Research Article

Genotoxic and Cytotoxic Effects of Different Xanthone Extracts of *Swertia* Species in *Pisum sativum.*

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ABSTRACT

Swertia is important medicinal plant in Ayurvedic system. The plant and its extracts have been evaluated for a number of activities like anti-inflammatory, anthelmintic, antimalarial, antidiabetic, anticancerous and antidiarrheal. In this study, the plant was subjected to genotoxicity studies in order to ascertain an aspect of the safety of the drug. The xanthone extracts of three *Swertia* species was evaluated in *Pisum sativum* root tips. Genotoxic effect was studied in three concentrations, 1%, 3% and 5%. Our findings indicated that exposure of xanthone extract inhibited the mitotic cell division at higher concentration when compared to the mitotic index (MI) in control. *S. densifolia* xanthone extract of 5% concentration was noted to be most effective in inducing retardation in MI compared to all other studied species. The chromosomal aberrations like condensed prophase, metaphase precocious arm, metaphase stickiness, chromosomal fragments, anaphase bridges (multiple bridge, double bridge and broken bridge), stickiness in anaphase, laggards and unequal distribution of chromosomes were also recorded in the treated root tips. The aberrations of most frequent occurrence were arisen as Anaphase bridge (46.43%), Metaphase stickiness and prophase condensation (30.77%) and Anaphase stickiness. The highest frequency (59.09%) of aberrations was recorded in 5% *S. densifolia* xanthone extract.

Keywords: xanthone, genotoxicity, chromosomal abnormality.

INTRODUCTION

Swertia is a large genus of herbs distributed in the mountainous regions of tropical Asia, Europe, America and Africa. About 40 species of the genus have been recorded in India. Out of these species *Swertia chirayita* is most important for its medicinal uses. The plants of the *Swertia* genus are rich sources of xanthones, flavonoids, irridoids and secoiridoids, glycosides, terpenoids and alkaloids. Xanthonoids are the major class of compounds among the chemical constituents of this genus, and since they often exhibit multidirectional biological activities, Whole plant of *Swertia* is important in Ayurvedic system as well as in Allopathic system of medicine. Numbers of *Swertia* plants have been used since the remote past for the treatment of various ailments, particularly in the Indian sub-continent. Some highly effective and useful traditional applications of *Swertia* species in the indigenous system of medicine have been described by Maninder.[1]

Popular medicines make use of many medicinal plants species. However, their indiscriminate and uncontrolled use can cause more harm to public health, thus the knowledge about these plants, from their cellular levels to their action on living organisms is important for making it a more affordable alternative form of therapy.

Cytogenetic studies of plant species provides clue for possible alterations of plant chromosomes caused due to mutagenic substances in their composition or resulting from their metabolism. The study of mutagens in eukaryotic nuclei has been observed by cytological methods and it is known that the mutation may result from the action of radiation, drugs and viruses, as well as the intrinsic stability of nucleic acids. Therefore, mutagens can be detected cytologically by cellular inhibition; disruption in metaphase; induction of chromosomal aberrations, numerical and structural, ranging from chromosomal fragmentation to the disorganization of the mitotic spindle, and consequently of all subsequent dependent mitotic phases. Among the various tests available for this purpose, those that uses root tips are extremely useful and can easily be handled. Regarding plant bioassays *Allium cepa*, *Lactuca sativa*, *Zea mays* and *Vicia faba* have been the most common species used for cytological as well as genotoxicity evaluation. Chromosomal abnormalities were observed at prophase, metaphase, anaphase and telophase of cell division. An attempt therefore has been made to study the cytological effects of *Swertia* species crude xanthone extract in root tips of *Pisum sativum.*

MATERIALS AND METHODS

Plant Material

The studied species of *Swertia* were collected from three different localities; *Swertia densifolia* from Kas-Satara Dist., *Swertia minor* from Sinhagad-Pune Dist., and *Swertia lawii* from Panhala-Kolhapur Dist.

Extraction of Crude Xanthone

Fresh and mature leaves of *Swertia species* were collected randomly, cleaned and shade dried. The dried leaves were powdered and macerated in

dichloromethane and methanol (1/1, v/v) for 48 hrs. filtered and the filtrate was concentrated using Rotary evaporator to obtain crude extract. The part of this extract was reextracted with ethyl acetate and stored in the fridge for further use.[2]

Cytological Studies

Pisum sativum seeds were soaked in tap water for 2 hours at 25°C. Blotted with blotting paper to remove water and were soaked in1%, 3% and 5 % (W/V) aqueous xanthone extracts of test *Swertia* species for 12 hrs. For control, the seeds were soaked in distilled water, allowed to germinate in moist filter paper containing petri plates. Seeds were germinated with 2-3 cm long primary roots which are a suitable length to be used in fixation. Root tips were cut, fixed in Cornoy's fluid (3: 1 (v/v) ethanol: glacial acetic acid) and stored overnight at 4°C. The next day they were placed in 70% (v/v) aqueous alcohol and refrigerated until used.

Averages of five slides were made for each concentration using ten root tips.

Slides were prepared by method described by Sharma and Sharma.[3] Root tips were hydrolyzed in 1 N HCl for 5 min at 60 °C and then thoroughly washed in double distilled water.

Then the roots were stained with acetocarmine to accomplish a stain, subsequently washed with water. Root tips were squashed with the help of cover glass and tapped with rubber hammer to spread the cells. Slides were made permanent by mounting in Canada Balsam, examined, and photographed.

The mitotic index (MI) and chromosome aberrations were determined by examination of cells.

Cytotoxicity was calculated from the MI. Genotoxicity was assessed by observing chromosome aberrations in mitotic cells.

The Mitotic index (MI) (%) is calculated by the following formula:

$$MI = \frac{Total\ number\ of\ dividing\ cells}{Total\ number\ of\ cells\ scored} \times 100$$

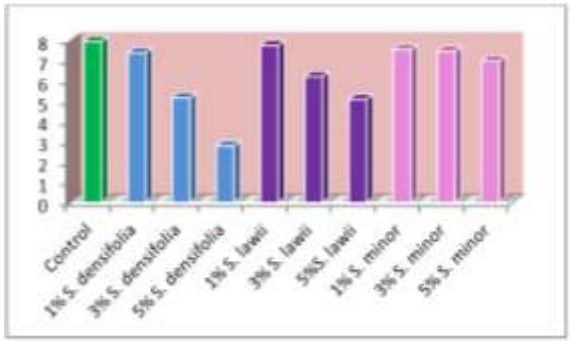
The Abnormality Index (%) is calculated by the following formula:

$$Abnormality\ Index = \frac{Total\ number\ of\ abnormal\ cells}{Total\ number\ of\ cells\ scored} \times 100$$

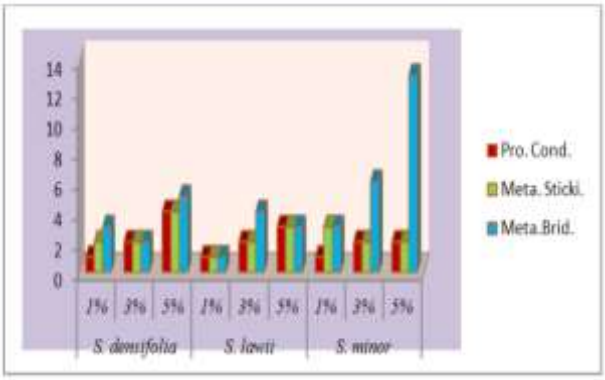
RESULTS AND DISCUSSION

Results of the current study reflected the utility of root tips cells of *P. sativum* for monitoring the genotoxic effects of *Swertia* xanthone extracts.

P. sativum assay enabled the assessment of different genetic endpoints, which are mitotic index and chromosome aberration. The results obtained are tabulated in Table-1 and 2.



Graph 1: Effect of *Swertia* crude xanthone extract on mitotic indices in *Pisum sativum*.



Graph 2: Chromosomal abnormalities induced by *Swertia* xanthone extracts in *Pisum sativum.*

Table 1: Effect of crude xanthone extract of *Swertia* species on mitotic index in *Pisum sativum* root tips.

S. No.	*Swertia* Species	Conc.(in %)	Total no. of cells screened.	Total no. of dividing cells.	% Mitotic index (MI)
1	**Control**	-	782	72	9.20
2	***S. densifolia***	1	651	48	7.37
		3	698	36	5.15
		5	790	22	2.78
3	***S. lawii***	1	698	54	7.73
		3	790	49	6.20
		5	904	46	5.08
4	***S. minor***	1	784	59	7.52
		3	993	74	7.45
		5	975	68	6.97

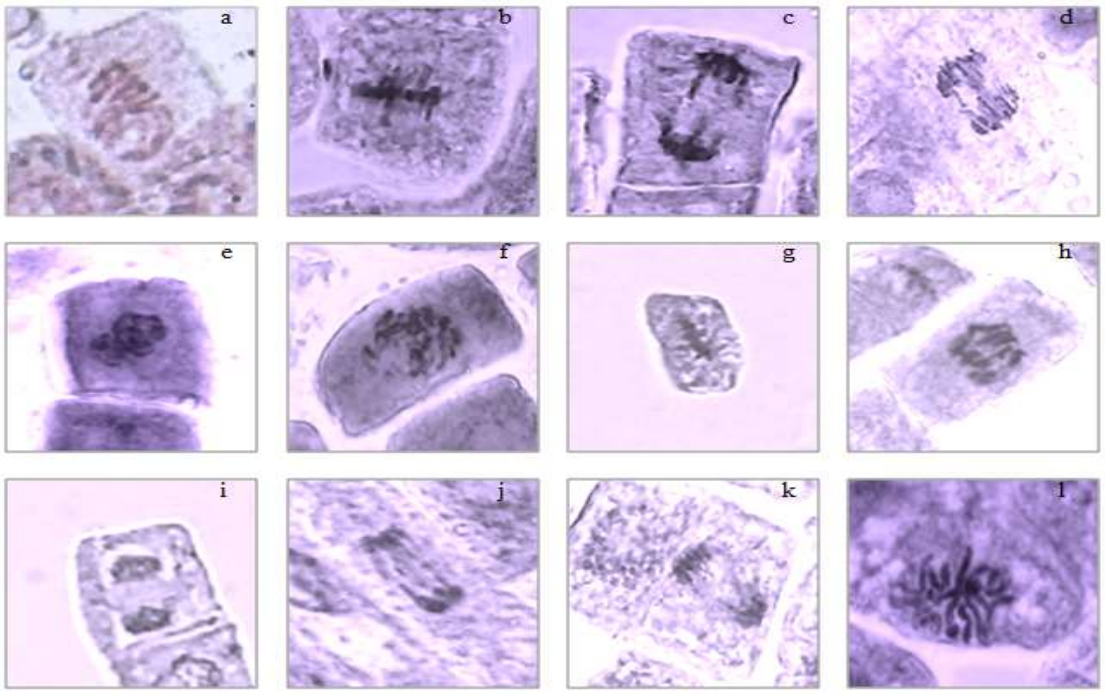


Figure 1 Chromosomal aberrarions observed in *Pisum sativum* root tips treated with crude xanthone extracts of *Swertia* species a-condensed prophase, **b-** precocious arms at metaphase, **c-** broken bridge, **d-** multiple broken bridge, **e-** sticky anaphase, **f-** chromosome fragments, **g-** diagonal metaphase, **h-** anaphase bridge, **i-** sticky anaphase, **j-** laggard, **k-** unequal distribution and **l-** C-mitosis.

Lower MI (2.78%) was recorded in *S. densifolia* 5 % concentrations while highest MI (7.73%) was observed in 1% *S. lawii* xanthone treatment. Exposure of *Swertia* xanthone extract inhibited the mitotic cell division at higher concentration when compared to the mitotic index of control group (Graph 1). *Swertia* crude extract exerted a genotoxic effect at 5 % concentration more pronouncedly.

Chromosomal abnormalities observed are condensed prophase, metaphase precocious arm, metaphase stickiness, chromosomal fragments, diagonal metaphase, anaphase bridges (multiple bridge, double bridge and broken bridge), stickiness in anaphase, laggards and unequal distribution of chromosomes. The types of abnormalities in all the treated samples were prophase condensation, metaphase stickiness and anaphase Bridge (Graph 2).

The highest degrees of chromosomal anomalies were induced by 5% *Swertia densifolia* xanthone treatment (59.09%). Types of abnormalities observed more prominently were anaphase Bridge (46.43%) in 5% *S. minor* concentration, prophase condensation and metaphase stickiness (30.77%) in 5% *S. densifolia* concentration, anaphase stickiness (23.07%) in 3% *S. lawii* concentration and precocious arms (17.86%) in 5% *S. minor* concentration.

Mitotic index is used as an indicator of cell proliferation biomarkers which measures the proportion of cells in the mitotic phase of the cell cycle. Hence, the decrease in the mitotic index in *P. sativum* somatic cells could be interpreted as retardation in mitosis or cellular death. Celik[4] studied extracts of *Plantago lanceolata* L. and their results showed that aqueous extracts reduced mitotic index and induces chromosome aberrations in treatment groups compared to controls. Similar results were obtained by Abraham and Nair in *vicia faba*.[5] Our results are also in accordance with these authors. The depression of mitotic index reflects negative impacts on somatic growth at initial stages as a primary effect in the development of plant.[6] The reduction in mitotic activity may result from a blocking of G1 stage suppressing DNA synthesis.[7] The lowering of Mitotic Index might be consequence of the inhibition of DNA synthesis at S-phase[8] and this inhibition could be due to blocking in G2 preventing the cell from entering mitosis.[9]

The abnormalities of chromosomes could be due to the blockage of DNA synthesis or inhibition of spindle formation, thereby causing structural chromosomal changes. Rehab and Salem,[10] tested genotoxicity of synthesized butenolide in *Allium cepa.* The bioassay test showed occurrence of sticky metaphase and anaphase bridges with decrease in MI. In all the studied concentrations anaphase bridge and sticky metaphase are most common compared to other types of abnormalities. Patil and Mendhulkar,[11] observed the cytomictic cells in *Desmodium tortuosum***.** Siddiqui,[12] evaluated the leaf extracts from four medicinal plants *viz., Azadirachta indica*, *Tectona grandis, Dalbergia sissoo* and *Eucalyptus tereticornis* using *Pisum sativum.* He observed abnormalities like c-mitosis, laggard, bridges, stickiness, precocious separation, vagrant and fragments with reduced mitotic index in a dose-dependent manner.

Table 2: Chromosomal aberrations in *Pisum sativum* root tips treated with crude xanthone extracts of three *Swertia* species.

SNo	*Swertia* Species	Xanthone Conc. (in %)	Total cells screened	Prophase		Metaphase								Anaphase								Total aberrent cells	Total % Abnorm.
				Condensation		Bridge		Stickiness		Precocious arms		Diagonal Meta.		Bridge		Stickiness		laggard		Unequal distribution			
				No	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No	%		
1	**Control**	-	782	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	***S. densifolia***	1	651	1	12.5	-	-	2	25	1	12.5	-	-	3	37.5	-	-	-	-	1	12.5	8	16.66
		3	698	2	28.57	-	-	2	28.57	1	14.29	-	-	2	28.57	-	-	-	-	-	-	7	19.44
		5	790	4	30.77	-	-	4	30.77	-	-	-	-	5	38.46	-	-	-	-	-	-	13	59.09
3	***S. lawii***	1	698	1	20	-	-	1	20	-	-	-	-	1	20	1	20	-	-	1	20	5	9.25
		3	790	2	15.38	-	-	2	15.38	1	7.69	-	-	4	30.77	3	23.07	-	-	1	7.69	13	26.53
		5	904	3	20	1	6.67	3	20	3	13.13	1	6.67	3	20	1	6.66	-	-	1	6.67	15	32.60
4	***S. minor***	1	784	1	7.14	1	7.14	3	21.43	2	14.29	1	7.14	3	21.43	2	14.29	1	7.14	-	-	14	23.72
		3	993	2	10	1	5	2	10	4	20	2	10	6	30	1	5	1	5	1	5	20	27.02
		5	975	2	7.14	1	3.57	2	10.71	5	17.86	1	3.57	13	46.43	2	7.14	2	7.14	1	3.57	28	41.17

Chromosome bridges may be due to the chromosomal stickiness and subsequent failure of free anaphase separation or may be attributed to an unequal translocation or inversion of chromosome segment.[13] The bridges, noticed in the cells are probably formed by breakage and fusion of chromosomes and chromatids.[14] The chromosomal fragments are more common in metaphase and anaphase. Chromosome stickiness reflects highly toxic effects, usually of an irreversible type probably leading to death. According to Ahmet and Grant,[15] stickiness of chromosomes might be resulted due to increased chromosome contraction and condensation or possibly from the depolymerisation of DNA. Babich[16] reported that metaphases with sticky chromosomes lose their normal appearance and appear to have a sticky "surface" which causes chromosome agglomeration, possibly due to effects on chromatin and chromosome organization. The chromosomal fragments also result from multiple breakages of the chromosome in which there is a loss of chromosome integrity.[17] Laggards may be attributed to hindrance of the prometaphase movement accompanied by the adhesion of the centromeres of one or more chromosomes to the inner surface of the plasma membrane and movement of the others towards the equatorial plate leading to the appearance of such lagging chromosomes.[18]

Due to the reduced number of dividing cells, it can be postulated that *Swertia* xanthone extract might exhibit a negative effect on cell division and possibly be involved in blocking the DNA or protein synthesis required for the normal cell division process.

CONCLUSION

The finding in present study unearthed that the high concentration of *Swertia* xanthone extract treatment induces gradual decrease in mitotic index and also induces chromosomal abnormalities. It is clearly evident that the *Swertia* xanthone extract have potential to induce cytotoxic and genotoxic effect. All the three *Swertia* species proved to have cytotoxic and genotoxic impact. *S. minor* showed maximum types of abnormalities compared to other two studied species. It may be possible to control weeds, pests and crop disease using novel agrochemicals based on natural products. The study encourages the use of botanical extracts for the biological control instead of synthetic chemicals. Many workers have proved that root tip assay is useful in initial screening for new plant extracts to validate their use as cytotoxic agents against malignant cells. This will greatly help in exploring new plant extracts as drugs for cancer treatment.

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