



Cytotoxic effect of *Acacia catechu* extract on the *Allium cepa* root system

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ABSTRACT

Evaluation of effect of different concentrations of catechu extract (*Acacia catechu*) on onion root system has been done. In the present study effect of catechu extract of different concentrations (0.02%, 0.04% , 0.06% , 0.08% , 0.1%) were compared with control treatment. . Results obtained indicated that the aqueous catechu extract of various concentrations showed a decrease in root length at higher concentration. Also, the various concentrations of the extracts had toxic effects on the cells, which caused general significant reduction in the mitotic index and increase in total abnormality percentage when compared with the control after 72hrs of incubation at room temperature. This study revealed that higher concentrations of catechu extract have affected the root tips of *Allium cepa* resulting in chromosomal aberrations. Further investigations are recommended to ascertain the mutagenic effect of the consumption of catechu on man.

Key words: Catechu extract, *Allium cepa*, Chromosomal aberrations

INTRODUCTION

Many of the drugs, which we use today, are based on folk remedies and subsequent ethno pharmacological studies. There are more than 100 drugs of known structure that are extracted from higher plants and used in allopathic medicine. More than hundred years old drugs like morphine, digitalis and atropine are the time honoured remedies.[1] Further, pharmaceutical preparations were discovered that were solid or aqueous, alcoholic or hydroalcoholic fluid extracts of soluble plant or animal constituents. During this period, the use of plants and

animals or their parts were abandoned for the more concentrated extracts. Plant extracts can be used for scientific testing, to find out which nutrients or chemicals are present in the plant. Plant extracts are also used in some beauty products (shampoos, soaps, perfumes), medicines, or food flavoring (like vanilla extract). Different pharmaceutical dosage forms or preparations were originally designed to extract and concentrate the active drug principles like alkaloids, glycosides and volatile oils primarily from plants and used for therapy.[2] The increasing use of medicinal herbs is a clear evidence of public interest in having alternatives to conventional medicine. However, despite the profound therapeutic advantages possessed by medicinal plants, some of their constituents have been found to be potentially toxic, mutagenic, carcinogenic and teratogenic.[3] Long-term use of herbs to treat or manage diseases can induce cellular damages and thus increase the side effects and potential toxicity of the medicinal plants, hence, the need to assess their potential toxicity.

Catechu (or Khair), a hot water extract of red heartwood of *Acacia catechu*, is a brown-coloured material with a bitter taste. It belongs to the family Leguminosae-mimosaceae.[4] It has some medicinal values and is abundantly grown in the region of South Asian countries. The solution of *Acacia catechu* is acidic in nature. *Acacia catechu* extracts contain from 13% to 33% catechin which is a polyphenolic compound and from 22% to 50% tannic acid, which is called catechu-tannic acid. Besides these, water-soluble extractive matter, gum, and mineral substances are also found to be present. Catechin and quercetin are polyphenolic compounds which also proved to increase the mechanical properties of the polymeric films. *Acacia* is a medicinal plant commonly used in Nigeria in the traditional management of cancer.[5] It been reported to have great medicinal value, with treatment of intestinal disorders, antioxidant, antiinflammatory, antiviral, antiparasitic, antibacterial, antimicrobial, antihepatotoxic, and antihelminthic activities.[6]

Toxicity of plant extracts

Medicinal herbs have been used since ancient times. However, the scientific study of their effects has flourished in the present age. Nevertheless, some of them can cause adverse effects or have the potential to interact with other medications. It is known that green plants in general are a primary source of antimutagens as well as natural toxic agents.[9] Recent investigations has revealed that many plants used as food or in traditional medicine have cytotoxic and genotoxic effects in vitro and in vivo assays. Many plants contain mutagenic and carcinogenic

substances which has been correlated with high rate of tumour formation in some human populations. *Glinus lotoides*, *Plumbago zeylanica*, *Rumex steudelii* and *Thymus schimperi* showed genotoxic effects in the earlier experiments.[10]

Catechu is POSSIBLY SAFE when taken orally and used appropriately Also catechu if used in larger amounts can cause liver problems, hypertension and even death.[11] This substance can also cause genetic defects in the course of time and can pass on to next generation. Hence it is necessary to check its effect at different concentrations on a medium that shows visible results. Since the most effective medium is onion root tip, *Allium cepa* assay is used.

Onion root tips

The plant's name comes from the Latin word unio, or annianus, and is associated with the Welsh onion, meaning anvil.[12] It has long been known that chemical agents as well as ionizing radiation can induce mutations and chromosome aberrations in both plant and animal cells. *Allium cepa* bulbs were selected for research material because it is easy to obtain root meristem and have a small number of chromosome. It has been shown that plant meristem have a well regulated structure of mitotic cycle. The first induction of this was discovering of the quiescent in root apices. The onion is a subspecies and primary member of the plant family. The aim of present investigation is to check the clastogenic effects of plant extract at different concentrations on mitosis of *Allium cepa L.*

The *Allium cepa* assay is an efficient test for chemical screening and in situ monitoring for genotoxicity of environmental contaminants and has been widely used to study genotoxicity of many plant extracts revealing that these compounds can induce chromosomal aberrations in root meristems of *A. cepa*.[13]

MATERIALS AND METHODS

1. Plant extract. Acacia catchu was procured from local shop in bulk for preparation of the extract.
2. Study model: Onion (*Allium cepa*) will be procured from local market. Equal sized bulbs were chosen from a population of the common onion *Allium cepa* ($2n=16$). Onions were stored under dry conditions. Small Onions having a diameter of (1.5-2.0 cm) were preferred.

3. Acetocarmine stain

The catechu sample was crushed using mortar and pestle into a fine powder. 1% of aqueous extract was prepared by mixing 1g of catechu powder in 100 ml of distilled water. By using this extract, following concentrations were prepared: 0.02%, 0.04%, 0.06%, 0.08% and 0.1%. (2 sets).

The experiment was conducted in two sets labeled as Set A and Set B. (Both of which were replicates.) Each set had a control test tube (containing distilled water) and a series of 5 test tubes containing different concentrations of the toxicant. i.e. 0.02%, 0.04%, 0.06%, 0.08% and 0.1%.

The outer scales in the bulbs and the brownish bottom plate was removed, keeping the ring of root primordia intact. The bulbs were then put into fresh clean water to ensure proper cleansing and to protect the primordia from drying. After removing the bulbs from the "pool", they were placed on a soft paper and then transferred to the toxicant/test liquid of varying concentration (set A & set B) and control tube (distilled water).

The onions were placed directly in the test liquids without the previous germination of the root tips. The evaporated amounts of test liquids were replaced regularly. Care was taken to keep all other parameters constant. The experiment was performed at room temperature and was protected from direct sunlight. Test liquid was stored in the refrigerator at 4°C and should be brought to room temperature about an hour before use. The root length was measured after every 24 hours up till 72 hours.[14] After treatment (for 72 hours), the bulbs were washed thoroughly under running tap water. The root tips from each bulb were plucked and fixed in carnoy's fixative for 24 hours.

Squash Preparation: For chromosomal analysis, the root tips were hydrolysed in 1N HCl at 60° C for 1 minute and transferred to a watch glass containing acetocarmine and 1N HCl (9:1). They were then heated for 4-5 minutes. The tip of the root was then cut with a sharp blade and placed on a glass slide in a drop of water and covered with a coverslip. The root tip was then squashed by tapping with the back of the dissecting needle and sealed. The cells were scored under the microscope for different types of chromosomal aberrations. Active Mitotic Index (AMI) % and Total Abnormality Percentage(TAP)% were calculated.[15]

Active Mitotic Index (AMI) % = $\frac{\text{Total number of dividing cells} \times 1000}{\text{Total number of cells observed}}$

Total number of cells observed

Total Abnormality Percentage (TAP) % = $\frac{\text{Number of abnormal cells} \times 100}{\text{Total number of cells observed}}$

Total number of cells observed

Results

Table 1: Effect of varying concentrations of catechu on root length of the onion

Concentration(%)	Mean root length (mm)
Control	2.75
0.02%	1.75
0.04%	1.1
0.06%	1.0
0.08%	0.88
0.1%	0.65

Concentration of catechu(%) vs mean root length (mm)

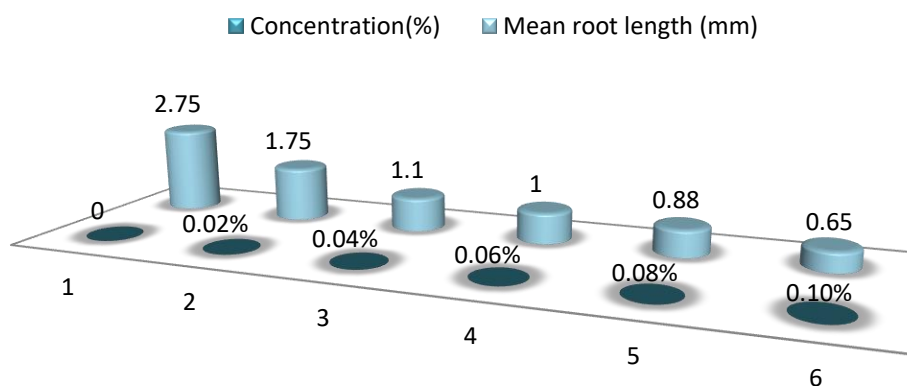


Figure 1: Graph of concentration of catechu extract (%) vs mean root length of the onion.

It is observed from the graph that as the concentration of catechu extract increases, the mean root length of *A. cepa* decreases.

Table 2a: Effect of varying concentrations of catechu on the colour of onion root tip

Concentration of Extract	Observations		
	Normal	Abnormal	
	White	Pale	Brown/Black
CONTROL (dw)	Yes	No	No
0.02%	Yes	No	No
0.04%	Yes	No	No
0.06%	Yes	No	No
0.08%	Yes	No	No
0.1%	Yes	No	No

Table 2b: Effect of varying concentrations of catechu on the shape of onion root tip

Concentration of Extract	Observations		
	Normal	Abnormal	
	Straight	Bulb	Broken tip
CONTROL (dw)	Yes	No	No
0.02%	Yes	No	No
0.04%	Yes	No	No
0.06%	Yes	No	No
0.08%	Yes	No	No
0.1%	Yes	No	No

Table 2a and 2b indicated that there was no change in the colour and the shape of the root tips, when the onion root tips were exposed to different concentrations of catechu extracts.

Table 3: Determination of the mitotic index among 100 cells scored following 72 h exposure of onion root tip cells to five different concentration each of catechu extract.

Concentration of Catechu extract (%)	Number of dividing cells	Mean MI (%)
Control	86.78	8.7
0.02%	63.00	6.3
0.04%	61.50	6.2
0.06%	50.20	5.0
0.08%	40.00	4.0
0.1%	8.62	1.57

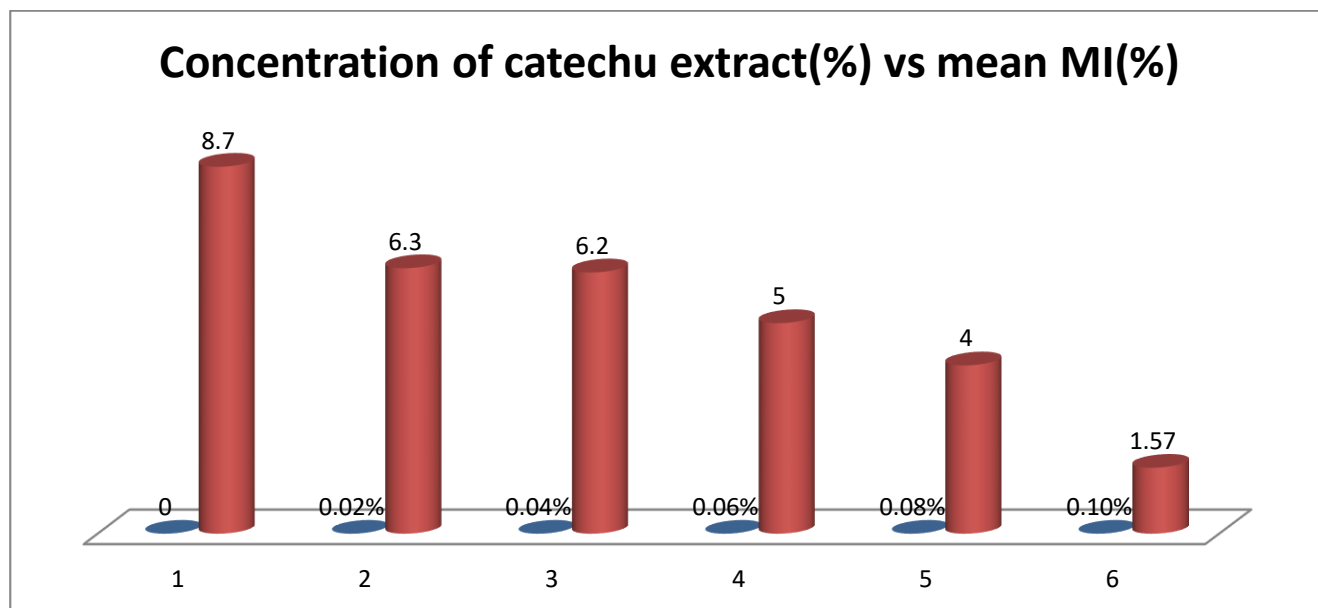


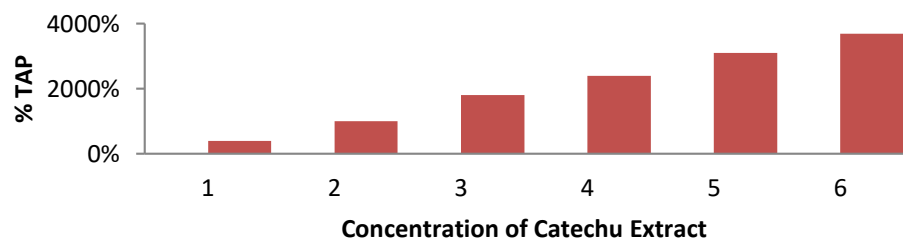
Figure 2:Graph of concentration of catechu extract (%) vs mean MI(%)

The graph indicate decrease in mitotic index with an increase in the concentration of catechu extract.

Table 4: Determination of the total abnormality percentage among 100 cells scored following 72 h exposure of onion root tip cells to five different concentration each of catechu extract.

Concentration of catechu extract	Observations		
	Total No of cells observed	No of chromosomal aberrations observed	Total Abnormality Percentage (TAP) (Mean)
CONTROL (dw)	100	4	4
0.02%	100	10	10
0.04%	100	18	18
0.06%	100	24	24
0.08%	100	31	31
0.1%	100	37	37

Effect of concentration of Catechu Extract on of TAP



From the graph it can be concluded that an increase in the catechu concentration causes an exponential increase in the total abnormality percentage of onion root tips.

Microscopic observations of the cells

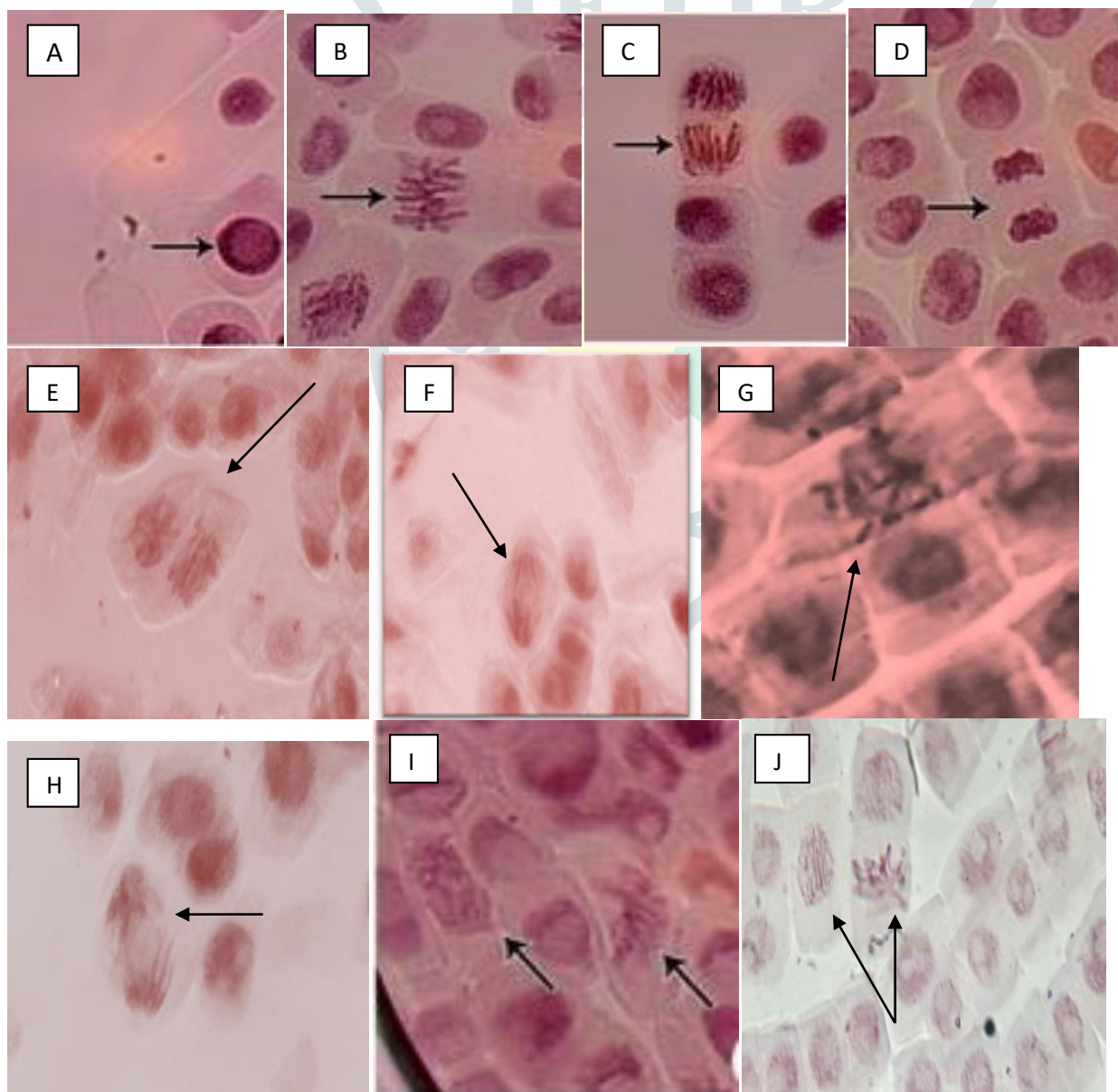


Figure 4 shows Chromosomal aberrations and nuclear irregularities observed in root tip cells of *A. cepa* following treatments with different concentrations of catechu extract

A. Normal prophase B. Normal metaphase C. Normal anaphase D. Normal telophase E. Sticky chromosomes F. Chromosomal bridge G. Chromosome break H. Disturbed anaphase and Chromosomal bridge I. Delayed anaphase and vagrant J. Chromosome laggards and c-mitosis

DISCUSSION

The *Allium cepa* test is important test in vivo, where the roots grow in direct contact with the substance of interest enabling possible damage to DNA of humans to be predicted.[16] Previous studies have also validated the use of plants for genotoxic studies .The higher plants *A. cepa* (onion) have relatively large monocentric chromosomes in reduced numbers and are accepted as suitable test organisms for the study of plant mutagenesis.[17] In this study, test enables the assessment of different genetic endpoints which occur as a result from exposure to catechu.

Catechu caused significant inhibition of MI in *Allium cepa* meristem cells and induced Chromosomal aberrations.

The mean number of mitotic index were analyzed for the homogeneity of the data collection and it was observed that all the data were homogenous and similar. The mitotic indices of the treated cells at each dose were compared with that of the control. It was found that there was a decrease in the MI as the concentration of catechu increased. Also, abnormal cells were observed as the concentrations increased which led to an increase in the total abnormality percentage with increasing concentration. The changes in MI and TAP of *Allium cepa* cells are indicators of cytotoxic and genotoxic potential and mitodepressive activity of catechu.[18] The number of aberrations increased as concentration of catechu increased.

The chromosomal aberrations such as chromosomal bridge,sticky chromosomes,laggards,,c-mitosis,breakage and vagrant were observed at all concentrations, except at the highest tested concentration. The results showed that higher concentrations were able to inhibit significantly cell division. Catechu cause lethal aberrations in *Allium cepa* at higher concentrations. Stickiness is due to inter-chromosomal linkages of sub-chromatid strands coupled with excessive formation of nucleoproteins and inappropriate protein-protein interaction.[19]

Laggards are formed as a result of a chromosome that did not overlap along the long axis of the spindle with any of the properly segregating chromosomes.

Nuclear abnormalities are characterized by morphological alterations in the interphase nuclei as a result from the exposure to catechu.[20]The number of cytological aberrations increased with increasing concentration.

Studies with higher plants have demonstrated that catechu induce mitotic abnormalities .It has been earlier reported that presence of catechu alters mitotic index and induces mitotic abnormalities in the root-tip cells of onion seedlings.[21] In the present investigation onion root-tips were utilized to study the effect of water-soluble extract of catechu upon the mitotic process. Since the plants are inexpensive, immobile, and can easily be grown in controlled conditions, they should prove excellent material as compared to animals.[22] In one study in 2007, however, catechu was genotoxic in tests with *Vicia faba*. Catechu extracts demonstrated mutagenicity in *Vicia faba* and was weakly mutagenic at two concentrations. These results are in agreement with our findings because *Vicia faba* is a plant system as well and shows the same results as the plant system of our study,*Allium cepa*. Also, it is more likely to mimic the effects in another system including the human system.

CONCLUSION



The present study has further demonstrated the usefulness of the *A. cepa* chromosome aberration assay in assessing the genotoxicity of plant extract (catechu).It was also observed that roots treated with different concentrations of catechu extract (0.02%, 0.04%,0.06%,0.08%,0.1%) showed a significant decrease in their length. Analyses of the mitotic index indicated differences among the various concentrations. The analyses indicated that as the concentration increased, the mitotic index was significantly reduced at all concentrations when compared with the control. The Chromosomal aberrations in *Allium cepa* root tip meristem cells after 72h exposure to catechu were stickiness,laggards, chromosomal breaks,c-mitosis,delayed and disturbed anaphase.

FUTURE PLANS

Medicinal plants have been widely used by both ancient and modern man of all cultures for treating different ailments. A single plant processed in different formulations can be used to cure a wide range of diseases. However, the historic role of medicinal herbs in the treatment and prevention of diseases and in the development of pharmacology do not assume their safety for uncontrolled use by an uninformed public. Studies of genotoxicity and anti-genotoxicity can help evaluate the safety and effectiveness of herbal health products.As catechu proves to

be a cytotoxic agent, its use can be minimised and avoided effectively. Also, its use in food additives and in medicines can be replaced by another non-toxic compounds. [23]

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