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Possible active molecule from Wrightia tinctoria and its effect on chromosome and nuclei and scope for Psoriasis

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Abstract

The plant Wrightia tinctoria is used widely in the treatment of psoriasis from AYUSH stream of medicine in India. Although a few clinical and laboratory experiments have been conducted but still the therapeutic value of Wrightia tinctoria is not clear and credible. We have taken the present study to understand the mechanism of action of Wrightia tinctoria on nucleus and chromosomes of the cells and how different stages of nuclear and chromosomal divisions are altered. The findings clearly show that the plant possess 'some activity' on nuclear and chromosomal division, does not possess cytotoxicity, does not interfere with any of the plant hormones and the probable bioactive molecule being Indirubin. Details are presented in the paper.

Keywords: Allium sativum, Indirubin, mitosis, Wrightia, chromosome aberration, plant hormones.

Introduction

Innumerable scientific studies done in the past have although shown that the plant Wrightia tinctoria possesses 'some' medicinal effect for psoriasis [1, 2, 3] but still how and what in the above plant act at cellular level beg scientific answer.

Indian traditional healing practice in the recent times with the advent of the research work of Dr. JR Krishnamoorthy has positioned the plant- Wrightia tinctoria as one of the important medicine for the treatment of Psoriasis but still an undeniable scientific credence to the above therapeutic claim is lacking although the above treatment option is widely followed across the globe. [4,5]

Several phyto- active constituents having cytotoxic activity mediated via DNA modification or annulling its function has been reported earlier.[6] Whether the above plant also may have similar activity and therefore its role in the treatment of psoriasis has been arrived as a consequence of the above 'trial and error' treatment practice is not clear.

If such possibility is bee extrapolated then such therapy on prolonged duration may likely to produce many harmful effects than any great treatment benefit.

In the present study we have tried to answer the question of whether the phyto-actives of Wrightia tinctoria interfere in the mitosis stage and if so, at what

stage including whether such intervention alter chromosome and nuclei.

We have also made earnest attempt to identify the possible candidate phyto-active constituent (s) of Wrightia tinctoria on cell cycle.

In order to establish the possible mechanism of action of the phyto-active constituent (s) outside of the cytotoxicity, we have studied the interference of the above phyto-actives on plant hormones such as auxin, gibberlin and cytokinin.

The findings clearly reveals that neither Wrightia tinctoria nor the phyto-active constituent- Indirubin has any cytotoxic effect or does interferes in plant hormones suggesting the great therapeutic scope of Wrightia tinctoria in the treatment of psoriasis. Details are discussed in the article.

Materials and methods

Garlic root elongation assay: Healthy Allium sativum (garlic) underground stem was procured. Weight and size matched cloves of garlic were separated individually and used for the study. Each garlic clove was positioned in such a way so as to touch the proximal end of the clove over the surface of water. The entire set up was incubated in dark environment for 10days with regular observation.[7]

Wrightia tinctoria treatment: Alcoholic extract of Wrightia tinctoria was prepared by keeping the ratio of solvent: solute at 1:10. The total extraction was dried to evaporate the solvent and the extract thus obtained was used for the study. 0.1µg/ml, 0.2µg/ml, 0.3µg/ml of the above extract was used for the study. The root growth; the number of roots formed, the length of the root, thickness and the time taken for root formation under various treatment conditions were studied and compared with the untreated control.

Treatment with Indirubin: Indirubin was extracted from Wrightia tinctoria using a standard protocol and the characteristics of Indirubin was confirmed both by TLC and spectrophotometry. In brief the shade dried leaves of Wrightia tinctoria was treated with chloroform for 48 hours and then it was filtered, the filtrate was dried to evaporate the solvent and thus obtained extract was subjected to TLC and accordingly Indirubin separated in the silica plate was confirmed by Spectrophotometry.[11]

For the present study, indirubin procured from elsewhere was used. Indirubin at 0.1µg/ml, 0.2µg/ml, 0.3µg/ml was used for the study.

The root growth; the number of roots formed, the length of the root, thickness and the time taken for root growth was studied.

Examination of root tip for various stages of mitosis: The root tip just above the root cap region (after removal of root cap) was selected, cut and then placed over a glass slide. Then the sample was immersed in a fixative solution (Carroy's fixative) which is composed of G- acetic acid and ethanol (1:3) for 30 minutes. After 30 minutes of incubation, the sample was again treated with maceration fluid (Ethanol: Hcl) and after 10 minutes of treatment, the sample was treated with 1% Hematoxylin solution and then gently the sample was mashed and then observed under a microscope.

Various mitotic events such as interphase, prophase, anaphase, metaphase and telophase in the root treated with Wrightia extract or Indirubin or Alprazolam were examined microscopically and compared with the untreated control. Alprazolam was used as a positive control for studying the alterations at chromosomal and nuclear level.

Effect of Wrightia tinctoria and Indirubin on plant hormones - garlic root elongation assay: Auxin, gibberlin and cytokinin were the three plant hormones used for the present study. All the above three hormones were tested at 3 separate concentration such as 0.01, 0.02 and 0.03 µg/ml respectively. The root elongation assay protocol was followed as described earlier. After the addition of hormones along with Wrightia tinctoria or indirubin, the root elongation, number of roots formed per clove, the length, thickness and the time were correlated between treatment and control. The above study was planned to understand whether Wrightia tinctoria or Indirubin alters the root elongation and associated features by affecting the plant hormone or by affecting the chromosome or nuclei.

Result

Wrightia tinctoria treatment has significantly reduced the length of the root and the reduction in the length of the root was directly proportional to concentration of the Wrightia tinctoria. A concordant pattern in the above phenomenon was observed between day 3 & day 6. The number of roots formed in the cloves treated with Wrightia tinctoria treatment was significantly lower when compared to the control. However, the thickness of the roots was higher in the case of treatment when compared to control.

Indirubin has significantly reduced the formation of roots and the length in a concentration dependent manner indicating the superiority of Indirubin over Wrightia tinctoria in altering root formation in Allium sativum. Table-1 & image 1

Table 1: Garlic root elongation assay

Treatment	Conc. µg/ml	Parameter- Day 3			Parameter- Day 6		
		Length in cm	Number	Thickness in mm	Length in cm	Number	Thickness in mm
Wrightia tinctoria	0.1	X= 1.5 ±0.3	X= 11	0.5	X= 2.3 ±0.3	X= 13	0.8
	0.2	X= 1.3 ±0.2	X= 9	0.6	X= 2.1 ±0.2	X= 9	0.8
	0.3	X= 1.1 ±0.3	X= 8	0.6	X= 2.0 ±0.3	X= 12	0.6
Indirubin	0.1	X= 0.8 ±0.2	X= 10	0.9	X= 1.5 ±0.2	X= 10	1.4
	0.2	X= 0.5 ±0.1	X= 7	1.0	X= 1.0 ±0.1	X= 8	1.8
	0.3	X= 0.2 ±0.1	X= 5	1.1	X= 0.4 ±0.1	X= 7	2.1
Control	-	X= 1.8 ±0.3	X= 15	0.3	X= 3.6 ±0.3	X= 16	1.3

Image1

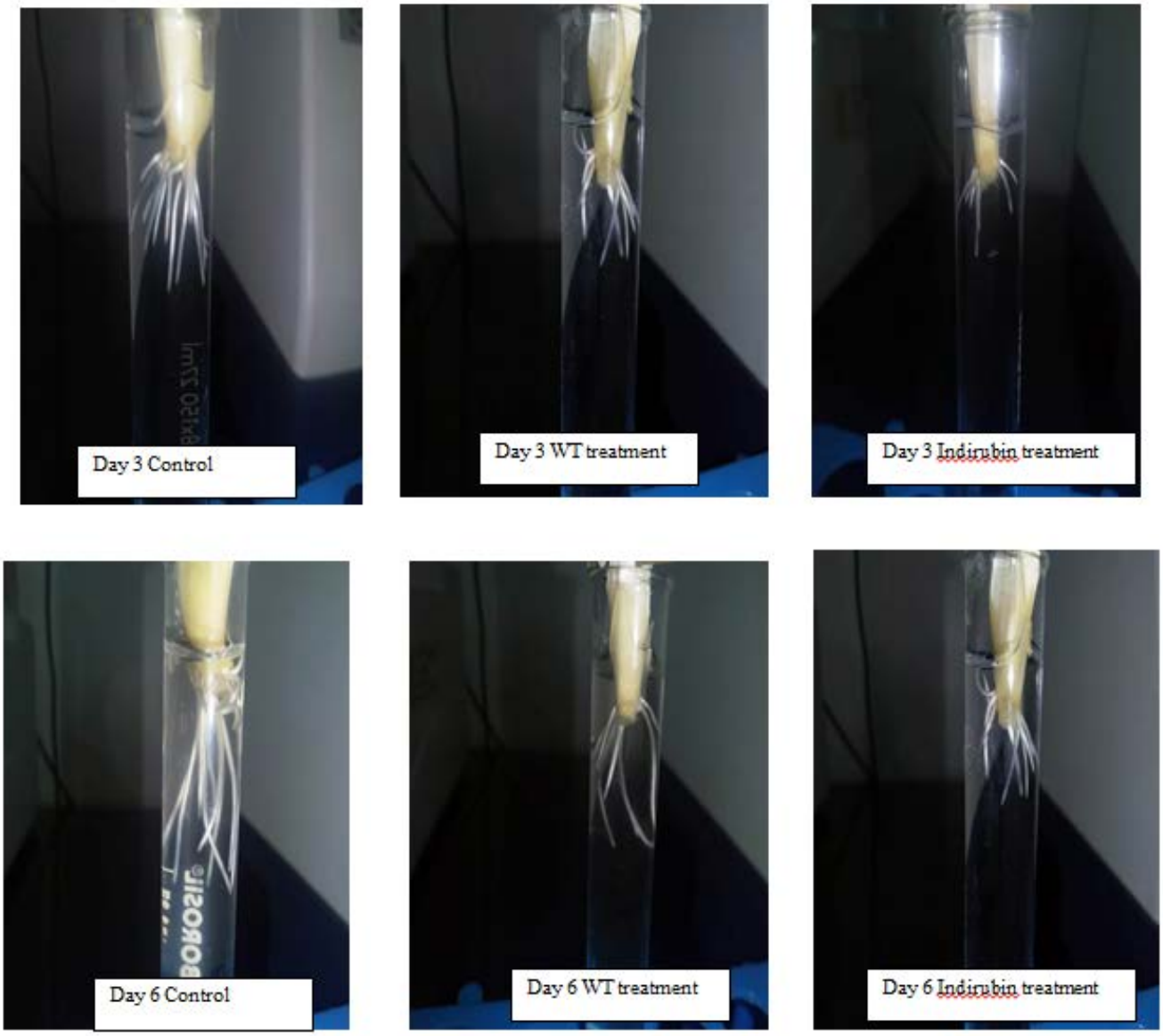

Table-2

Treatment	Conc.	Duration in days/ + indicates the presence									
		3					6				
		I	P	M	A	T	I	P	M	A	T
Wrightia tinctoria	0.1	+++	+++	++	-	-	+++	++	++	-	-
	0.2	+++	+++	++	-	-	+++	++	++	-	-
	0.3	+++	+++	+	-	-	+++	+	+	-	-
Indirubin	0.1	+++	++	-	-	-	+++	++	+	-	-
	0.2	+++	++	-	-	-	+++	++	+	-	-
	0.3	+++	+	-	-	-	+++	+	-	-	-

Control		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Effect on chromosome and nuclei

Neither Wrightia tinctoria nor Indirubin at 0.3% has induced any alterations at chromosomal or nuclear levels, whereas 0.05% of alprazolam has caused significant alterations at both chromosomal and nuclear level. Table -3 & Fig 1 -13

Table 3

Test	Conc.in µg/ml	Chromosomal and Nuclear alteration/Present (P) or absent (A)									
		VAP	IrrMB	SM	MBA	SBLC	LCA	IrrM	DBLC	LCFM	SMMN
WT	0.3	P	A	P	A	A	A	P	A	A	A
IR	0.3	A	A	P	A	A	A	A	A	A	A
ALP	0.05	P	P	P	P	P	P	P	P	P	P

VAP- Vacuolated nucleus at prophase , IrrMB- irregular metaphase and binucleated cells, SM- Sticky Metaphase, MBA- multibridge anaphase, SBLC- Single bridge lagging chromosome, LCA- lagging chromosome at anaphase, IrrM- irregular metaphase, DBLC- Double bridge with lagging chromosome, LCFM- Lagging chromosome with fragment at metaphase, SMMN- Sticky metaphase with micronuclei WT- Wrightia tinctoria extract, IR- Indirubin, ALP- Alprazolam

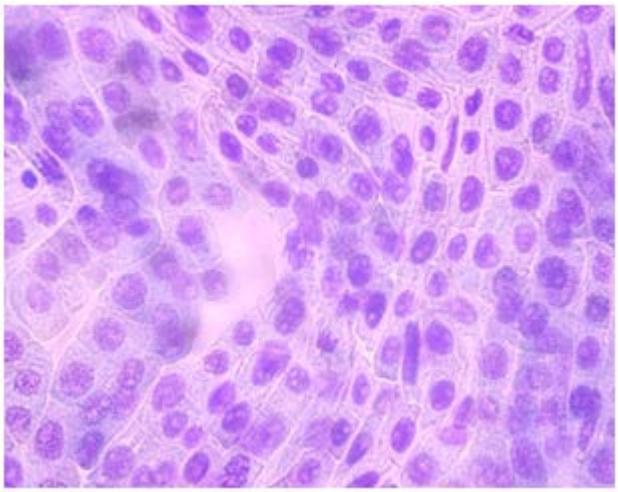

Fig. 1: Vacuolated Prophase

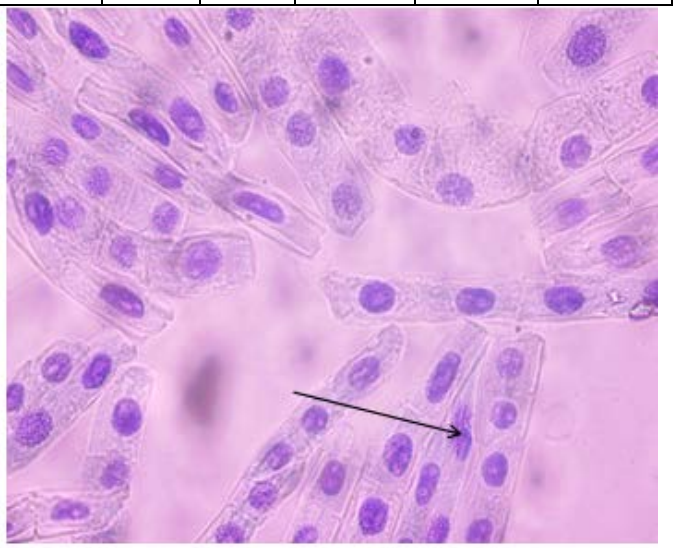

Fig. 2: Irregular metaphase & binucleated cells

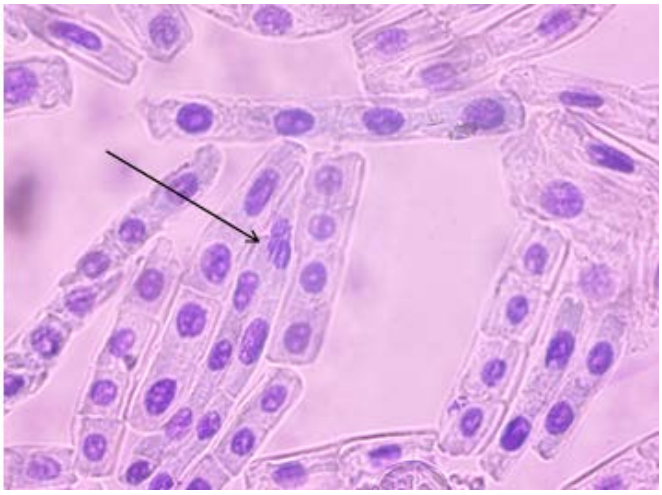

Fig. 3: Sticky metaphase

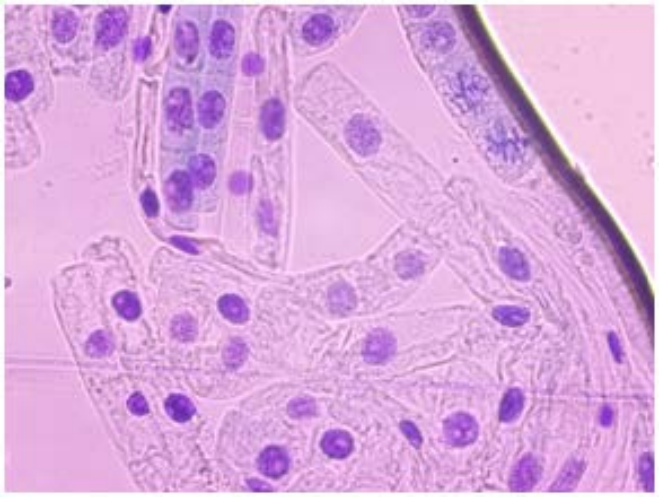

Fig. 4: Multi bridge Anaphase

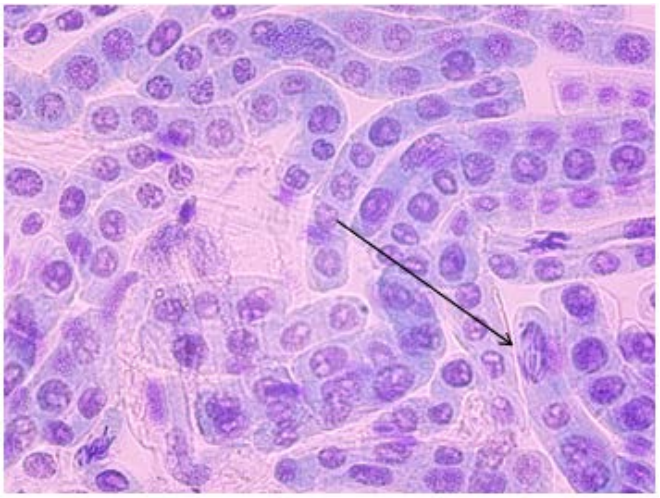

Fig. 5: Single bridge lagging chromosome

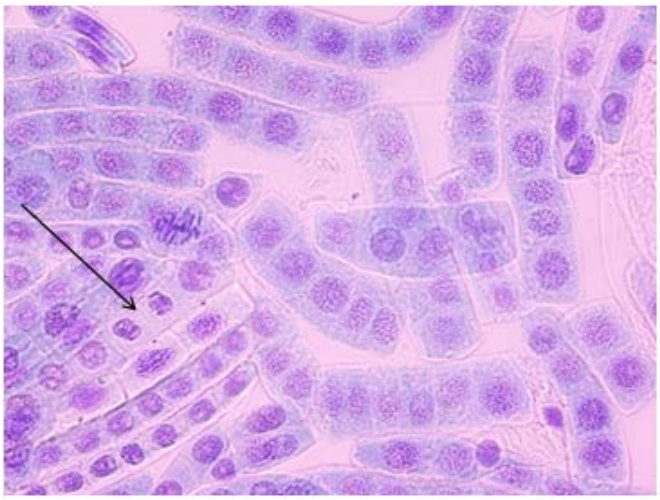

Fig. 6: Lagging chromosome at anaphase

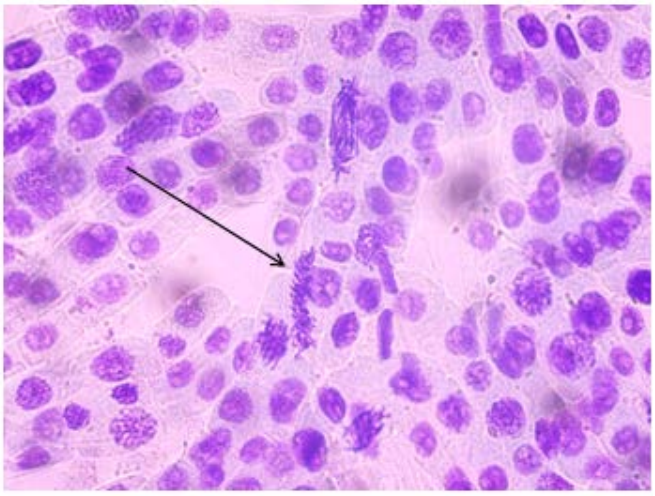

Fig. 7: Irregular metaphase

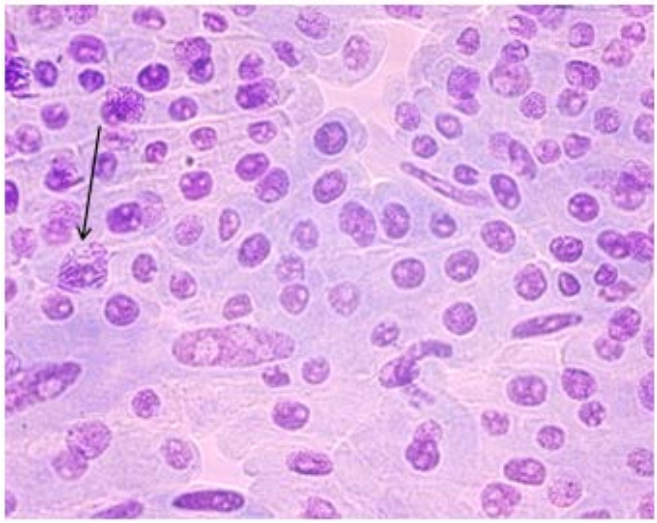

Fig. 8: Double bridge with lagging chromosome

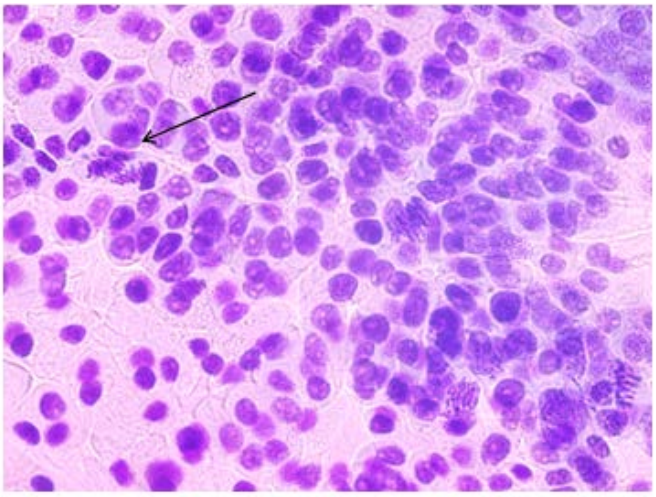

Fig. 9: Lagging chromosome with fragment at metaphase

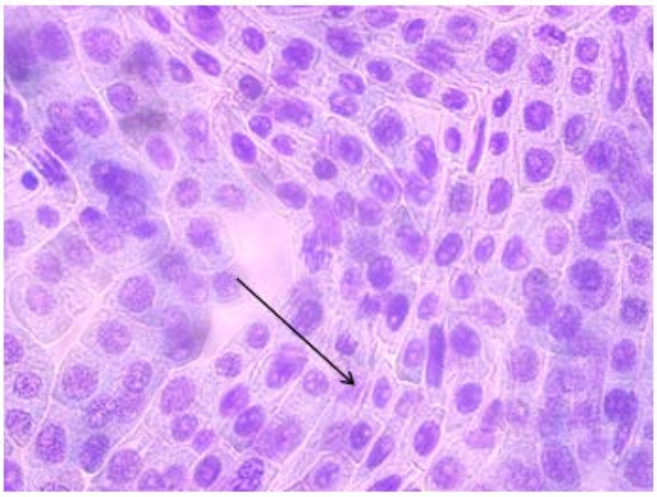

Fig. 10: Sticky metaphase with micronuclei

Effect of test compounds on plant hormones

Both Wrightia tinctoria and Indirubin at 0.3 µg/ml respectively has significantly reduced the elongation of roots, formation of number of roots and the thickness despite the apical root region was treated with 3 plant hormones such as auxin, gibberlin and cytokinin along with the above test compounds.

The treatment of garlic clove in hormone supplemented set up has affected the root length, number of roots formed and thickness. The above findings showed statistically comparable concordance between day 3 and day 6 of the experiment.

Table 4

Conc. µg/ml	Parameter- Day 3			Parameter- Day 6		
	Length in cm	Number	Thickness	Length in cm	Number	Thickness in mm
WT (0.3)	X= 1.1 ±0.3	X= 8	0.6	X= 2.0 ±0.3	X= 12	0.6
WT+ Auxin (0.3+0.03)	X= 2.6 ±0.2	X= 11	0.4	X= 3.2 ±0.8	X= 14	0.6
WT+ Gibberlin (0.3+0.03)	X= 2.4 ±0.2	X= 12	0.4	X= 3.7 ±0.5	X= 13	0.5
WT+ Cytokinin (0.3+0.03)	X= 2.9 ±0.4	X= 13	0.4	X= 3.5 ±0.6	X= 14	0.6
IR (0.3)	X= 0.2 ±0.1	X= 5	1.1	X= 0.4 ±0.1	X= 7	2.1
IR+Auxin (0.3+0.03)	X= 0.5 ±0.2	X= 7	1.0	X= 1.1 ±0.2	X= 9	1.2
IR+Giberllin (0.3+0.03)	X= 1.1 ±0.3	X= 9	0.8	X= 2.0 ±0.2	X= 12	1.1
IR+ Cytokinin (0.3+0.03)	X= 2.1 ±0.2	X= 7	0.6	X= 3.0 ±0.3	X= 11	1.2
Auxin (0.03)	X= 3.2 ±0.5	X= 18	0.5	X= 4.6 ±0.4	X= 20	0.8
Gibberlin (0.03)	X= 3.5 ±0.4	X= 17	0.6	X= 5.1 ±0.1	X= 19	0.9
Cytokinin (0.03)	X= 4.1 ±0.2	X= 15	0.4	X= 6.1 ±0.1	X= 18	0.7
Control	X= 1.8 ±0.3	X= 15	0.3	X= 3.6 ±0.3	X= 16	1.3

Discussion

Wrightia tinctoria has significantly reduced the root formation and elongation in Allium sativum when compared to the untreated control. The Wrightia tinctoria treatment has caused thickness in the root when compared to control.

We initially presumed that Wrightia tinctoria may affect the cell division as an 'in consequential cytotoxicity' and thereby hinders the root formation and elongation.

To understand the effect of Wrightia tinctoria on chromosome and nuclei of Allium sativum root, we have examined the treated root cells for various anomalies at chromosome and nuclei level.

To gain confidence and clarity, we have used alprazolam, a known allopathic preparation as positive control.[8] Interestingly we observed that Wrightia tinctoria did not cause any alterations in chromosome or nuclei whereas, alprazolam has caused various cytogenetic aberrations such as vacuolated nucleus at prophase, irregular metaphase and binucleated cells, sticky metaphase, multibridge anaphase, single bridge lagging chromosome, lagging chromosome at anaphase, irregular metaphase, double bridge with lagging chromosome, lagging chromosome with fragment at metaphase and sticky metaphase with micronuclei. Therefore we hypothesize that Wrightia tinctoria may not possess any cytotoxic effect and its effect on mitosis may be therapeutic in nature.

To understand the possible candidate phyto-constituent(s) that are responsible for the inhibition of mitosis, we have reverse engineered the research work done in the past and arrived that Indirubin[9, 10,11] might be the possible phyto-active molecule in Wrightia tinctoria for the above effect.

We have established the presence and relative concentration of Indirubin in the extract of Wrightia tinctoria that we have used in the study by the conventional method and UV spectroscopy.

After confirmation of Indirubin, we have evaluated the effect of Indirubin on mitosis and whether such an effect really alters the chromosome and nuclei, resulting in the manifestation of various cytogenetic anomalies.

Surprisingly Indirubin did not produce any alterations at chromosomal or nuclear level up to a concentration of 0.3µg/ml.

Inhibition of mitosis without causing any abnormalities at the genetic level raised our curiosity further to postulate whether Wrightia tinctoria and Indirubin may be affecting the plant hormones such as auxin, gibberlin and cytokinin and thereby retards the root formation.

In order to validate the above postulate, we have studied the effect of Wrightia tinctoria and Indirubin on root elongation in presence of the above hormones. The above hormones have significantly increased the root elongation as expected when compared to the untreated control but in presence of either Wrightia tinctoria or Indirubin the root formation and elongation got limited significantly. Whether Wrightia tinctoria or Indirubin inactivate the hormones and thus countermand root formation and elongation we have co-incubated Wrightia tinctoria or Indirubin with the hormones individually and kept for 5 days to study the UV spectroscopic profile of the mixture.

UV spectroscopy has revealed clearly that the spectroscopic profile of the hormone+ Wrightia tinctoria, hormone +Indirubin mixture remains stable and unaltered from beginning to end of 5 day incubation.

The above findings strongly suggest that Wrightia tinctoria or Indirubin does not seem to affect any of the 3 hormones. Further Wrightia tinctoria and Indirubin does not seem to affect the viability of apical root meristem as revealed by live cell exclusion assay.

Although our investigation has revealed the effect of Wrightia tinctoria and Indirubin on mitosis in non-

cytotoxic manner, the exact mechanism of action seems far from near to us.

Neither hormone inactivation nor the loss of meristematic property of the root apical cells due to Wrightia tinctoria and Indirubin are responsible, however Wrightia tinctoria and Indirubin effectively arrest the root elongation.

Our present investigation and the availability of various scientific findings on Wrightia tinctoria and Indirubin for the treatment of Psoriasis suggest that the 'safe participation' of Wrightia tinctoria and Indirubin in abrogating mitosis at cellular level may be the reason why the above plant is useful in psoriasis.

Although several more miles we have to travel in research to undoubtedly establish all subtle aspects of Wrightia tinctoria; the active constituent(s), concentration, mechanism of action, target site, safety profile, pharmacokinetic details, drug- drug interactions etc., but still our investigation clearly suggests the therapeutic scope of Wrightia tinctoria for Psoriasis strongly. Further our study has also indicated the therapeutic effect of Wrightia tinctoria and associated safety profile.

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