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Research Article

EFFECT OF BUTYLPARABEN ON TOTAL UTERINE TISSUE PROTEIN IN ADULT C3H ALBINO MICE

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ABSTRACT

In recent years, many environmental pollutants are found to posses estrogenic properties and thus these compounds are known as xenoestrogen or environmental estrogen. These environmental pollutants are present in wide variety of daily used products of human including variety of pharmaceutical and personal care products, foodstuff and products for children. Butylparaben is used in various cosmetics as a preservative like eye care make up products, sunscreen, facial products and skin anti aging products. Many experimental studies in in vivo and in vitro system have shown that butylparaben shows estrogenic activity. In this experiment the effect of butylparaben on studied the total uterine tissue protein in adult C3H albino mice was was studied by lowry method. Four doses of butylparaben 10 mg/Kg body weight/day, 50 mg/Kg body weight/day, 100 mg/Kg body weight/day and 500 mg/Kg body weight/day was administered for 7 consecutive days through subcutaneous route of administration. In the experiment olive oil was used as vehicle control and 17β estradiol was used as positive control. After 7 days of exposure butylparaben was found to increase total uterine tissue protein in adult C3H albino mice.

Keywords: 17β estradiol; Butylparaben; Preservative; uterine protein; Xenoestrogen.

INTRODUCTION

Certain chemicals in the environment to which human in daily exposed are found to adversely affect his health [1]. These chemicals includes phthales, triclosan, bisphenol a, parabens etc[1, 3]. Parabens includes a group of compounds that have good antimicrobial activity because of which it is widely used as preservative in many products of human daily use like a large range of pharmaceutical and personal care products, foodstuff and products for children[2, 4]. These chemicals enter into the human in the body through inhalation, ingestion and dermal absorption. Among the large group of parabens most widely used parabens are methylparaben, ethylparaben, propylparaben and butylparaben[2, 4, 5]. Parabens has drawn attention of the scientific community after its presence has been established in breast tumor tissue[8]. Studies have shown that butylparaben exhibits reproductive toxicity affecting organs like uterus, ovary, testis and mammary glands. It shows uterotrophic response, reproductive and developmental toxicity in rodents[4]. However butylparaben is found to be weakly estrogenic as it its potency is found to be 10000 fold less than 17β estradiol, a natural estrogen produced by the ovaries present in the female's body[9].

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.

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 and 500 mg/Kg body weight. Due to solubility constraint butylparaben was first dissolved in ethanol and than in olive oil.

Preparation of 17β estradiol

500ng of estradiol was prepared by dissolving estradiol first in ethanol than in olive oil.

Administration of dose

Adult female albino mice of C3H strain 8 weeks of age group and of average body weight 23±3 g were selected for the experiment. The mice were grouped into four groups (n=6) and were administered with 20µl olive oil (vehicle control group), 500ng estradiol (positive control group) and 3 doses of butylparaben of 10 mg/Kg body weight, 50 mg/Kg body weight, 100 mg/Kg body weight daily and 500 mg/Kg body weight.

After 24 hrs of last dose the mice were weighed and sacrificed by cervical dislocation under mild anesthesia (di ethylether). And uterus was collected, cleared of fats and vascular tissue, if any and weighed.

Preparation of tissue homogenate

5% homogenate of the tissue in distilled water was prepared from the uterus collected and protein was precipitated with 10% trichloroacetic acid (TCA) on ice and centrifuged at 1000 r.p.m for 10 mins. The supernatant was discarded and 5% trichloroacetic acid (TCA) was added and again centrifuged at 10 00 r.p.m for 10 mins. The supernatant was discarded and alcoholic ether was added and again centrifuged at 4000 r.p.m for 10 mins. The supernatant was discarded and 0.1 N NaoH was added and left for 3-4 hrs at room temperature.

Determination of protein in uterine tissue homogenate: Colorimetric estimation of protein was done considering 0.1% BSA (Bovine serum albumin) as standard (Lowry et al, 1951).

Table 1: Effect of Butylparaben on total uterine tissue protein of C3H mice

Compound	Dose(mg/kg bw)	Route	Total uterine tissue protein in mg/g tissue
Oil	20µl(per animal)	sc	11.4 ± 0.012
E_2	500ng	sc	31.8 ±0.125
BuPben	10	sc	11.4 ±0.25
BuPben	50	sc	13.8 ± 1.23
BuPben	100	sc	18 ± 1.23
BuPben	500	sc	18 ± 1.20

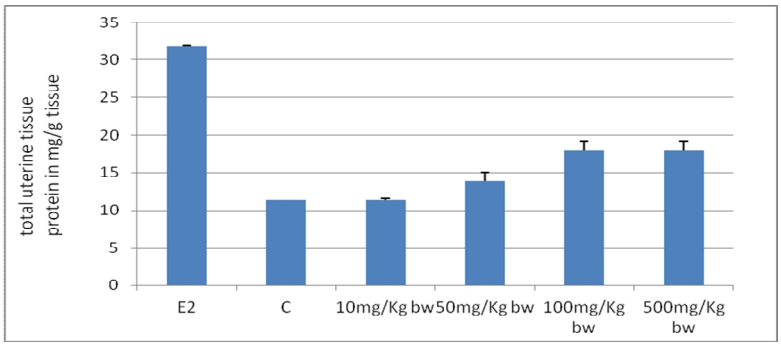

Figure 1: Butylparaben is found to show dose dependent change in total uterine tissue protein of C3H mice even though potency lowers than estradiol (p<0.1%).

RESULTS

The treatment of adult C3H mice with estradiol and four different dose level of butylparaben of 10 mg/Kg body weight, 50 mg/Kg body weight, 100 mg/Kg body weight daily and 500 mg/Kg body weight for 7 consecutive doses showed change in total uterine tissue protein. The estradiol treated group showed significant increase in total uterine tissue protein of 31.8 ±0.125 mg/g uterine tissue ($p<0.1$) compared to vehicle control group (olive oil) of 11.4 ± 0.012 mg/g uterine tissue.

10 mg/Kg body weight/day showed same level of total uterine tissue protein of 11.4 ±0.25 mg/g uterine tissue compared to vehicle control group (olive oil) of 11.4 ± 0.012 mg/g uterine tissue. 50 mg/Kg body weight, 100 mg/Kg body weight daily and 500 mg/Kg body weight showed significant increase in total uterine tissue protein of 13.8 ± 1.23 mg/g uterine tissue, 18 ± 1.23 mg/g uterine tissue and 18 ± 1.20 mg/g uterine tissue.

Results are shown in Table 1 and Figure1.

DISCUSSION

Adverse effect on the reproductive tract of female in rodents has also been reported but the effects are found to be shown in different doses in different strain of mice showing sensitivity differences. Butylparaben have been of recent concern because of its existence in low concentration in breast tumors[8]. In many experimental studies including both in vivo and in vitro studies butylparaben is found to mimic estrogen activity, thereby acting as potential xenoestrogen[9]. Studies show that butylparaben exerts reproductive, developmental as well as terratogenic toxicity in experimental animals[6, 7].

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