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CYTOTOXICITY OF SEED KERNEL WATER EXTRACT OF *CAESALPINIA BONDUC* (L) ROXB. ON ROOT MERISTEM CELLS OF *ALLIUM CEPA* L.

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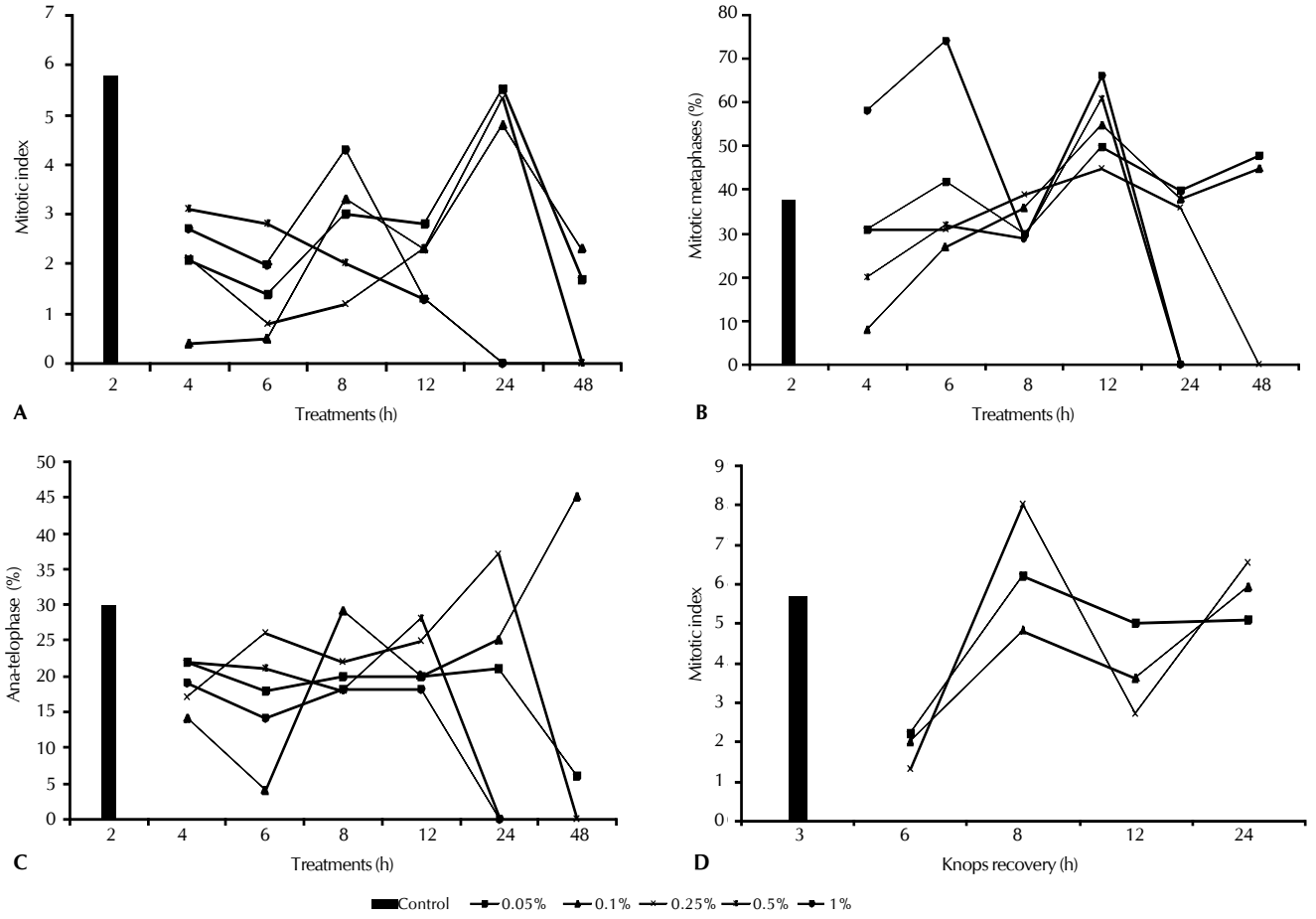
ABSTRACT
Cytotoxicity of seed kernel water extracts of *Caesalpinia bonduc* (L) Roxb. was studied in root meristem cells of *Allium cepa* L. The root tips of *A. cepa* were treated with different concentrations (0.05%, 0.1%, 0.25%, 0.5%, 1%) of crude extract for 4h, 6h, 8 h, 12h, 24h and 48h along with control. All the concentrations of water extracts treatment significantly decreased the mitotic index compared to control in all concentrations of treatments. Cytological effects included various levels of metaphase arrest along with clumping tendency of chromosomes. Selective concentrations (0.05%, 0.1% and 1%) of treatment also produced pre-treatment effects with well spread and condensed metaphase chromosomes. Infrequent incidence of unequal separation of chromosomes at anaphase was also observed. Recovery experiment clearly suggests that the present mito-inhibition and metaphase arrest are temporary and recovered within 24 h of incubation.

INTRODUCTION

Caesalpinia bonduc (L.) Roxb. [Syn. *Caesalpinia bonducella* (L) Fleming] is an ethnomedicinal species belonging to the family Caesalpiniaceae. The shrub is prickly and widely grows in Tripura (Deb, 1981). The seed of the plant is commonly known as natphal to the rural people of Tripura, which is extremely bitter in taste. *C. bonbuc* is used in traditional medicine like Ayurveda, Siddha, Unani and Homoeopathy (Kirtikar and Basu, 1988; Suryawanshi and Patel, 2011). Pharmacological activities of the seeds have been reported by several workers in controlling diabetes, trichuriasis, malarial fever without any known side effect (Burkill, 1995; Quisumbing, 1978; Chakraborty *et al.,* 2004 and Khan *et al.,* 2012). Phytochemical analysis of the seed also revealed to contain alkaloids, flavonoids, glycosides, saponins, furano di terpenes etc. (Moon *et al.,* 2010; Pillaia and Suresh, 2011; Khan *et al.,* 2012). The use of medicinal plant extracts for the treatment of human diseases is considered to be safe and dependable. According to WHO about 80% of people living in developing countries rely upon traditional medicine (Farnsworth *et al.,* 1985; Mukharjee, 2002). In view of the wide pharmacological activities of seed kernel of *C. bonduc,* it has been necessitated to study the influence of crude water extract of *C. bonduc* on root meristem cells of *Allium cepa* L.

MATERIALS AND METHODS

The *Allium* test introduced by Levan (1938) was used as plant assay system in the present experiment. The bulb of *Allium cepa* of same size were selected for test material and allowed to grow in pots containing sand. Fresh roots measuring 2-3cm long of 3-4 days old bulbs were taken out, washed and kept on the mouth of the experimental tubes containing different concentrations (0.05%, 0.10%, 0.25%, 0.05% and 1%) of seed kernel water extract of *C. bonduc*. All the experimental roots were properly immersed in the test solutions. Control was kept in tap water. Different duration of treatments (4, 6, 8, 12, 24 and 48h) was carried out for respective concentrations of water extracts. Root tips measuring 6-8 mm in size were cut from the respective treatments along with control and fixed in acetic alcohol (1:3) for overnight. Cytological preparations were made according to aceto-orcein squash technique (Sharma and Sharma, 1980). The fixed root tips in treatments and control were treated with 45% acetic acid for 5 minutes and stained with acetic acid (N) HCL mixture (9:1) for 1h. Stained root tips were squashed in 45% acetic acid and temporary slides were prepared for cytological observation under the microscope. At least 2500 cells from 15 random observations of 5 root tips were scored to study the mitotic index and percentage of different divisional stages of control and individual treatments. The bulbs with root of selected seed kernel extract treatments (0.05%, 0.1%, 1%) were allowed to recover in Knop's solution for 6, 8, 12 and 24h respectively. Cytological data recorded in the present investigation were presented in graphical form. Micro- photograph of significant divisional stages were taken with the help of SONY DSC-WX7 camera.



Figuren 1(A-D): Effects of *C. bonduc* extract on cell division of *A. cepa* L. A. Mitotic indices during different duration of treatments of respective concentration. B. Pattern of mitotic metaphase arrest during different hours of direct treatments. C. Percentage of Ana-telophase incidence at different duration of treatments. D. Recovery pattern in Knop's solution of selected treatments

RESULTS AND DISCUSSION

Different cytological parameters were studied both in control and treated root meristem cells of *Allium cepa* L. The control mitotic index of *A. cepa* was 5.91 ± 1.77 with 37.30% of metaphase and 30.24% ana-telophase respectively. Different degrees of mitotic inhibition were recorded depending on concentration and duration of *C. bonduc* seed kernel extract treatment. However, level of mitotic inhibition was contradictory among the treatments of varying duration (Fig. 1A). The mito- inhibition is maximum and effective in 0.05% of treatment. Varying frequencies of metaphase arrest was recorded among the treatments (Fig. 1B). High level of metaphase arrest (58.33%) with very low MI 2.74 ± 1.14 was observed in 1% extract during the short duration of treatments (4 h). Among the different concentrations and duration of treatments, 1% seed extract produced maximum metaphase arrest (74.78%) during 6 hrs of treatment (Fig. 2). Infrequent mitotic polyploid cells were also observed in 1% treatment for 4 h (Fig. 8). However, metaphase arrest produced in 0.25% of 6 hrs treatment resulted in better chromosome morphology and with well spread metaphases (Fig. 3). Gradual decline in mitotic indices was associated with varying degrees of mitotic metaphase arrest during long term treatment with or without chromosomal condensation and proper separation of chromosomes (Figs. 4, 5 and 6). Majority of metaphases recorded in 8 and 12h treatments, revealed chromosomal clumping tendency. Chromosomal clumping at metaphase was also observed in control. Prolong duration of treatments for 24 and 48h resulted in complete inhibition of mitotic process leading to well stained nucleated interphase cells (Fig. 7). The present study clearly indicated the clastogenic and spindle arrest properties of the seed kernel extract, which is evident from the MI values and manifestation of spindle abnormalities. Mito-inhibiton has been attributed to blocking of mitotic cycle during interphase that may result from a prolong G2 period or to the DNA synthesis (Kumar and Gupta, 2008). Mito- inhibition effect of various plant extracts or products have also been reported by several workers (Raj and Reddy, 1971; Kabarity and Malallah, 1980; Ene-Obong and Amadi, 1987; Kaushik, 1996). Analysis of different mitotic metaphase arrest produced during the present study suggests that extract of *C. bonduc* has significant effect on mitotic spindle function. This observation is in corroboration with previous plant extract studies by other workers (Ene-Obong and Amadi, 1987; Borooah, 2011; Akaneme and Amaefule, 2012). Knop's experiment with selected concentrations clearly indicated recovery of mito-depression to normal during 24h of incubation

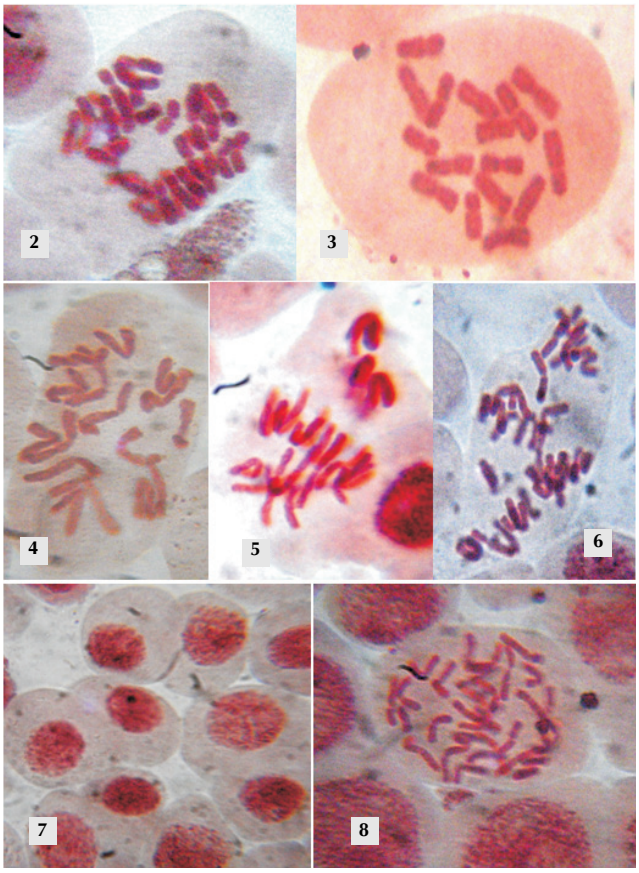


Figure 2-8: Mitotic stages recorded after different concentrations and durations of *C. bonduc* water extract treatments. 2. Diplotised mitotic metaphase. 3. Condensed and well spread mitotic metaphase with 16 chromosomes. 4. Irreegular condensation of mitotic chromosomes. 5 and 6. Irregular separation of mitotic chromosomes. 7. Mitotic inhibition at interphases. 8. Infrequent occurrence of mitotic polyploid cells

(Fig. 1D). This indicated that different mito-inhibition produced by the seed kernel extract of *C. bonduc* is not permanent and possibly not having any adverse effect since no cytological abnormality is recorded in the present study.

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