Journal of Advanced Scientific Research

Available online through https://sciensage.info

ISSN

0976-9595

Research Article

Destructive Effects of Mawa and Pan Masala on Somatic Cells of *Allium cepa* L.

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Received: 10-03-2025; Accepted: 12-03-2025; Published: 18-03-2025

https://doi.org/10.55218/JASR.2025160303

ABSTRACT

Mawa & Pan masala are Smokeless tobacco products consumed by people on a large scale. These materials consist of ingredients that cause carcinogenic effects. A comprehensive analysis was undertaken to elucidate the cytotoxic and genotoxic potential of the aqueous extracts obtained from these products. Three concentrations such as 10, 20 and 30% were prepared and assessed utilizing the *Allium cepa* model. The root tips were subsequently treated with different concentrations of extracts for 144 hours. The results revealed a significant decrease in the mitotic index, particularly in root tip cells exposed to 30% mawa extract at 20.53 and to 30% pan masala extract at 48.91%. The treated cells show different types of chromosomal aberrations like vacuolated cells, fragmented chromosomes, disturbed metaphase, laggards, binucleated cells, cells with damaged cell walls, disturbed telophase, etc. From the results, it can be concluded that smokeless tobacco products have cytotoxic and genotoxic effects. It causes abnormalities in cellular structure which may result in tissue or organ malformation. Care must be taken while consuming it.

Keywords: Mawa, Pan masala, Chromosomal aberrations, Cytotoxic, Genotoxic, Mitotic index.

INTRODUCTION

In the early 17th century European influences led to the introduction of tobacco in South Asia, primarily for the purpose of pipe smoking with possible additional use as a nasal snuff. In several countries of the Far East and Middle East, chewing tobacco is used widely. Epidemiological studies have revealed a significant global escalation in the oral consumption of smokeless tobacco products over the past few years. In many nations, such as India, eating tobacco is marketed as "gutka," which is made up of several components, such as areca nut, chewing tobacco, catechu and lime T. Askın C¸ *et al* (2001). In addition to showing secondary associations with many other kinds of malignancy, the oral use of wet smokeless tobacco products has been definitively associated with a higher risk of malignancies affecting the nasal cavities, oral cavity, lips, stomach and esophagus (Manashi Bagchi *et al*, 2009).

Globally tobacco is predominantly utilized for smoking purposes, whereas, in India its consumption is more diverse, encompassing both smoking and various smokeless forms. Notably India ranks 4th worldwide in terms of total tobacco consumption, with the majority being utilized for smoking & oral use while nasal use is relatively limited. A significant proportion of the total tobacco produced in the country is allocated towards various consumption formats. Approximately 48% is utilized in the form of chewing tobacco, 38% as bidis, & only 14% as cigarettes. Smokeless tobacco (SLT) products, which comprise a substantial segment of the market, contain numerous chemical substances. The present incidence of smokeless tobacco usage is 25% in Bangladesh and 27% in India, according to the "National Report of Global Adult Tobacco Survey". These nations offer 30 various kinds of smokeless items, such as zarda, which is made of dried and boiled tobacco leaf, lime, areca nut, additions, spices, and tannins (Kamal Niaz *et al*, 2017). Notably, between 30 and 40 percent of instances of cancer in India are oral cancer, and the most obvious cause of this disease is the extensive use of tobacco products, either through smoking or smokeless chewing (Ahmad Fazila*et al*, *2015*). Tobacco is the dried & cured leaves of *Nicotiana tobacum* L. belonging to the family Solanaceae. Mawa is made by combining processed tobacco, slacked lime, and tiny bits of areca nut. While pan masala, lime, catechu unidentified spices, flavoring agents and a dry powdered mixture of areca nut has become popularized in India as a chewing replacement, all the components are rubbed for a predetermined amount of time before being consumed.

In many experimental settings, areca nut, the main ingredient in pan masala, has been shown to have clastogenic, genetically toxic, and malignant potential (Gupta &Reyces 2003). Determining the effects of way of life, hereditary, and environmental variables on the stability of genomics in populations of humans is a growing global endeavor. Tobacco and betel quid chewing habits have increased globally as a result of fast globalization and shifting social attitudes.

The *Allium cepa* assay is a useful in vitro model for examining the cytotoxic and genotoxic effects of various compounds in this regard. Here, the roots can develop in close proximity to the experimental materials, allowing for the prediction of potential harm to the DNA and eukaryotic cells.

MATERIALS AND METHODS

Preparation Of The SLT Extract

The Smokeless tobacco products (Mawa & Pan Masala) were purchased from a local shop & a fine powder of them was prepared by using the grinder. Then 10 g of powder is taken to prepare 10% of the SLT extract. This powder was extracted by maceration in 100 mLD/W. The mixture was filtered with the help of Whatman No. 1 filter paper in a porcelain dish. This porcelain containing the mixture was kept in a water bath at 70°C in order to evaporate the D/W. After evaporation, the mixture was reconstituted by 100 mL D/W then once again the mixture was filtered & the extract was collected in a beaker which was labeled as 10%. Likewise, 10, 20, 30% extract for both the products (Mawa & Pan Masala) were prepared and stored in a bottle and kept at 4°C temperature for further use.

Plant Material Used

The onion bulbs of *A. cepa* L. were obtained from the local market of Bhiwandi. The bulbs were carefully descaled, sun-dried for 2 days and then scraped to remove existing roots, leaving the primordial root intact to facilitate the emergence of new roots. The bulbs were subsequently treated with varying concentrations (10, 20, 30%) of the Mawa & Pan Masala extracts, and placed on thermocol sheets positioned on trays filled with the respective extracts. Control used was distilled water. The onion bulbs were placed a room temperature to promote growth, with the test sample being replaced every day. After 144 hours, newly emerged roots were harvested, fixed in acetic alcohol, and subjected to hydrolysis and staining. The root tips were then squashed onto glass slides and examined under a light microscope at 45X magnification with a digital camera–equipped microscope (Exel Super TMC-220 Micron Optik) utilized to capture high-quality images of chromosome aberrations. Differences between the treatment and the control concentration of every extract were analyzed by mean of % of Mitotic index by using the following formula:

$$Mitotic\ Index = \frac{No.of\ cells\ undergoing\ mitosis}{Total\ No.of\ cells} \times 100$$

Chemical Analysis Of SLT Product

Determination of nor nicotine, total alkaloids and nicotine in tobacco present in mawa and pan masala powder is carried out. In 2.5 g of finely ground test material, 15 mL of barium hydroxide solution and 1 g of granular barium hydroxide were added. Until the test substance was completely soaked, the flask was swirled. 100 mL of benzene-chloroform solution was pipetted into the flask & agitated vigorously for 15 minutes using a shaking machine. Approximately 2 g of celite was added and the flask was again swirled until the filter aid was well dispersed. The two liquid phases were allowed to separate. Most of the hydrocarbon layer was transferred to an additional flask by filtering it with Whatman No. 1 filter paper. Two 125 mL conical flasks were filled with a 25 mL aliquot of the filter using a pipette. To get rid of any free ammonia that might be in the extract, a stream of air was run over the solution's surface in the first flask for 5 minutes. To the second container, 0.5 mL of acetic anhydride was added. One drop of crystal violet marker to each flask was added, and then 0.025N perchloric acid was used to titrate to a green endpoint. Another 25 mL portion of the filtrate should be acetylated if the nicotine level corresponds to or more than 24%. To get the comparable mark, 25 mL of acetic acid was added and titrated potentiometrically (Mammen Daniel, 1991).

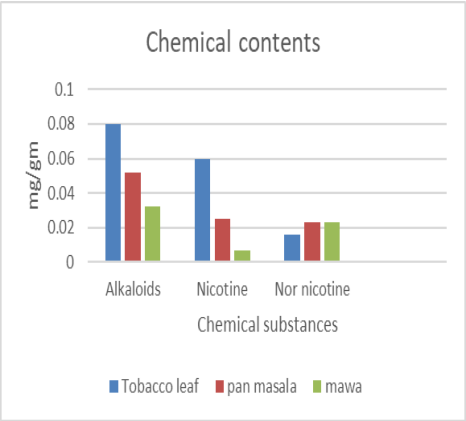
RESULTS AND DISCUSSION

When exposed to varying concentrations (10, 20, 30%) of mawa and pan masala resulted in a significant, dose-dependent inhibition of "the mitotic index in root tip cells of *A. cepa* L". Notably, all treatment groups exhibited a substantial reduction in mitotic index values examine with the control 57.57% (Table 1), with the lowest dose initiating this effect. The highest concentration of Mawa (30%) at 144 hours induced a pronounced decrease in mitotic index (20.53%), whereas the same concentration of pan masala resulted in a decrease to 48.91%.

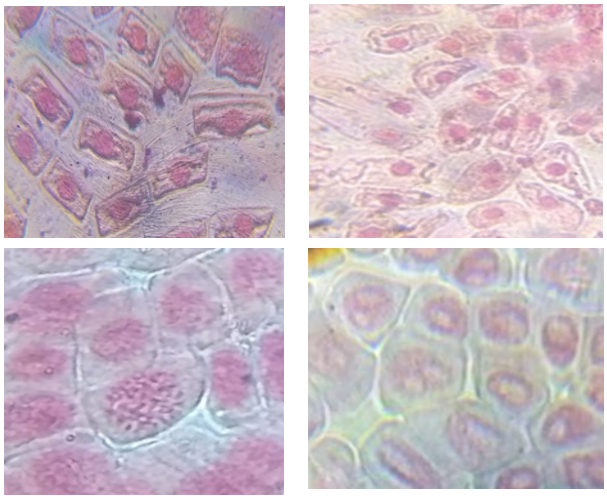
The data presented in plates 1, and 2 illustrate an ascending relationship between the percentage of chromosomal aberrations and the concentrations of the test compounds. Notably both the test compounds exhibited a dose-dependent increase in the percentage of chromosomal aberrations across all concentrations. The frequency of mitotic aberrations was remarkably elevated as the concentration of the test materials increased from 10 to 20 and 30%. The highest incidence of mitotic aberrations was observed in the root tip subjected to 30% of mawa *i.e.* 44.02% whereas 30% of pan masala could induce 68.75% at the same dose and duration of treatment.

Table 1: Mitotic index for mawa graph no 1: chemical analysis of substances and pan masala

% of extract	*Mawa (%)*	*Pan masala (%)*
control	57.57	57.57
10%	35.62	55.35
20%	25.5	50
30%	20.53	48.91



graph 1: Chemical analysis of SLT



Source: Damage cell wall shrunk cells fragmented chromosome bivacuolated cells

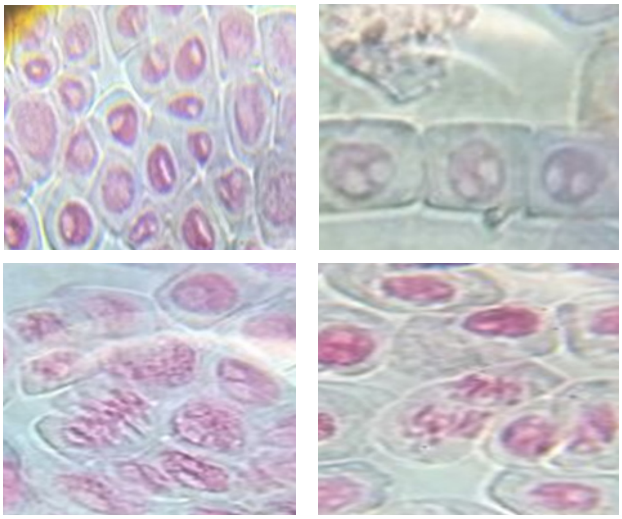
Plate no 1: Chromosomal aberrations observed in root tips treated with mawa extract

The most common abnormalities in the chromosome noted were vacuolated cells, bivaceoulated cells, fragmented chromosomes, disturbed metaphase, laggards, binucleated cells, a cell with a damaged cell wall, shrunk cells, scattered chromosomes, disturbed telophase, etc. In the root tip of *A. cepa* treated with mawa extract with different concentrations, the abnormalities noted were shown in plate 1.

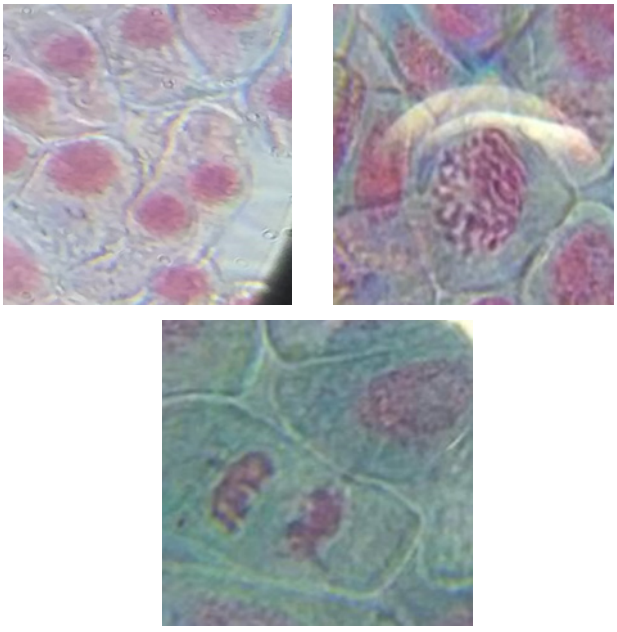
The destructive effect observed on the cells by Pan masala extracts was explained in plate 2 where vacuolated nucleus, binucleated cells, scattered chromosomes, laggard chromosome, and disturb telophase were clearly seen.

The quantity of total alkaloids, nicotine, and nicotine was estimated by using the chemical assay. The results show that the total amount of alkaloids present in the tobacco leaf powder is 0.08 mg/gm as compared to pm 0.032 mg/gm, & Mawa 0.0516 mg/gm (Graph 1). The amount of nicotine was calculated by using the standard formula which is found to be 0.06 mg/g in standard solution whereas in test solutions like mawa the amount of nicotine was 0.0076 mg/g after the same duration of extraction & in the pan masala 0.02596 mg/g. From the same acetylated aliquot, the amount of nor nicotine present in the standard tobacco leaf powder and test compound was calculated with the help of the formula. The amount of nor nicotine, in tobacco leaf powder was found to be 0.0164 mg/g, whereas it was 0.0236 & 0.0236 mg/g in Mawa & pan masala extract, respectively.

The toxic effects of carcinogenic substances present in mawa and pan masala were evaluated by analyzing the mitotic index, chromosomal aberrations and chemical constituents. The cytotoxicity of any substance can be based on its effect on the mitotic index. The increase or decrease in MI indicates a cytotoxic effect (Adgbite & Sanyaolu, 2009). The results unequivocally demonstrate that the Mawa & Pan masala extracts, across various concentrations, remarkably reduced the mitotic index, indicating a pronounced cytotoxic effect on *A. cepa* cells. Microscopic evaluation of squashed *A. cepa* L. root tip meristem cells revealed that Mawa & pan masala extracts induced several mitotic abnormalities when compared with control. Chromosomal aberrations are widely regarded as a critical cytogenetic parameter for assessing genotoxicity. The rise in amounts was correlated with a rise in mitotic defects. These all may be due to the presence of the alkaloid 'Nicotine' which brings about binucleate cell and chromosomal fragmentation. The alkaloid affects cells that undergo C-mitosis and exhibit chromosomes that divide lengthwise and remain attached only at the region of the centromere, as a result considerable shortening of chromosomes (Ali Irfan *et al.* 2012). Many researchers believe that defects like multipolarity and c-mitosis stickiness that result from the inhibition of spindle formation indicate significant pollutant toxicity (Lazareva *et al*., 2003). The clustering of chromosomes that results in aberrant chromosomal configurations is known as chromosomal stickiness. Chromosomal



Source: Vacuolated nucleus, bivacuolated nucleus, disturbed metaphase, disturbed telophase



Source: Binucleated cells, scattered chromosome, laggard chromosome

Plate no 2: Chromosomal aberrations observed in root tips treated with pan masala extract

stickiness and clumping can be induced by a combination of genetic and environmental factors. Various agents have been identified as causative factors contributing to chromosomal stickiness, highlighting the complexity of this phenomenon (Panneerselvam *et al.*, 2012). Chromosomal abnormalities were noted by (Nafea *et al.*, 2022) while studying the effect of heavy metals on the root tip of *Allium*.

CONCLUSION

It is concluded from the above study that both the SLT products contain high amounts of Nicotine and nor nicotine. These alkaloids have genotoxic and carcinogenic effects on the human body if consumed regularly. This leads to different health problems. The findings demonstrate that both mawa and pan masala has a chance to cause genotoxicity because they are both strong inducers of chromosomal abnormalities in *A. cepa* L. root tip cells. One of the serious health problems brought on by using SLT products is oral malignancy. There is an urgent need for communication, awareness, and knowledge regarding the harmful effects of pan masala and mawa due to their genetically toxic and carcinogenic characteristics.

ACKNOWLEDGMENT

The authors are thankful to the G. M. Momin Wome's College for providing laboratory facilities.

CONFLICT OF INTEREST

None

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HOW TO CITE THIS ARTICLE: Gupte A, Ansari NA. Destructive Effects of Mawa and Pan Masala on Somatic Cells of *Allium cepa* L. *J Adv Sci Res*. 2025;16(03): 15-18 **DOI:** 10.55218/JASR.2025160303