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Full Length Original Research Paper

In Vivo Genotoxic studies of β-Asarone in Mice Bone Marrow

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Abstract:
Present study was carried out to ascertain whether β- asarone, a constituent of herbal medicinal oil exert genotoxic effects in mouse bone marrow. For this study Swiss *albino* mice (five male and five female) were treated with maximum tolerated dose (MTD) of β-asarone as single *i.p.* dose, corresponding to the LD_{50} of β- asarone (182.4 mg/kg body wt) for the mice. The micronucleus test was conducted using a method based on OECD and European Union guidelines to observe the effects on mouse bone marrow. The results obtained suggest that β-asarone did not increase the incidence of micronucleated polychromatic erythrocytes (MPE) in bone marrow. However, cyclophosphamide used as positive control had increased the incidence of MPE in bone marrow. Our findings revealed that β- asarone did not have genotoxic or mutagenic effects for the tested dose.

Keywords: *Acorus calamus,* micronucleus, cyclophosphamide, beta asarone.

Introduction

β-asarone (cis-isomer of 2,4,5-trimethoxy-l-propenylbenzene) is a constituent of oil derived from the dried rhizome of *Acorus calamus* Linn. and *Acorus europaeum* which is used as herbal medicine. Percentage of β-asarone content depends on ploidy condition of the plant. The volatile oil from the tetraploid form of calamus rhizome contains up to 95 to 96% β- asarone as a major component, on the other hand oil from the European or triploid form contains less than 10% of β- asarone. A diploid form of the plant contains virtually no asarone [1]. The rhizome oil is strong and fragrant, pungent and aromatic. Calamus oil is used as flavouring agent. Oil is used in foods such as frozen desserts, yoghurts, cakes, confectionery and desserts [2]. Calamus oil is also largely used in the production of alcoholic beverages such as bitters, liqueurs and vermouths [3]. Oil also possesses antigonadal activity in insects [4, 5]. The root containing β- asarone is used in traditional medicine to treat diabetes [6]. A very recent study by Geng *et al.*, have shown β-asarone as a potential candidate for development as a therapeutic agent to manage cognitive impairment associated with conditions such as Alzheimer's disease [7]. But β- asarone in pre-weanling (12 day old) mice given either single i.p. injections of 52 mg/kg bw β- asarone or successive injections on days 1, 8, 15 and 22 (total dose approximately 1 mg), an increased incidence of hepatomas was observed [8]. In another chronic feeding study, β- asarone produced a dose-dependent increase in leiomyosarcomas in the intestine of male rats [9]. As a consequence of these findings, the use of β-asarone is restricted to 1 ppm in alcoholic beverages and snack foods [10]. In human the

maximum intake of β- asarone from food and alcoholic beverages can be restricted to be approximately 115 µg/day *i.e.*about 2 µg/kg body weight/day. But most of the biological activity of *Acorus calamus* is considered due to presence of β- asarone. In a number of *in vitro* experiments β-asarone was found to have anti-cholinergic activity and was reported to stop frog hearts in diastole and to reduce the tone and longitude of intestinal movement [11]. The rhizomes, roots and essential oils extracted from rhizomes of *A. calamus* are reported to possess several important activities including antimicrobial [12], anticellular and immunosuppressive [13] and allelopathic [14]. Aromatic oils obtained by alcoholic extraction of the rhizome are used in the pharmaceutical and oenological industries [15].

The mutagenicity of the β- asarone has been investigated in various studies in mammalian and sub-mammalian test systems, both *in vivo* and *in vitro*, covering a variety of end points. But there is no report present in the literature at the chromosomal level. The purpose of the present study was to assess whether β- asarone has the genotoxic effect on Swiss *albino* mouse bone marrow. It was designed to provide a rigorous examination of the *in vivo* activity of β- asarone in the micronucleus test, using bone marrow sampling. Swiss *albino* mice and the *i.p.* route were used to reproduce the conditions to provide the largest increases in MPE following treatment with β- asarone. Killing time was chosen 18, 24 and 36 h, at which the highest incidence of MPE was previously reported [16].

Materials and Methods

Materials

β- asarone, cyclophosphamide were purchased from Sigma-Aldrich (USA), Dimethyl sulfoxide (DMSO), Grunwald-geimsa stain were obtained from Himedia (India).

Animals

Group of Swiss *albino* mice (five male and five female) was chosen for the study. Swiss *albino* mice were issued from the VIT University animal house. Experiments were conducted in male and female weighing 25-30 g. Different groups of mice were maintained in different cages with food and water *ad libitum* condition. All the animals were maintained in the animal house at 24 ± 1°C with a 12-h light-dark cycle. During the time period of experiments mice were kept free from any pathogenic infection. Animals were 5 weeks old when used for micronucleus tests.

Methods

The micronucleus test was conducted using a method based on OECD and European Union guidelines [10, 17]. Briefly, groups of five male and five female mice were treated with isomer mixture containing 70% β-asarone at MTD corresponding to LD 50 value (LD50 182.4 mg/kg body wt *i.p.*). DMSO was used as a vehicle for β-asarone so the control animals were treated with the vehicle alone. Positive controls received cyclophosphamide (40 mg/kg body wt *i.p.*). All doses were administered at 3 ml/kg body wt.

Bone marrow preparation

For the determination of bone marrow effects, separate groups of animals were sacrificed at 18, 24 and 36 hours after dosing by an overdose of anaesthetic (halothane). All animals receiving β-asarone underwent gross pathological examination. Femurs were removed and stripped clean of muscle. The iliac end of the femur was removed and bone marrow sampled with a fine brush

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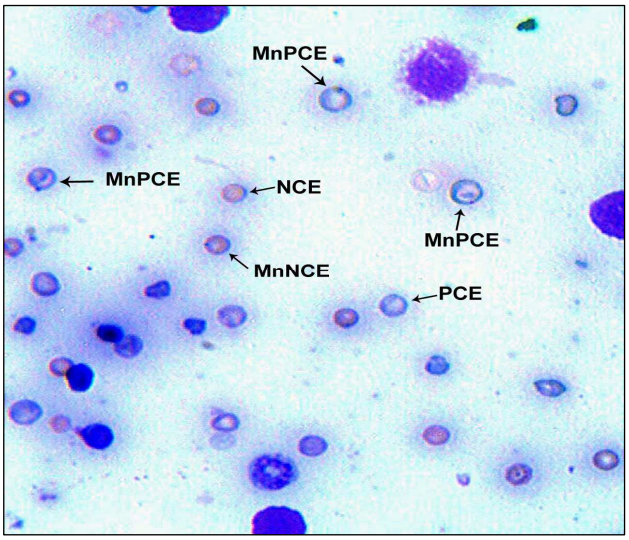

Figure 1: Bone marrow smear of Swiss *albino* mice showing PCEs (Polychromatic Erythrocytes), MnPCEs (micronucleated polychromatic erythrocytes), NCEs (normochromatic erythrocytes) and MnNCEs (micronucleated normochromatic erythrocytes).

(rinsed in 0.9% saline). Two smears were prepared and allowed to air dry, prior to staining with May Grunwald-geimsa stain (Figure 1). Slides were coded and scored blind, 2000 polychromatic erythrocytes (PCE) were examined for the presence of micronuclei [18].

Results

Beta asarone has been evaluated for its ability to induce MPE in the Swiss albino mice bone marrow. The procedures and experimental design employed complied with the recommendation of the OECD and EU guidelines except that a single dose level was used [10,17]. Beta asarone was tested at the MTD in both male and female mice, as shown by adverse clinical reactions to treatment. The results of this study states that the PCE in bone marrow was not affected by treatment with β- asarone. A statistically non-significant increase in the incidence of MPE in the bone marrow of β- asarone-treated females at 24 hours (5.8) sampling time (Table 1), compared with the vehicle control (5.2) was observed. This increase suggests that the bone marrow had been subjected to stress at MTD of β- asarone. The increase is considered not biologically important, since the value (5.8) lies within the range of control values (5.0-15.5 MnPCEs) reported for this strain [19-22]. Therefore, a value of 5.8 MPE/2000 PCE is clearly not a biologically important increase over controls for this mouse strain. No other statistically significant increases in the incidence of MPE, compared with the control values, were seen in the bone marrow or of any β- asarone treated mice at any of the sampling times investigated. However, there is statistically significant MPE induction in cyclophosphamide treated animals.

Table 1: Incidence of MN in the bone marrow of male and female Swiss *albino* mice at various times after administration of a single dose of β-asarone.

Treatment	Total No. of MnPCEs of 2000PCEs		
	18 h	24 h	36 h
Male			
Vehicle control	4.2 ± 0.44	4.6 ± 0.54	4.6 ± 0.54
β-asarone	4.0 ± 0.70	4.4 ± 0.54	4.6 ± 0.54
Cyclophosphamide	51.4 ± 1.34***	54.8 ± 0.83***	65.8 ± 1.30***
Female			
Vehicle control	5.0 ± 0.70	5.2 ± 0.44	5.6 ± 0.54
β-asarone	4.8 ± 0.40	5.8 ± 0.44	5.8 ±0.44
Cyclophosphamide	48 ± 1.58***	51.8 ± 1.78***	61.6 ± 1.14***

Discussion

Till date no *in vivo* genotoxic studies were carried out in β- asarone. But i*n vitro* unscheduled DNA synthesis assay was done in isolated rat hepatocytes treated with 10^{-6} to 10^{-3} M (equivalent to 209 µg/l - 209 mg/l and 0.8 to 836 µg/plate) of pure β- asarone. No unscheduled DNA synthesis was detected [23]. However recently genotoxicity of β- asarone (unknown source) was

determined by unscheduled DNA synthesis assay in cultured rat hepatocytes treated with 5 x 10^{-4} M (equivalent to 104.5 mg/l and 4.18 µg/plate), but the effect was abolished by co-treatment with cimetidine suggesting that activation by cytochrome P450 was necessary to achieve the genotoxic effect of β- asarone [24]. In another study, Ames test with *Salmonella typhimurium* was performed to check the genetoxic potential of the β- asarone. Beta asarone was found to be non mutagenic in *Salmonella* strains TA98, TA100, TA1525, TA1537 or TA1538 at concentrations of 2, 20 and 200 µg/plate in the presence of S9 activation [25]. In another tests β-asarone was not found to be mutagenic at concentrations of 0.2 to 200 µg/plate in strains TA97, TA98, TA100, TA1535, TA1537 or TA1538 in either the presence or absence of S9 activation. Thus the genotoxic potential of β- asarone is unclear since only few data were available. There is only one study has been conducted so far in the isomers of alpha asarone involving micronucleus test. Our results represented here indicate that β -asarone is not genotoxic in mouse bone marrow at MTD. However, further studies will be much needed to know and explore the side effects of the β-asarone on prolong or chronic exposure.

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