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Research Article

Anti-mitotic Activity of Methanolic Extract of *Thevetia peruviana* fruits

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Abstract

Thevetia peruviana plant, a member of the Apocynaceae family, has a long history in traditional medicine for treating various ailments such as amenorrhea, malaria, jaundice, hemorrhoids, constipation, headaches, and skin issues. It contains significant amounts of cardiac glycosides in both its roots and seeds, which are known for their cytotoxic effects similar to digoxin. Given its potential toxicity and widespread use, current research is focused on assessing the antimitotic properties of the methanolic extract derived from the fruits of *Thevetia peruviana*. In the pursuit of potential pharmaceutical findings from plants, the antimitotic properties of the extracts were investigated through the *Allium cepa* assay. The findings indicated a noteworthy decrease in the mitotic index of root tips compared to the control, with the degree of mitotic suppression escalating alongside the concentration of the extracts. Therefore, the study suggests that the methanol extract derived from T. peruviana fruit has notable inhibitory and mitodepressive impacts on cell division in *Allium cepa* L. meristems.

Keywords: *Thebetia peruviana*, Anti-mitotic Activity, *Allium cepa* assay, mitotic index

INTRODUCTION

Thebetia peruviana, a member of the Apocynaceae family, is commonly referred to as the yellow oleander, has a history of traditional medicinal use in managing a range of health conditions, including amenorrhea, malaria, jaundice, hemorrhoids, constipation, headaches, and various skin disorders[1]. The plant is reported to exhibit cytotoxic properties attributed to its significant cardiac glycoside content found in both its roots and seeds[2]. These glycosides produce effects akin to digoxin and primarily function by inhibiting the Na+-K+ ATPase enzyme within the cardiovascular system[3,4]. The plant has been reported cytotoxic due to the presence of a high amount of cardiac glycoside, with intermediate poisoning potentially manifesting as a first-degree atrioventricular (AV) block that may progress to AV dissociation[5]. Furthermore, the seeds are the most toxic part of the plant as it contains glycosides thevetin A, B and nerifolin, followed by the leaves and fruit[6]. Apart from that, the plant contains different secondary metabolites like alkaloids, flavonoids, steroids, terpenoids, tannins, saponins etc[7]. Due to the potential toxicity and popularity of the plant, the current research focuses on the evaluation of antimitotic activity of the methanolic extract of *Thevetia peruviana* fruits.

Chemotherapeutic compounds currently utilized in anti-cancer treatment owe much of their success to drugs that disrupt the typical course of mitosis. These drugs, predominantly microtubule-binding agents, work by impeding the activity of the mitotic spindle, thus arresting the cell cycle during mitosis and prompting apoptosis in cancerous cells[8]. Cancer is a multifactorial disease due to its being adaptive in that it can promote proliferation and invasion through an overactive cell cycle, which in turn causes cellular division, which is targeted by antimitotic drugs, which are highly effective chemotherapeutic medications[9].

To explore potential drug discoveries from plants, the antimitotic effects of the extracts were assessed using the *Allium cepa* assay. This root meristem assay is recognized as a dependable method for screening environmental mutagens and carcinogens[10,11]. The *Allium cepa* species, commonly referred to as onion, is a highly effective option for bioassays. It has been widely utilized to identify cytostatic, cytotoxic, and mutagenic properties in diverse compounds, including those derived from plants with potential anticancer effects.[10] Thus the current research aims to determine antimitotic activity of methanolic extract of *Thevetia peruviana* fruits.

MATERIAL AND METHODS

Collection and Authentication of plant material

Based on the literature survey, plant named *Thevetia peruviana* was selected for study. The aerial of *Thevetia peruviana* was collected in the month of March, 2023 from various areas of Haldia, Purba Medinipur Dist., West Bengal and Authenticated by Scientist-in-charge, Central National Herbarium, Botanical Survey of India, Kolkata, West Bengal, India. Plant Herbarium was prepared and preserved in the

department of pharmacognosy, Haldia Institute of pharmacy, Haldia, Purba Medinipur, West Bengal, India.

Preparation of extract

The selected plant products were extracted by cold maceration method[12]. 60.6 g of dried (fruit pulp) powder, of *Thevetia peruviana* was extracted with 300 ml methanol, then the product is filtered. A dry powder weighing 60 g was combined with 300 ml of 70% methanol in a mixing process, and the resulting mixture was carefully poured into a 500 ml volumetric flask. The solution was allowed to stand for a duration of 4 hours with continuous shaking to facilitate thorough dissolution. Following this, the concoction was set aside for a period of 3 days to ensure optimal extraction. Subsequently, the mixture underwent filtration using filter paper to separate the liquid filtrate from any remaining solid residue. And after that the extract was dried and kept for further use.

Anti-mitotic activity study

For mitotic studies five healthy medium sized onion bulbs weighing 25-28 g were taken with 2-3 cm root length. Their outermost brownish scaly skin and dead roots were scraped off near the disc. They were left in tubes filled with tap water to grow for 3 days so that their discs were submerged in water. They were left to grow at average temp. 24-25˚C and 46.6% avg. humidity and partial exposure to sunlight until their roots were about 1 cm long. The bulbs that developed uniform root were selected for further studies[13] (Fig. 5). These roots of onions were placed on beaker filled with phosphate buffer solution (Fig. 6), methotrexate 0.1 mg/mL (Fig. 1) and *Thevetia peruviana* fruit extracts (1.5 mg/mL) (Fig.2). *Thevetia peruviana* fruit extracts (1 mg/mL) (Fig. 3), *Thevetia peruviana* fruit extracts 0.5 mg/mL (Fig. 4), and *Thevetia peruviana* fruit extracts 0.1 mg/mL (Fig. 5) for 24 h. PBS was used for dilution as well as control and methotrexate used as a standard for study. Set up for all three plant extracts along with control (PBS Solution) and standard (methotrexate) was done separately in triplicate. For each bulb the Mitotic Index was determined.

Squash Preparation

The final 2-3 millimetres of the root meristems were trimmed and subjected to heating in a solution comprising Acetocarmine and N/10 HCl in a 9:1 ratio[14]. The watch glass holding the root tips was heated until the tips were soft and darkly stained. A tip was then taken and squashed in a drop of fresh acetocarmine on a clean slide after a cover slip was put. The slide was wrapped in 2 layers of filter paper and squashed by the application of direct vertical pressure of the thumb. The slides of mitosis thus prepared were scanned under the microscope at 10 X. Cells showing various stages of mitosis and non-dividing cells were counted. 40 cells per onion bulb were counted.

Mitotic Index (MI) formula

Mitotic Index (MI) % = No of dividing cells/ Total no of cells × 100

Statistical analysis

The data were expressed as the mean of three replicates with standard deviation. GraphPad Prism software was used for analysis of p value. One-way analysis of variance (ANOVA) was used to analyze the significant difference between controls, standard and methanolic extracts of *T. peruviana* fruits extracts. $P < 0.05$ was considered as significant.

Fig No 1: Root tip treated with Standard Solution of Methotrexate (0.1 mg/ml)

Fig No 2: Root tip treated with test solution (1.5 mg/ml)

Fig No 3: Root tip treated with test solution (1 mg/ml)

Fig No 4: Root tip treated with test solution (0.5mg/ml)

Fig No 5: Root tip treated with test solution (0.1 mg/ml)

Fig No 6: Root tip treated with control solution

RESULT AND DISCUSSION

Antimitotic assay

Microscopic observation: After staining, the root tip cells were observed under light microscope by using acetocarmine stain. The microscopic view of stained root tip cells at 10x is shown in Fig. 7. The results of effect of *T. peruviana* fruit extract with different concentration on mitotic index (MI) of *Allium cepa* root tip cells are given in Fig. 8.

Observation Table: The Mitotic Index (MI) is described as the proportion of cells within a population that are currently undergoing mitosis in relation to the overall number of cells in that population[13]. A heightened mitotic index signifies a greater number of cells undergoing division, and this is a crucial prognostic factor that predicts both overall survival and responsiveness to chemotherapy in the majority of cancer types. The Mitotic index depressant activity of the drug is shown on Table no. 1 from which it is evident that the extracts derived from *Thevetia peruviana* fruit demonstrate a notable decrease in the mitotic index of root tips compared to the control group. Additionally, the suppression of mitosis increases with higher concentrations of the extracts.

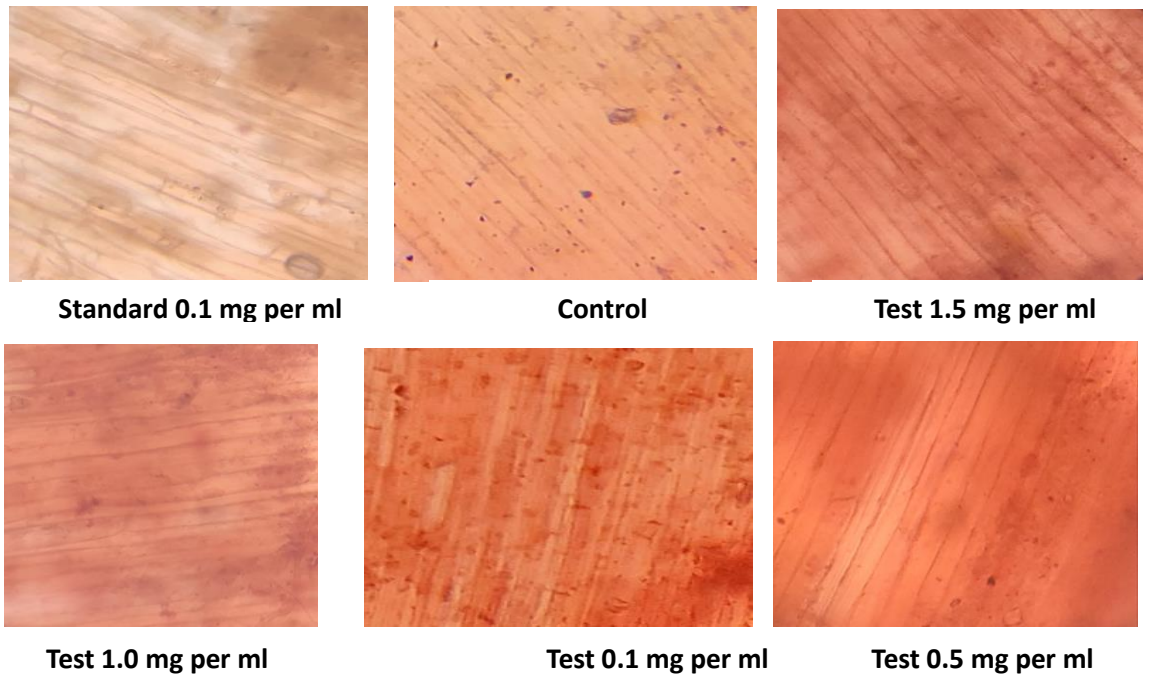

Figure 7: Microscopic observation of Squashed root tips of *Allium cepa*

Table 1: Mitotic Index calculation of *Allium cepa* root tip

SL No.	Groups	Experiment No	Total number of cells	No. of dividing cells	No. of non-dividing cells	Mitotic Index	Average Mitotic Index (MI)
1.	Control (Phosphate Buffer)	1.	40	25	15	62.5%	70.833±7.217
		2.	40	30	10	75%	
		3.	40	30	10	75%	
2.	Standard (Methotrexate) (0.1 mg/ml)	1.	40	3	37	7.5%	6.667±1.443
		2.	40	3	37	7.5%	
		3.	40	2	38	5%	
3.	Test (*Thevetia peruviana* extract 1.5 mg/ml)	1	40	3	37	7.5	8.33±1.443
		2	40	4	36	10	
		3	40	3	37	7.5	
4.	Test (*Thevetia peruviana* extract 1mg/ml)	1.	40	7	33	17.5%	17.500±2.500
		2.	40	8	32	20%	
		3.	40	6	34	15%	
5.	Test (*Thevetia peruviana* extract 0.5mg/ml)	1.	40	13	27	32.5%	28.33±3.819
		2.	40	11	29	27.5%	
		3.	40	10	30	25%	
6.	Test (*Thevetia peruviana* extract 0.1mg/ml)	1.	40	21	19	52.5%	50.00±2.500
		2.	40	19	21	47.5%	
		3.	40	20	20	50%	

All data expressed as mean values ± SD (n = 3) represented by error bars

Statistical analysis

Statistical analysis was conducted utilizing the GraphPad Prism software. The graph showed comparative analysis of mitotic index of phosphate buffer solution, methotrexate (0.1 mg/mL) and *Thevetia peruviana* fruit extracts 0.1 mg/mL, *Thevetia peruviana* fruit extracts 0.5 mg/mL, and *Thevetia peruviana* fruit extracts 1 mg/ml, *Thevetia peruviana* fruit extracts 1.5 mg/ml treated *Allium cepa* root tips. Extract of *T. peruviana* fruit extracts showed significant antimitotic activity, by decreasing rate of mitosis in comparison to Control. Methotrexate (0.1 mg/mL) was used as a standard and shows highest antimitotic activity.

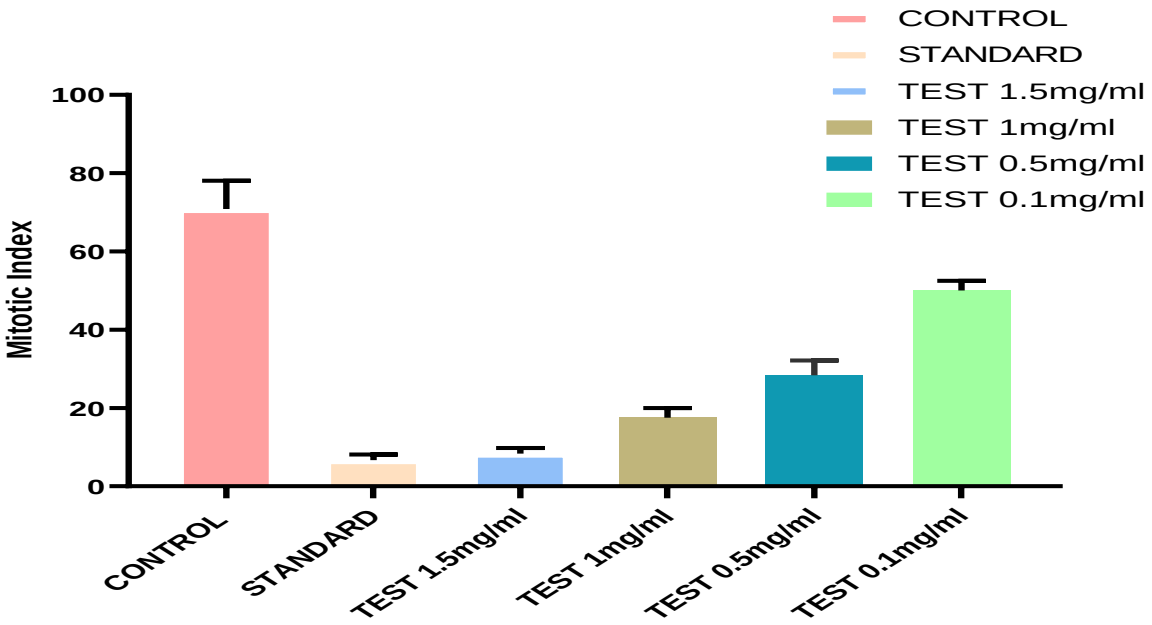

Figure 8: Mitotic index (MI) comparison between PBS, Methotrexate and *Thevetia peruviana* fruit extracts 0.1 mg/mL, *Thevetia peruviana* fruit extracts 0.5 mg/mL, and *Thevetia peruviana* fruit extracts 1 mg/mL, Thevetia peruviana fruit extracts 1.5mg/ml

CONCLUSION

The present study was an attempt to evaluate the antimitotic activity of methanol extract of *T. peruviana* fruit on *Allium cepa* L. root tip cells. The findings indicated that the methanolic extract from *T. peruviana* fruit led to a substantial decrease in the mitotic index of root tips compared to the control group. Moreover, the suppression of mitosis was observed to escalate with increasing concentrations of the extracts. Consequently, the study suggested that the methanolic extract of *T. peruviana* fruit possesses notable inhibitory and mitodepressive effects on cell division in *Allium cepa* L. meristems. Hence these plants may be useful for their anti-cancer properties. Further studies have to be conducted to obtain more detailed mechanism of action of plant material in view of its antimitotic activity. Moreover, additional in vivo research is imperative to demonstrate the complete potential of utilizing *T. peruviana* fruit extract in cancer therapy. In conclusion, the aforementioned discovery highlights the significance of *T. peruviana* fruit as a reservoir of bioactive compounds with potential anticancer properties. *T. peruviana* fruit extract, however, is poisonous. Therefore, additional toxicology research is needed to determine the safe dose, and additional investigation is required to determine the mechanism by which phytoconstituent is responsible for mentioned anti-mitotic activity.

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Conflict of Interest

None.

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