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Extraction of *Solanum Trilobatum* and the Effect of Sobatum on Chromosomes

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Petroleum ether, chloroform, ethyl acetate and ethanol were used for the extraction of Solanum trilobatum. The partially purified component of the plant named as sobatum was obtained from the petroleum ether:ethyl acetate (75:25) extractable portion. Apart from plant extraction, an effort was also made to evaluate the effect of sobatum on chromosomes. Groups of mice were administered sobatum (100, 200 and 400 mg/kg) or cyclophosphamide (100 mg/kg) followed by colchicine, which was administered 90 minutes before sacrificing the animals for arresting mitosis. It was shown that there was no variation in the chromosome number or significant abnormalities in the sobatum and DMSO treated groups, where as cyclophosphamide induced remarkable chromosomal abnormalities. This investigation revealed that the sobatum did not have the potential to induce any chromosomal aberrations in the bone marrow erythrocytes of swiss mice.

CHROMOSOMAL aberrations are microscopically visible changes in the chromosome structure, which may be due to complete breaks of a single chromatid resulting in the loss or deletion of a part of the chromosome. The deleted material may appear as fragments in metaphase preparations[1]. The incidence for double stranded breaks being the ultimate lesions for chromosomal aberrations was reported by Obe et al.[2]. In routine cytogenetic screening of chemicals, according to the basic requirement of many regulatory authorities, bone marrow test is sufficient and in addition, several researchers conducted prominent work using human lymphocytes[3,4] and rodent lymphocytes[5].

Sobatum was obtained from petroleum ether:ethyl acetate (75:25) extractable portion by fractionation of the petroleum ether extract of the plant Solanum trilobatum. Sobatum produced a high degree of cytotoxicity in Dalton's lymphoma ascites cells (DLA), Ehrlich ascites cells and tissue culture cells [L929 and vero cells]. it was also found to inhibit the peritoneal and Solid tumours induced by DLA and EA tumour cell lines[6]. On exposure to DMBA, sobatum significantly reduced the papilloma formation and thereby inhibited the skin carcinogenicity[7]. This may due to the significant free radical scavenging activity of sobatum[8]. In addition to these, there was a lack of induction of micronucleus in the bone marrow cells of swiss mice[9]. The present study focuses on the extraction of sobatum and evaluation of its cytogenetic potential and its utility to induce chromosomal aberrations after 24 and 36 hours of exposure.

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MATERIAL AND METHODS

Petroleum ether, chloroform, ethyl acetate, ethanol were obtained from SD Fine Chemicals, Madras, India. Giemsa stain was obtained from Indian drugs and pharmaceuticals Ltd., Hyderabad, India, cyclophosphamide, colchicine were purchased from Sigma, USA. *Solanum trilobatum* was collected from the hilly area of Nagarcovil forest (Tamil Nadu, India) during February-March and was authentically identified by a qualified Botanist.

Betasitosterol was obtained from Regional Research Laboratory, Trivandrum.

Extraction and fractionation of the plant

Whole plant was cleaned, washed and cut into small pieces, dried, powdered and used for extraction. The plant powder (100 g) was filled in a Soxhlet extractor and extracted exhaustively with petroleum ether(60-80°), chloroform (60.5-61.5°), ethyl acetate (76-77°) and ethanol (78-79°) sequentially. The petroleum ether (60-80°) extract was concentrated and chromatographed batch wise over silica gel (113.16 g) column and eluted with different solvent systems sequentially. The petroleum ether:ethyl acetate (75:25) extractable portion, which was named as sobatum on concentration and crystallization from methanol yielded a single crystalline colourless compound which was identified as betasitosterol by direct comparison (melting point, mixed melting point and superimposibable IR) with an authentic sample of betasitosterol.

Chromosomal studies

Weaning swiss albino mice of body weight range between 16 and 20 g, were maintained at the animal house under standard conditions of room temperature (25±2°), Humidity, and were supplied with rat feed (M/s Lipton India Ltd., Calcutta, India) and water *ad libitum*. All the animals were weighed and grouped according to the nearest body weight[10]. The animals were assigned to five groups of 6 animals each. Group II, III and IV received different doses of (100, 200 and 400 mg/kg) sobatum[11]. Group I and V were given vehicle (DMSO 2 ml/kg) and cyclophosphamide (100 mg/kg) as a positive control, respectively. Animals were administered intraperitoneally with a single injection of sobatum, vehicle and positive control samples[12]. Colchicine (10 mg/kg) was administered 90 minutes before sacrificing the animals for arresting mitosis. Three animals each from test, control and positive control groups were sacrificed at the end of 24 hours and the remaining 3 animals in each groups were sacrificed at the end of 36 hours by cervical dislocation. Both femora were removed through the pelvic bone, just below the knee. The bones were freed from muscles, and proximal end of the femora was carefully shortened with scissors. Saline (0.5 ml) was aspirated into a disposable syringe, subsequently the needle was inserted to a few millimetre into the bone marrow

Table 1: Yield of different fractions of *Solanum trilobatum*

Solvent	Dried extract(g)
Petroleum ether	2.480
Chloroform	0.790
Ethyl acetate	0.512
Ethanol	0.212

The plant material is sequentially extracted with petroleum ether, chloroform, ethyl acetate and ethanol. The extracts were evoporated to dryness and weighed.

Table 2. Yield of different fractions from 10 g of petroleum ether extract of Solanum trilobatum

Solvent	Quantity of extract obtained (g)
PE : EA (95:5)	3.51
PE : EA (90:10)	1.35
PE : EA (75:25)	1.61
EA alone	1.91

PE = petroleum ether, EA = ethyl acetate

Table 3. Yield of different fractions from 1.6 g petroleum ether:ethyl acetate (75:25) extractable portion (sobatum)

Solvent	Quantity of extract obtained (g)
PE alone	0.41
PE : EA (90:10)	0.37
PE : EA (75:25)	0.14
EA alone	0.53

PE = Petroleum ether, EA = Ethyl acetate

Table 4 - Chromosomal abnormalities observed at 24 and 36 hours of treatment with the sobatum and cyclophosphamide

Group	Dose mg/kg	Average abnormalities/ 100 plates									
		Chromatid				Chromosome				Others	
		Gaps		Breaks		Gaps		Breaks			
		24	36	24	36	24	36	24	36	24	36
I	2 ml	1	2	1	0	1	0	0	0	7	5
II	100	1	1	1	1	0	0	0	0	5	4
III	200	2	0	1	0	0	2	0	0	2	4
IV	400	1	1	1	0	0	2	0	0	2	2
V	100	9	13	3	5	3	3	4	3	12	17

Group I received DMSO, group II, III and IV received sobatum and group V received cyclophosphamide which served as positive control.

canal. The bone marrow was flushed into the centrifuge tube and mixed thoroughly. All the tubes were centrifuged at 1000 r pm for 5 minutes. Cell button was collected, mixed with hypotonic solution (0.075 M KCl) and incubated for 20 minutes at 37°. Tubes were centrifuged again, and the cell button was fixed with fixative (3:1 methanol-acetic acid) for 3 changes. Slides were prepared by flame drying, stained with Giemsa stain and were subjected to light microscopic evaluation for the evidence of chromosomal abnormalities[1].

RESULTS AND DISCUSSION

Exhaustive extraction with organic solvents of increasing polarity afforded about 24.8 % extractable residue on removal of solvent from petroleum ether extract under vacuum (Table 1).

Petroleum ether extractable portion of Solanum trilobatum on further fractionation by adsorption chromatography showed that about 35.1 % extractable portion obtained by petroleum ether and ethyl acetate in the ratio of 95:5, 13.5 % and 16.1 % extractable portions was obtained by petroleum ether and ethyl acetate in the ratio of 90:10 and 75:25, where as 19.1 % extractable portion was obtained by ethyl acetate alone (Table 2).

The most active petroleum ether:ethyl acetate (75:25) extractable portion of *Solanum trilobatum* named as sobatum (an anticancer agent, proved by *in vitro* and *in vivo* methods) on further fractionation recovered about 4.1 % petroleum ether extractable portion, 3.7 % and 1.4 % petroleum ether:ethyl acetate (90:10, 75:25) extractable portions and 5.3 % ethyl acetate extractable portion (Table 3). Sobatum, on concentration and crystallization from methanol gave a single crystalline pure compound with a melting point of 138° and was identified as betasitosterol.

There are two main types of chromosomal abnormalities, 1. Variation in the number of chromosomes, 2. Changes in chromosomal structure. In the first case, the abnormalities such as autosomal and polyploids are found and in the second case gaps, breaks and translocations are found. The chromatid and chromosomal abnormalities such as gap, break and translocations (by the abnormal pairing configuration as rings or chains) were scored in 100 well spreaded metaphase[13] plates per animal in sobatum, DMSO and cyclophosphamide treated groups. It was found that cyclophosphamide-induced 9 and 13 chromatid gaps, 3 and 5 chromatid breaks at the end of 24 and 36 hours (Table 4). The induction of chromatid type of abnormalities were minimum in all dose groups of sobatum (100, 200 and 400 mg/kg), which was similar to that of DMSO control group. It was also shown that there was no variation in the chromosome numbers or significant abnormalities in the sobatum and DMSO treated groups, where as cyclophosphamide caused remarkable chromosomal abnormalities.

The administration of sobatum at different dose levels and time periods did not increase the frequency of structural chromosomal aberrations like gap, breaks in bone marrow cells of swiss mice, which indicates that sobatum did not have any effect on the bone marrow cell proliferative activity. From this cytogenetic studies, it can be suggested that the anticancer agent sobatum[6, 7] at different concentrations and at different time periods fails to influence the induction of chromosomal abnormalities confirming that sobatum used even at 400 mg/kg body weight concentration was found to be absolutely safe/non genotoxic.

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