



EFFECT OF SHORT TERM EXPOSURE OF BUTYLPARABEN ON UTERINE WEIGHT OF OVARIECTOMISED C3H ALBINO MICE

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ABSTRACT:

Butylparaben is a commonly used anti microbial preservative in cosmetics, pharmaceuticals and food. 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. 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. In this experiment sensitivity of butylparaben exposure on uterus of ovariectomised C3H albino mice was studied through uterotrophic assay considering 5 different doses of 10 mg/Kg body weight/day, 50 mg/Kg body weight/day, 100 mg/Kg body weight/day, 500 mg/Kg body weight/day, 1000 mg/Kg body weight/day for 7 consecutive days through subcutaneous route of administration. The ovariectomised C3H albino mice were grouped as vehicle control (olive oil), positive control (estradiol) and the above five different doses of Butylparaben. After the short term exposure of 7 days butylparaben is found to exert a dose dependent change in uterine weight of ovariectomised C3H albino mice.

Keywords: Butylparaben, estrogen, uterotrophic assay, reproductive toxicity

INTRODUCTION:

Certain chemicals present in the environment have recently drawn attention of the scientific community in possessing endocrine disrupting property. Human exposure to these chemicals occurs through inhalation, ingestion and dermal absorption when these chemicals are released from various sources like pesticides, medical wastes, industries, agriculture, pharmaceutical and personal care products (PPCPs)¹ and some other household products. Butyl ester of p- hydroxy benzoic acid (Butylparaben) is commonly used as antimicrobial preservatives in cosmetics, pharmaceuticals, and food and is of extensive use in many PPCPs due to their heat stability and antimicrobial activity. Butylparaben is of growing concern as recent studies have revealed that in *in vitro*⁴ and *in vivo* models, BP mimics estrogen activity², thereby acting as a potential xenoestrogen. Studies showed that BP exerts reproductive, developmental as well as teratogenic toxicity in experimental animals^{2,3}. Butylparaben is widely used by manufacturing companies for its low cost and efficiency as a microbial agent⁸. Butylparaben is a very popular preservative because of its ability to inhibit DNA and RNA synthesis like ATPase and phosphotransferase in some bacterial species and disrupt their membrane transport proteins⁸. It is used in various cosmetics as a preservative like eye care make up products, sunscreen, facial products and skin anti aging products. Butylparaben has also been of recent concern because of its existence in low concentration in breast tumours^{6,7}.

MATERIALS AND METHODS:

Animals and Housing:

For the experiment female albino mice of C3H strain were selected from Animal house facility of department of zoology, Gauhati University. 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.

Preparation of doses of butylparaben:

Butylparaben (Sigma Aldrich) 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. 500ng of estradiol was prepared by dissolving estradiol first in ethanol than in olive oil.

Experiment design:

Female mice of 8 weeks of age group and of average body weight 25±2g were selected for the experiment. The mice were subjected to complete bilateral ovariectomy following the method used by Kalita et al.,(1998)⁹. Ovariectomy leads to removal of the major source of estrogen hormone in the blood and thus the estrogen sensitive tissue in the body remains in there basal state. Prior to surgery the proposed ovariectomised mice were deprived of feed for 12 hours. For ovariectomy the mice were anesthetized by intra peritoneal injection of ketamine Hcl (50 mg/Kg body weight, Parke-Davis) and Xylazine 2% (100mg/kg body weight, Bayer)[Karnam et.al,1993]. Bilateral ovariectomy were done in experimental mice using a dorsal approach in a sterile surgical theatre. After wiping the mice back with 90% ethanol, a small dorsal midline incision (1-1.5 cm) was made in the skin below the last rib. The ovary was removed by cutting the oviduct as close to the ovary as possible. The remaining oviduct and uterus were replaced into the body. After bilateral ovariectomy the mice are allowed to recover for 12 days.

The ovariectomised 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.

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

RESULTS:

The treatment of ovariectomised adult C3H mice with estradiol and five different dose level of butylparaben for 7 consecutive doses showed changes in the uterine weight. The estradiol treated group showed significant increase both in uterine weight ($p<0.1$) as well as increase in body weight. Butylparaben showed a dose dependent effect on uterine weight in C3H mice. 50 mg/Kg body weight/day showed a significant increase in uterine weight ($p<0.1$) compared to vehicle control group (olive oil). 10 mg/Kg body weight/day showed increase in uterine weighed but is statistically insignificant. 100 mg/Kg body weight/day and 1000 mg/Kg body weight/day showed a significant decrease in uterine weight compared to vehicle control group. Results are shown in Table 1 and Figure1.

DISCUSSION:

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 CD1 and CF 1 mice thus explaining a great intraspecific variation. 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 100 mg/Kg body weight). Even though this preservative to which human is widely exposed possess low estrogenic activity, the broad used of it by human is a matter of concern. Thus the safety of the wide use of chemical should be reassessed.

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Compound	Dose (mg/kg bw)	Route	Uterine weighed (in mg)
Oil	10 ml	sc	91.6 ± 0.6
E ₂	500ng	sc	190.2± 7.26
BuPben	10	sc	108.6± 4.47
BuPben	50	sc	107.6± 14.6
BuPben	100	sc	75.8± 5.72
BuPben	500	sc	65.2± 2.35
BuPben	1000	sc	59.8± 10.55

TABLE I: SHOWING EFFECT OF BUTYLPARABEN ON UTERINE WEIGHT OF C3H MICE.

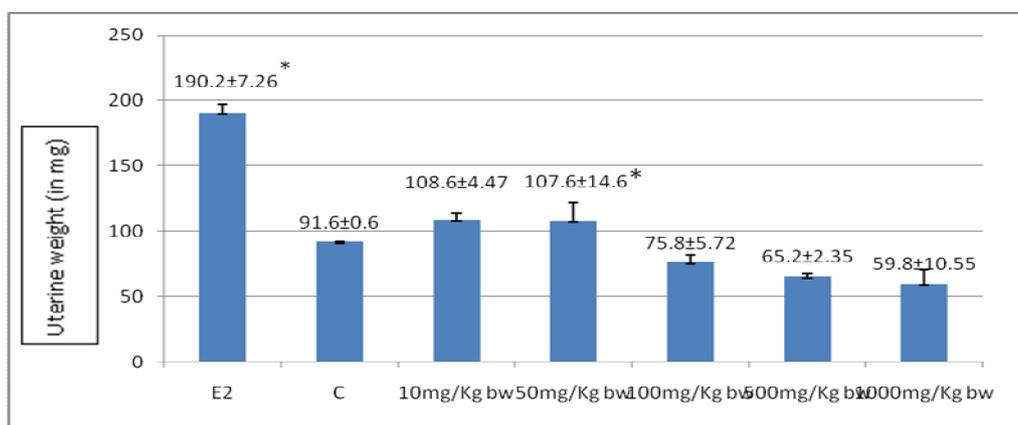


Fig 1: Butylparaben is found to show dose dependent change in uterine weight of C3H mice even though potency lowers than estradiol ($p<0.1\%$)

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