

Cytotoxic and Genotoxic Effects of Crude Leaf Extract of *Momordica balsamina* (L.) on the Root-Tips of *Capsicum Frutescence* (Linn)

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ABSTRACT

Cytotoxic and genotoxic potential of crude extract of *Momordica balsamina* was evaluated *in vivo* using the root-tips of *Capsicum frutescence*. The test concentrations were determined according to the doses which were suggested for utilization in alternative medicine i.e. 7g with its graded multiples of 2, 3, and 4 respectively. *In vivo* test showed that crude extract of *M. balsamina* plants induced some chromosomal aberrations in the genome of *Capsicum frutescence*. Cytotoxicity and genotoxic was found to increase with increased extract concentrations; this was followed by a decreased number of meristematic cells as the concentration of the extract increased to 28%. The results showed that the extract of *M.balsamina* administered at 14, 21, and 28grams increased the inhibition of cell division and induced DNA damage which were detected in the forms of segmental agglutination, chromosomes dissolutions, laggard polyploidy and stickiness. However, lower concentrations of 7g/7% were not effective. This suggests that the leaf extract *M. Balsamina* is probably safe for consumptions at the recommended dose of 7g. However, the possible DNA damage and segmental changes seen as the concentrations increased to 28grams suggest that higher doses above the recommended 7g could be dangerous to human cells at the long run. This therefore calls for caution in the consumption of raw herbal products prepared from *M. balsamina*. This study recommends that Low concentration of the leaf extract and wide spacing of dosage is therefore suggested for use if *M.balsamina* is to be used in traditional Medicare.

Keywords:Cytotoxic, genotoxic, crude extract, *Momordica balsamina* and *Capsicum frutescence*.

INTRODUCTION

Medicinal plants have increasingly become an integral part of the human society in combating various diseases ranging from skin infection to gastro-intestinal problems, since the down of civilization [1]. Of which *momordica balsamina* is one. The plant is a tendril bearing annual vine native of tropical region.

The crude extracts of many plant constitute the essential ingredients of many herbal drugs. Frequent use of these drugs in their crude form may, however, be likely to produce adverse side effects [2,3]. Hence the need to ensure quality and safety of herbal products [2]. Medicinal herbs have been used in folk medicine for millennia and in recent times,

scientific study of their effects has flourished. Nevertheless, some of them can course adverse effects or have the potential to interact with other medication [4,5]. Moreover, there is little information on the potential risk to health of such herbs [6,7].

Despite that the medicinal use of plants is probably as old as human kind itself. More than 150,000 plants species have been studied, and many of them contain therapeutic substances. These substances can be extracted and used in the preparation of drug or the plant can be used directly as a medication. Medicinal plants synthesize toxic substances which serve as defence against infections, insects and herbivores, but often affects the organisms that feed on them. This is because some constituents of medicinal plants have been

shown to be potentially toxic, carcinogenic, mutagenic and teratogenic [8,9].

Herbal preparations in general contain active ingredients which act on the ailments while others do not. The non-essential ingredients in the herbal preparation which are consumed along with the essential ones, are accumulated in the human system [10,11]. Recent investigations have revealed that many plants used as food or in traditional medicine have cytotoxic and genotoxic effects *In-vitro* and *In-vivo* [12,13]. These raise concern about the potential mutagenic or genotoxic hazards resulting from the long-term use of such plants [14]. The present study investigated the effect of crude extract of *Momordica balsamina* (L) on cytotoxic and genotoxic effects of mitotic chromosomes on *Capsicum frutescens* (L) root-tips.

MATERIALS AND METHODS

Collection of Materials

Ripe fruits of *Capsicum frutescens* (Linn.) were purchased from the Mubi main market. Seeds from these fruits were pried out and air dried in the laboratory at room temperature so as to maintain optimum performance. The leaves of *Momordica balsamina* were collected from Samunaka residences in Mubi Adamawa state Nigeria; the plant was identified using their preserved specimen vouchers in biological science laboratory, Federal Polytechnic Mubi. The leaves were washed thoroughly with clean water and dried at room temperature after which it was ground into powder. The pulverized leaves were measured out into 7g, 14g, 21g and 28g. The desired quantity of this plant was prepared using 100ml of lukewarm tap water at 45°C as solvent. After 24h of soaking of the pulverised leaves, the plant extract was sieved and filtered into beakers to get the crude extract.

Planting of Seeds

The seeds were soaked in the filtered extracts for 72 hours in a beaker, changing the filtrates every 24 hours so as to discourage fouling of

the filtrates. Another set in tap water and EMS as controls. The beakers were labelled accordingly. Seeds were planted in a separate germination pot and each was labelled as well. Watering of the pots was done using the extracts and tap water every day or alternately. Alternate watering was done for four days and after that, watering of the pots was done using tap water only. This was to prevent too much coloration of the root tips and avoid serious concentration of the plant extracts.

Harvesting and Pre-treatment of Root-tips

After 8 days from the date of planting, and when the roots were 2 - 2.5cm long, 2 cm from the growing root-tip were carefully excised using forceps and scissors giving consideration to roots with bulgy and creamy tips. This was done between 7.30 - 8.00am during which the metaphase spreads of the cells of the growing roots were active [15]. The selected and excised good root-tips immediately placed into 0.05 % colchicine for 5 hrs. This was done to arrest mitosis at metaphase and which may also further contract the chromosomes. It is also intended

to clear the cytoplasm making it more transparent [16]. Aeration was achieved using a battery operated aerator so as to replenish lost oxygen.

Fixation of Root-tips

The method of [17] with little modifications was employed. The roots were washed thoroughly in fast running tap water so as to remove dirt and sand, and fixed in 1:3 v/v glacial acetic alcohols for 12 hrs. This was intended to kill the plant material rapidly in such a way that the internal structures are preserved in a life-like form [10]. The roots were later washed in 30 % ethanol for 3hrs, transferred into absolute ethanol for 1 hour for hardening, then stored in 70 % ethanol at 4°C in refrigerator until required for use.

Cytological Analysis:

Hydrolysis

When required for cytological analysis stored root-tips were washed thoroughly in several changes of distilled water for 15 minutes these

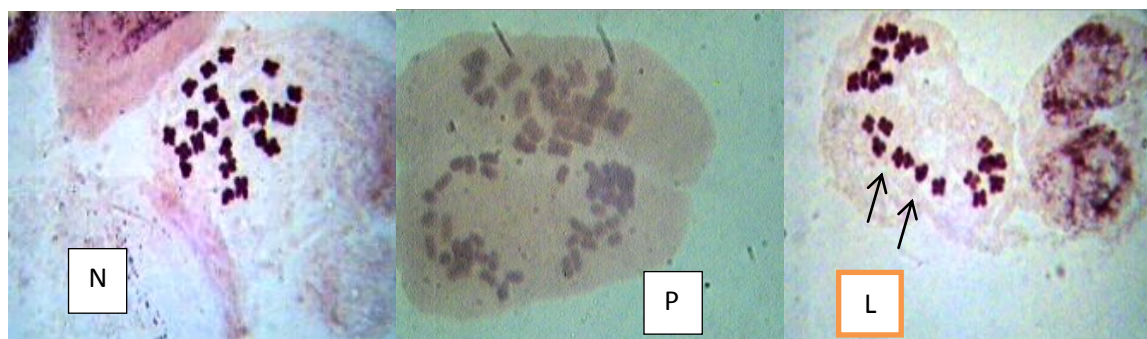
were then rinsed in cold and warm 1 N HCL in quick succession and hydrolysed in 1N HCL for 8 - 10 minutes in a water bath maintained at 60°C.

Staining

Using a pair of forceps, the roots were picked and place on a glass slide and the meristematic tip measuring about 1.5 mm from the tip were cut and the longer end discarded. A drop of 2 % acetic orcein was added and covered with a

cover slip. Squashing techniques modified by [1,2] was used to prepare slides. The squashing was done using the blunt end of a mounting pin; these ensure a single layer spread of cells. Excess stain was drain using a blotting paper carefully placed at the edges of the cover slip. Observation under microscope was done using the x10, x40, x100 oil immersion objective respectively. Slides showing chromosomal abnormalities were sealed using ladies white vanish. These were analyzed and micro graphed using a digital camera Motic ML 2.0.

RESULTS



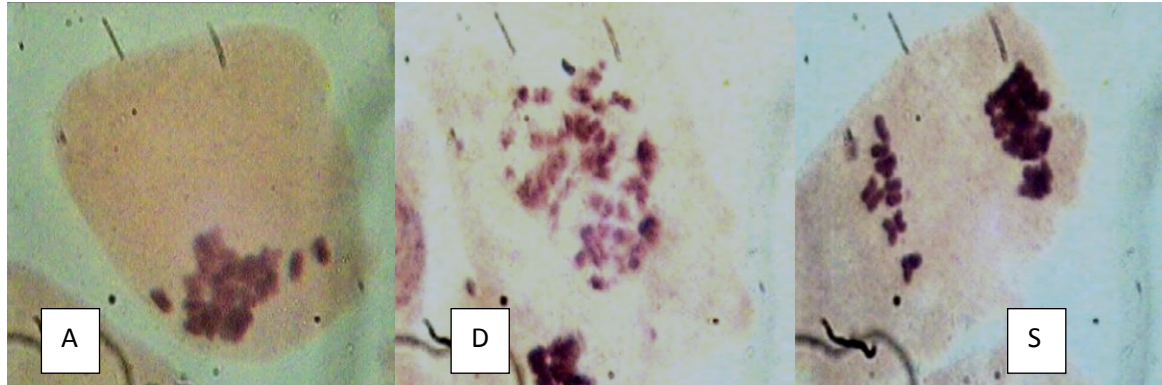


Plate1: photomicrographs of the treated *capsicum frutescence* root tips cell showing different chromosomal aberrations.

KEY: A-Agglutination, S-Stickiness, P-Polyploidy, D-Dissolution, L-Laggard, N-Normal Bivalent 2n=24.

The different types of aberrations induced by the addition of different concentrations of *Momordica balsamina* crude extracts are shown in Plate1. The aberration observed in this study are Agglutination, Chromosomes stickiness, Polyploidy, Laggard and Chromosome Dissolution. The cytotoxic and genotoxic effects of different concentration of

the crude extract of *Momordica balsamina* on the root-tips of *Capsicum frutescence* is shown in Table1. The result showed increase in the number of aberrant cells as the concentration of the crude extracts of *Momordica balsamina* increased.

Table 1:Cytotoxic and genotoxic effects of *M. balsamina* (L) on the root tips of *C. frutescence* (Linn)

Taxon	Conc.of crude extracts (g)	No. of MI plates scored	% of MI scored with normal bivalents (%)	(% of MI plates with aberrations)				
				A	S	L	D	P
<i>M.basalmina(Linn)</i>				A	S	L	D	P
	7	150	100	-	-	-	-	-
	14	150	90	6	-	-	4	-
	21	150	85	5	7	3	-	-
	28	150	65	-	10	15	11	9

KEY: A-Agglutination, S-Stickiness, P-Polyploidy, D-Dissolution, L-Laggard

DISCUSSION

In this study, *Capsicum frutescence* was used as the experimental material to determine the cytotoxic and genotoxic effects of the crude extract of *Momordica balsamina*, because chromosomal aberration observed in plants according to [4] shows resemblance with the aberration produced in human cells. And also plant cells as in man contain the same blue prints [7].

The use of chromosomes in studying the possible harmful effects of plant extracts on human is rooted on the fact that chromosomes are the vehicles which convey genetic or hereditary unit called gene. Thus any material damage or disposition caused to the chromosomes is bound to affect the function of the genes which they convey in turn and affect the individual or offspring of the individual in question.

In vivo testing of the cytotoxic and genotoxic effects of the crude extracts of *Momordica balsamina* on the root tips of *capsicum*

frutescence revealed that the extract is not cytotoxic and genotoxic at the recommended dose of (7% or 7g). However, at higher concentration, the plant extracts showed the potency to induced genetic damage at the levels of chromosomes and its spindle apparatus in *capsicum*. Thus causing different types of aberrations, the aberration were chromosomes agglutination, chromosomes stickiness, laggard, chromosomes dissolution and polyploidy. These conforms with similar effect reported by [1,2], that there is induction of polyploidy cells, laggard, anaphase bridge, chromosomes breakage at Anaphase when onion root tip cells were treated with neem leaf extract. [11], also reported stickiness of chromosomes on *Crinum jagus* root tips. According to [2], the chromosomal aberrations associated with herbal medication were traced due to the presence of phytochemicals such as alkaloids, flavonoids and terpenoids found in the plant.

CONCLUSION

This study revealed that the used of *Momordica balsamina* crude extract on the root tips of *Capsicum frutescence* at high concentration has cytotoxic and genotoxic effects on the mitotic chromosomes of *Capsicum frutescence* cells. Therefore it can be concluded that the use of crude extracts of *Momordica baslmina* should be used with caution since it has been indicated to be

cytotoxic and genotoxic on the root tip of *Capsicum frutescence*. However the findings in this study cannot be overlook because of the toxicological target in the DNA, which exists in all cellular forms including humans. Further studies should therefore be directed towards considering the application of *Momordica balsamina* extracts for different duration and its cytological effects on man.

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