m among the sections was kept. Endometrium (Luminal epithelium + glandular epithelium) and myometrium widths were measured in all slices. All measurements were made in 40 x using light microscope (Leitz, ortolux II, Germany).

Quantification of uterine glands in adult cyclic mice

Uterine endometrial glands were classified as method given by Gunin et al., 2001⁹. Uterine endometrial glands were subdivided into a four morphological types - Normal glands (simple tubular glands with no branches), cystic glands (Glands of round size which are of more than average or large size), glands with daughter glands, conglomerate of glands.

Estimation of total uterine tissue protein

Colorimetric estimation of total uterine tissue protein in normal adult cyclic mice and ovariectomised mice was done considering 0.1% BSA (Bovine serum albumin) as standard (Lowry et al, 1951)¹⁰. Absorbance was read at 670 nm against blank on a colorimeter (Systronics 106).

Statistical analysis

Statistical analyses for all the data of animal experimentations were performed using MS Office Excel 2007 and Window's Statistical Package for Social Sciences (SPSS). The results were expressed as mean $\pm$ standard error (SE) of mean. The means in both negative as well as positive control versus treated animals were analyzed for significant by Student's independent t- test distribution. A value of $p < 0.05$ was considered statistically significant for all the tests.

RESULTS

Effect of BuPben on uterine wet weight in adult mice

Exposure to 100 mg BuPben for seven days caused significant ($p < 0.01$) increase in uterine weight compared to both control and vehicle control group. In 50 mg BuPben uterine weight showed significant increase ($p < 0.05$) compared to both control and vehicle control group. No significant change was observed in BuPben dose of 10 mg. The results are shown in Figure 1.

Effect of 21 days exposure of BuPben on uterine wet weight was also studied in adult mice. Both BuPben dose of 100 mg/Kg bw and estradiol treated group showed statistically significant increase ($p < 0.01$) in uterine wet weight when compared to both control and vehicle control group. In 50 mg/Kg bw BuPben the uterine weight showed significant increase ($p < 0.05$). Results are shown in figure 2.

Morphometric analysis of uterus following exposure to BuPben in adult mice

In 100 mg/Kg bw BuPben and estradiol treated group significant increase in thickness of endometrium ($p < 0.05$) observed when compared to control and vehicle control group. However, no significant change in thickness of endometrium was observed following exposure of mice to BuPben dose of 10 mg/Kg bw and 50 mg/Kg bw when compared with control and vehicle control group.

Significant increase ($p < 0.05$) in thickness of myometrium was also observed following BuPben exposure at dose 100 mg/Kg bw when compared to the control groups. However, no significant change in thickness of myometrium was observed following exposure of mice to BuPben dose of 10 mg/Kg bw

and 50 mg/Kg bw when compared with control and vehicle control group. Results are shown in figure 3.

Effect of exposure of BuPben on number of uterine glands in adult mice

Study on change in number of uterine glands was studied following BuPben exposure for 7 days. In the study four different types of uterine glands were studied which included normal gland (A), cystic gland (B), gland with daughter gland (C) and glands forming conglomerate (D). No cystic gland and glands forming conglomerate were found in both control and vehicle control group. In 10 mg/Kg bw dose of BuPben the number of normal glands showed a significant decrease ($p < 0.05$). Significant increase ($p < 0.01$) in number of glands forming conglomerate was found in 10 mg/Kg bw dose of BuPben when compared to control and vehicle control group. No significant change in number of gland with daughter glands was observed in 10 mg/Kg bw dose of BuPben. No cystic gland in uterus was found in 10 mg/Kg bw dose of BuPben. In BuPben dose of 50 mg/Kg bw significant decrease ($p < 0.01$) in number of normal glands was observed compared to control and vehicle control group. Significant increase ($p < 0.01$) in number of gland with daughter glands and glands forming conglomerate was observed in 50 mg/Kg bw dose of BuPben.

In 100 mg/Kg bw dose of BuPben the number of normal glands showed a significant decrease ($p < 0.01$). Significant increase ($p < 0.01$) in number cystic gland and glands forming conglomerate was observed in 100 mg/Kg bw dose of BuPben. Significant increase ($p < 0.05$) in number of gland with daughter gland which was 15 ± 1.43 was also observed in 100 mg/Kg bw dose of BuPben. Similar to BuPben treated group significant change in number of uterine gland was observed in estradiol treated group.

Effect of exposure of BuPben on total uterine tissue protein in adult mice

The results of the total uterine tissue protein content in BuPben treated group and estradiol treated group of mice were compared with control and vehicle control group. In 100 mg/Kg bw dose of BuPben significant increase ($p < 0.05$) in total uterine tissue protein content was observed. Similar to 100 mg/Kg bw dose of BuPben, estradiol treated group showed significant increase ($p < 0.01$) in total uterine tissue protein content.

However, BuPben dose of 10 mg/Kg bw and 50 mg/Kg bw did not showed any significant change in total uterine tissue protein content.

Effect of exposure of BuPben on uterine histology in adult mice

Histological architecture of uterus of mice treated with BuPben and E2. In the present study normal histoarchitecture with uniform, uninterrupted columnar epithelium was observed in the control groups and in BuPben dose of 10 mg/Kg bw. Well-developed endometrial glands and small uterine lumen (UL) were also observed in control groups and BuPben of 10 mg/Kg bw.

Significant change was observed in E2 treated group and in BuPben dose of 50 mg/Kg bw and 100 mg/Kg bw with enlarged uterine lumen, increase in thickness of endometrium (E) and myometrium (M). The columnar epithelium in these groups was also found to be distorted.

DISCUSSION

BuPben is a major concern to the scientific community for its adverse effects on the female reproductive tract. Estrogen is reported to induce increase in uterine weight¹¹. As BuPben at dose 50 mg and 100 mg shows similar results, it describes the

estrogenic potential of BuPben. Xenoestrogens zearalenone and ethinyl estradiol are reported to cause significant increase in uterine weight in immature Wister rats similar to BuPben¹². Routledge et al., 1998 reported increase in dry weight of uterus following administration of BuPben at dose 200 mg bp/kg/day⁴. Parameters like increase in uterine wall thickness are considered as indicators of estrogenic activity¹³. Vo et al., 2010 reported myometrial hypertrophy in Sprague–Dawley rat following BuPben exposure similar to the results obtained in the present study. In the present study BuPben is found to cause significant increase in total uterine protein content. Ireland et al., 1980 have reported in a study on female rats that exposure to estradiol can cause significant in total uterine tissue protein content¹⁴. Significant increase in uterine glands was reported in mice following exposure to phytoestrogen genistein and estradiol similar to the present study¹⁵. Thus the above study shows the estrogenic potential of BuPben.

CONCLUSION

The major concern over the use of environmental chemical with estrogenic property like BuPben is the fact that effects of exposure to the chemical have not been restricted to human but also other non target animals. Adverse effects of the chemical to the reproductive system may lead to sterility. There have also been concerns over the long term constant exposure of human to chemical due to the presence of the chemical in wide variety of human used products. These chemical tends to persist in the environment for longer duration of time as they do not decay easily. Adverse effects of the chemical to the reproductive system may lead to sterility.

Figure 1: Effects of 7 days exposure of BuPben, 17β estradiol, control and vehicle control on uterine wet weight 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).

Figure 2: Effects of 21 days exposure of BuPben, 17β estradiol, control and vehicle control on uterine wet weight 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)

Figure 3: Effects of exposure (7 days) of BuPben on morphometry of uterus in adult mice. Data are expressed as Mean $\pm$ SEM. (n=5/group). Asterisks denote significant relationship with control groups (*p<0.05).

Figure 4: Effects of exposure (7 days) of BuPben on number of uterine glands in adult mice. Data are expressed as Mean $\pm$ SEM. (n=5/group). Asterisks denote significant relationship with control groups (*p<0.05) (** p<0.01)

Figure 5: Effects of 7 days exposure of BuPben on total uterine tissue protein in adult mice. Data are expressed as Mean $\pm$ SEM. (n=5/group). Asterisks denote significant relationship with control groups (*p<0.05) (** p<0.01)

Plate 1: Effect of 7 days BuPben treatment on uterine histology in adult mice. Significant modification in E2 treated group and BuPben dose of 50 mg/Kg bw and 100 mg/Kg bw was observed when compared to the control groups and BuPben dose of 10 mg/Kg bw. Abbreviations: UL – Uterine lumen; E- Endometrium; M- Myometrium. Magnification: 10x

REFERENCES

1. Soni MG, Carabin IG, Burdock GA. Safety assessment of esters of p-hydroxybenzoic acid (parabens). *Food and Chemical Toxicology* 2005; 43 Suppl 7: 985-1015.
2. Masten, S. A. "Butylparaben [CAS No. 94-26-8]." Review of Toxicological Literature, National Toxicology Program 2005.
3. Darbre PD, Aljarrah A, Miller WR, Coldham NG, Sauer MJ, Pope GS. Concentrations of parabens in human breast tumours. *Journal of applied toxicology* 2004; 24 Suppl 1: 5-13.
4. Routledge EJ, Parker J, Odum J, Ashby J, Sumpter JP. Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. *Toxicology and applied pharmacology* 1998; 153 Suppl 1: 12-19
5. Oishi S. Effects of butylparaben on the male reproductive system in rats. *Toxicology & Industrial Health* 2001; 17: 31-39.
6. OECD. Giving knowledge for free. The emergence of open educational resources. Paris: OECD Publishing 2007.
7. Lemini C, Hernandez A, Jaimez R, Franco Y, Avila ME, Castell A. Morphometric analysis of mice uteri treated with the preservatives methyl, ethyl, propyl, and butylparaben. *Toxicology and industrial health* 2004; 20 Suppl 6: 123-132.
8. Markey, C. M., Wadia, P. R., Rubin, B. S., Sonnenschein, C. and Soto, A. M. (2005). Longterm effects of fetal exposure to low doses of the xenoestrogen bisphenol-A in the female mouse genital tract. *Biology of Reproduction*. 72, 1344–1351
9. Gunin AG, Mashin IN, Zakharov DA. Proliferation, mitosis orientation and morphogenetic changes in the uterus of mice following chronic treatment with both estrogen and glucocorticoid hormones. *Journal of endocrinology* 2001; 169 Suppl 1: 23-31
10. Lowry OHN, Rosenbough J, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry* 1951; 193: 261-275

11. Grunert G, Fernandez S, Tchernitchin AN. Methods for the evaluation of responses to estrogen in individual cell types or regions of the uterus. *Hormone Research in Paediatrics* 1984; 19 Suppl 4: 253-262
12. Ireland JS, Mukku VR, Robison AK, Stancel GM. Stimulation of uterine deoxyribonucleic acid synthesis by 1,1,1-trichloro-2-(p-Chlorophenyl)-2-(o-Chlorophenyl) ethane (o, p'-DDT). *Biochemical Pharmacology* 1980; 29: 1469-1474.
13. Branham WS, Zehr DR, Sheehan, DM. Differential sensitivity of rat uterine growth and epithelium hypertrophy to estrogens and antiestrogens. *Experimental Biology and Medicine* 1993; 203 Suppl 3: 297-303
14. Ireland, J. S., Mukku, V. R., Robison, A. K. and Stancel, G. M. (1980). Stimulation of uterine deoxyribonucleic acid synthesis by 1,1,1-trichloro-2-(p-Chlorophenyl)-2-(o-Chlorophenyl) ethane (o, p'-DDT). *Biochemical Pharmacology*. 29, 1469-1474
15. Debnath N, Kalita JC, Ghosh SK, Dhar B. Uterotrophic responses and modulation of uterine gene expression induced by combinations of genistein and coumestrol in ovariectomized mice. *The Bioscan* 2015; 10 Suppl 2: 553-561.

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