Research Article

Impact of Butylparaben on Uterine Weight of C3H Albino Mice

***Pallabi Goswami, J. C. Kalita**

Animal Physiology & Biochemistry Laboratory, Department of Zoology, Gauhati University, Assam, India.

ABSTRACT

Parabens are alkyl ester compounds of p-hydroxy benzoic acid and are widely used as preservatives in many pharmaceuticals and personal care products (PPCP), foodstuffs etc. Four parabens methyparaben, ethylparaben, propylparaben and butylparaben are found to be most widely used parabens in products of daily human use. Butylparaben is found to show stronger estrogenic response compared to rest of the widely used parabens. Many *in vivo* and *in vitro* studies have shown that parabens are weak estrogenic chemicals including butylparaben. Butylparaben is found to be 10,000 fold times less potent than 17β estradiol. In this experiment, adult C3H albino mice of 8 weeks of age were exposed to both high and low dose of butylparaben and the effect of butylparaben on uterine weight was studied through uterotrophic assay. For the experiment five different doses of butylparaben mg/Kg body weight of 10 mg/Kg body weight/day, 50 mg/Kg body weight/day, 100 /day, 500 mg/Kg body weight/day, 1000 mg/Kg body weight/day was considered. The adult C3H albino mice were grouped as vehicle control (olive oil), positive control (estradiol) and the above five different doses of butylparaben and were administered with the doses for seven consecutive days through subcutaneous route of administration. After the short-term exposure of 7 consecutive days, butylparaben was found to show a significant increase in uterine weight at doses 50 mg/Kg body weight/day and 500 mg/Kg body weight/day.

Keywords: 17β estradiol, butylparaben, pharmaceuticals and personal care products, preservative, uterotrophic.

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Address for correspondence:
Pallabi Goswami
Animal Physiology & Biochemistry Laboratory, Department of Zoology, Gauhati University, Assam, India.
Email: goswami.pallabi29@gmail.com

INTRODUCTION

In recent years it is found that a wide variety of synthetic chemicals present in the environment are able to function like 17β estradiol which is a natural estrogen produced by ovary [1]. These chemicals also known as xenoestrogens are found to have certain degree of structural similarity with 17β estradiol because of which they have the potential to disrupt normal endocrine function resulting in disruption of normal reproductive function by affecting both male and female reproductive organs and increasing susceptibility to various dangerous diseases like cancer [1- 5]. Parabens are group of synthetic chemicals of which methyparaben, ethylparaben, propylparaben and butylparaben are found to be most widely used as a preservative in many cosmetics, pharmaceuticals and in food stuffs [9].

It is estimated that parabens were used in 13200 different cosmetic formulations and independent analyses of cosmetic products found parabens in 99% of leave on products [3-9]. Butylparaben is widely used as preservative in many preservatives in many pharmaceuticals and personal care products (PPCP), foodstuffs as it acts as a good antimicrobial agent showing broad spectrum of antimicrobial activity. Butylparaben have been of recent concern because of its existence in low concentration in breast tumors [9]. In many experimental studies including both in vivo and in vitro studies butylparaben is found to mimic estrogen activity, thereby acting as potential xenoestrogen [6- 8]. Studies show that butylparaben exerts reproductive, developmental as well as terratogenic toxicity in experimental animals [3]. Human

exposure to xenoestrogens can adversely affect reproductive function and reproductive cycle [3]. Even though butylparaben shows weak estrogenic response of 10,000 fold less than 17β estradiol, its long term human exposure to this chemical is a matter of concern [3- 9].

Materials and Methods:

Chemicals: Butyl p- hydroxybenzoate (Butylparaben) was obtained from Sigma Aldrich. It purity, guaranteed by manufacturer was at least 99%.

Animals and Housing:

For the experiment female C3H albino mice were selected from Animal house facility of department of zoology, Gauhati University (Animal ethical clearance number: 902/AC/05/CPCSEA). The animals were housed in wire mesh plastic cages with solid bottom containing saw dust and maintained under uniform condition of natural photoperiod (12 hr light/dark cycle), relative humidity(75%-87%) and temperature(30±2°c). The mice had free access to water and commercially available animal diet, vitamins and mineral supplement (purchased from Agrivet Farm Care Division, Glaxo Smithkline, Chennai, India) and were fed ad libitum.

Estrous cycle was observed everyday by microscopic examination of vaginal smear. Only mice showing four consecutive cycles were consider for the experiment.

Body weight and clinical signs were recorded on a daily basis throughout the period of the experiments.

Preparation of doses of butylparaben:

Butylparaben was prepared in doses of 10 mg/Kg body weight, 50 mg/Kg body weight, 100 mg/Kg body weight, 500 mg/Kg body weight,1000 mg/Kg body weight. Due to solubility constraint, butylparaben was first dissolved in ethanol and than in olive oil and 500ng of 17β estradiol was dissolved first in ethanol than in olive oil.

Test compounds administration:

Female mice of 8 weeks of age group and of average body weight 25±2g were selected for the experiment. The mice were grouped into 7 groups (n=6) and were administered with 20µl olive oil (vehicle control group), 500ng estradiol (positive control group) and 5 doses of butylparaben of 10 mg/Kg body weight, 50 mg/Kg body weight, 100 mg/Kg body weight, 500 mg/Kg body weight, 1000 mg/Kg body weight daily through subcutaneous route of administration. The doses were administered at 24hour interval.

After 24 hrs of last dose, the mice were weighed and sacrificed by cervical dislocation under mild anesthesia (di ethylether).

RESULTS AND DISCUSSION

Table 1: Showing Effect of Butylparaben on Uterine Weight of C3H Mice. *indicates significance at p<0.01 compared to olive oil (vehicle control group)

Compound	Dose (mg/kg bw)	Route	Uterine weighed (in mg)
Oil	20 µl (per animal)	Sc	91.6 ± 0.6
E_2	500ng	Sc	190.2± 7.26*
BuPben	10	Sc	101.4± 8.15
BuPben	50	Sc	157.2± 9.28*
BuPben	100	Sc	95.6± 4.22
BuPben	500	Sc	116.8± 2.35*
BuPben	1000	Sc	89.8± 9.70

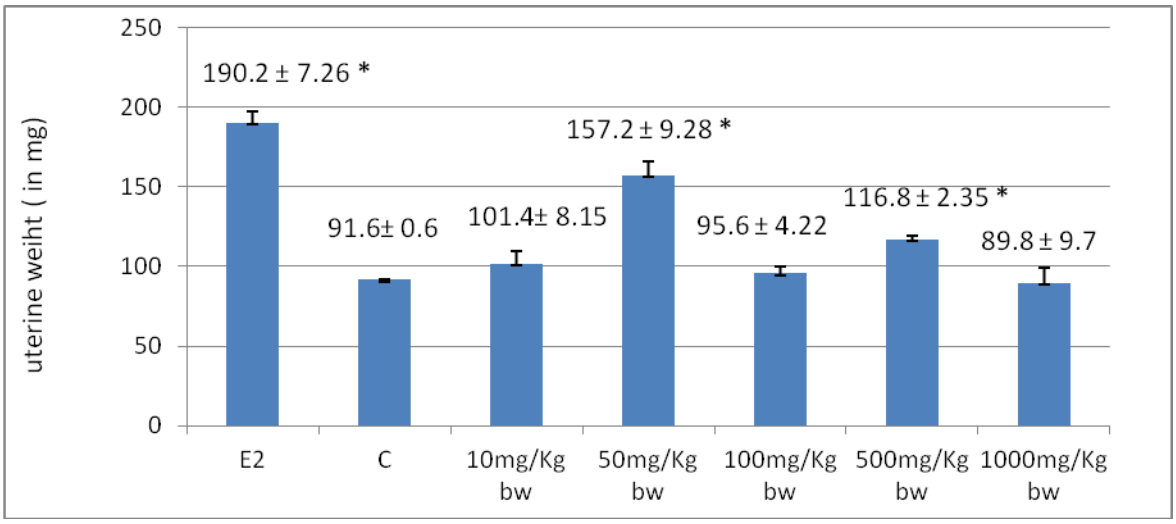

Fig 1: Butylparaben is Found To Show Dose Dependent Change in Uterine Weight Of C3H Mice Even Though Potency Lowers Than Estradiol(P<0.01%).

The treatment of adult C3H mice with estradiol and five different dose level of butylparaben for seven consecutive doses showed changes in the uterine weight. The estradiol treated group showed significant increase in uterine weight of 190.2 ± 7.26 mg ($p<0.01$). Butylparaben showed a dose dependent effect on uterine weight in C3H mice. 50 mg/Kg body weight/day and 500 mg/Kg body weight/day showed a significant increase in uterine weight of 157.2 ± 9.28 mg ($p<0.01$) and 116.8 ± 2.35 mg ($p<0.01$) compared to vehicle control group (olive oil) of 91.6 ± 0.6 mg. 10 mg/Kg body weight/day showed statistically insignificant increase in uterine weight of 101.4 ± 8.15 mg. 100 mg/Kg body weight/day showed a statistically insignificant increase in uterine weight of 95.6 ± 4.2 mg. 1000 mg/Kg body weight/day showed a statistically insignificant decrease in uterine weight compared to vehicle control group of 89.8 ± 9.70 mg. Results are shown in **Table 1** and **Figure1**.

The high rate of human exposure of butylparaben has been of growing concern as it is found to mimic estrogen activity in *in vitro* and *in vivo* system [3]. Studies showed that butylparaben exerts reproductive toxicity in experimental animals. Several studies report *in vivo* estrogenic effect in CD1 and CF1 mice and there are known strain differences in sensitivity to endocrine disruption.

CONCLUSION

The data shown here confirms the estrogenic potential of butylparaben as it shows a positive uterotrophic response at certain doses (50 mg/Kg body weight and 500 mg/Kg body weight). Evaluation of the activity of butylparaben in mice have shown significant differences in the sensitivity of the uterus of C3H mice strain with those reported with CD1and CF 1 mice thus explaining a great intraspecific variation [3].

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