Detection of anti mitotic potential of Linum usitatissimum L water extract using plant bioassay

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Abstract:

The cell cycle control system is arrested when cell detect damage. Cell death may takes place at any mitotic phases and decrease cell division rate or stop it as result of genotoxicity action of tested material such as plant extracts. In this study ant mitotic action potentials of flaxseed water extract was investigated using *Allium cepa* bioassay. Different concentration of crude extract (2.5%, 5%) were tested at different periods (2, 4, 6hours). Number of chromosomal behavior aberrations in different mitotic phases and mitotic index was calculated at different treatment time and concentration.

The result showed a significant decrease in mitotic index in treated cells in comparison with control. This cell division inhibitory effect is time and concentration dependent. The result showed also that grounded seed extract has more mitodepressant action than whole seed extract. Microscopic examination of orceinz stained cells showed high cell accumulation in interphase, no micronucleus or chromosome fragmentation where observed , C-metaphase was the higher frequency type of chromosomal aberration. The data suggest physiogenic mitodepressnt effect of Flaxseed seed water extract.

Keywords: Mitodepressant, genotoxicity, plant bioassay, Flaxseed.

Introduction

Herbal medicines are being used by about 80% of the world population in developing countries for heath care without toxicity test application . Several medicinal plant are mutagenic, clastogenic and carcinogenic (Jeeva ,et al.,2013; Tawab,et al., 2004 ;seham,et al., 2014 ; Soliman,M .2001).The cell cycle control system is arrested when cells detect damage. Cell death may takes place at any mitotic phases. The change in the rate of cell division (MI) or chromosome behavior and structure is known as genotoxic effect of tested material such as plant extract. *Allium cepa plant bioassay was recommended for genotoxicity detection and evaluating because of its simplicity ,inexpensiveness and its several merits namely (,Grant,1982;* Antonise-Wiez,*1990 ;* Basbülbül, et *al*., 2008 ; Peters and Amon (2013).

Flaxseed*(Linum usitatissimum*L) is composed of multiple chemical constituents such as measurable concentrations of lignans and isoflavones (Muir, 2006; Bommaredds, et al., 2006; Abazzua, *et al*., 2007). The mechanisms of Flaxseed biological activity have not been fully elucidated. The present study was conducted to evaluate the genotoxicic activity of aqueous extract of flaxseed in the root-tip cells of *Allium cepa* .

Material and Method:

Plant extract:

For water extract ,a weight of 50g of air dried seeds were soaked in 100ml water at 60 ^{0}c. The collected extract were filtered and concentrated. The stock solution was diluted to constriction 2.5 and 5%.

Cytological studies

Allium seed or bulbs were germinating in tap water at room temperature. When the roots reaches 3-5cm they treated with tested extract for 2 and 4 hours for each concentration. Control roots were a kept in tap water without treatment. Untreated and treated root detached, fixed in Carnoy`s solution (3 :1 ethanol to acetic acid) for 24 hours. Root tips were hydrolyzed with 1N HCL at 60 ^{0}c . Orcein stain in squash technique was carried out with modification (Soliman,M. 2001) .

Five temporarily slides were prepared for each treatment and concentration and the experiment repeated 3 times. A least 1000 cells per slide were examined under 40x magnification. MI was determined by counting the number of mitotic cells among the total amount of scored cells per experiment. % of abnormality and abnormality in each mitotic phase was determined. The most common abnormalities were pictured under light microscope. The significance of difference between the mean results and control was determined by AWOVA test using SPSS software.

Result and Discussion:

Water extract of whole flaxseed extract showed no significant inhibitory effect on cell division of treated cells with low and high concentration for 2hours (Figure 1). By increase time of treatment, the potential of cell inhibitory effect enhanced significantly comparing to the control .This inhibitory action is concentration and time of treatment dependent. The MI of cells treated with high(5%) and low (2.5%) concentration were 5.63 and 82.8 respectively comparing to the control 11.36 . The anti mitotic potential of grounded flaxseed on divided *Allium* root tips was also investigated . Data in figure2 showed that ground plant seed has more inhibitory effect on cell division . At 2hour treatment there was significant decrease in MI. This Mitodepressant effect was significant between the two treatment and the control . Maximum inhibitory level was recorded in cells treated with high concentration of whole seed extraction (56.3) and with high conc. of ground seed(5.86) at 6 hours treatment (p= 0.008 and p= 0.013 respectively). The decrease in cell division rate is result of in appropriable cell events have potential to arrest the cell cycle progression in treated cells (Nair , *et al* 2011; McCollum *et al*,2005 ;Chin and Yeong,2010).

In present study the mitodepressant potential of flax seed was proved by using *Allium cepa* plant assay. The morphological characterization of chromosome behavior abnormalities indicted a positive correlation between decrease in mitotic index , abnormalities and metaphase arrest .The result in Fig 3&4 showed different level of abnormality which increase at low concentration and short treatment time and decrease at high concentration because of cell death which was accompany with

decrease in MI . No mutation was observed in cells treated with high concentration of the extract for 6 hr ,because cells prevented from entry to M phase and accumulated in

inerphase ,fig.5 . Decrease in mitotic index previously reported after treatment with plant extracts (Tawab,*et al*.,2004;Park, 2001;McCollum, *et al*,2005) and herbicide (El-Gharmery, *et al*.,2000).

Microscopic examination of stained treated cells showed high accumulation of cells in interphase which indicate that flaxseed water extract interfere with DNA replication and repair that lead to stop cell division at G2 phase (McCollum, *et al*.,2005). The data showed high frequency of c- metaphase chromosomal aberration (figure 3). High frequency c-metaphase in treated cells indicate deficiency in microtubules depolimerization and polymerization (Murata, et al., 2013) which observed by several aurthors (Tawab,*et al* 204 ;Bekir, et al.,2013). No micronucleus or chromosomal fragmentation was observed in treated cells which suggest the non-classtogenic , physiogenic type of genotoxicity. Data in this study confirm that both entry into and exit from mitosis is blocked in treated cells suggesting the flaxseed water extract may interfere with regulatory proteins and checkpoints of cell division (Morgan ,1997; Tawab ,2004). Accordingly it may be concluded the non-clastogenicity anti mitotic potential of *Linum usitatissimum* which make it save to use in cancer treatment . In fact Flaxseed anticancer activity cited in many studies (Jeeva ,2013; Jenab and Thompson ,1996).

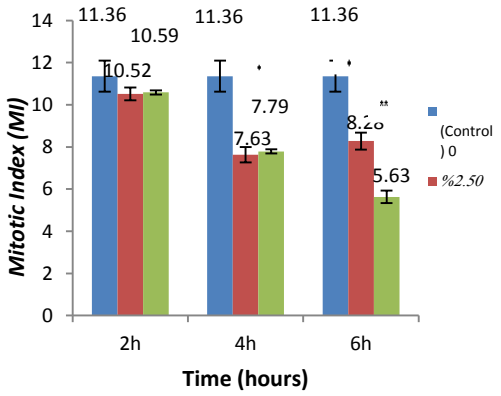

Figure 2. Mitotic Index of Allium cepa treated with grouned flaxseed extract at different treatment time and concentration.

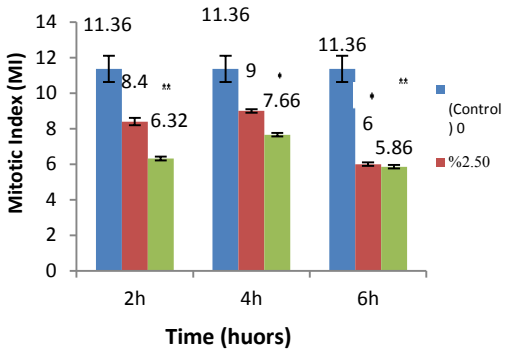

Figure 1. Mitotic Index of Allium cepa root tip treated with whole flaxseed extract at different concentration and time .

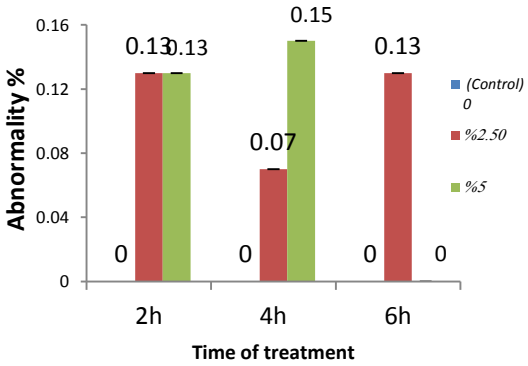

Figure 3. Abnormality% of cells treated with whole flaxseed extract at different concentration and treatment time

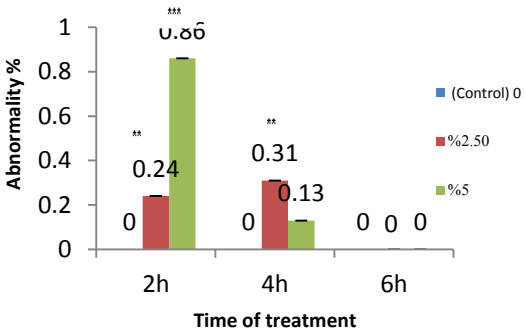

Figure 4. Abnormality% of cells treated with grounded flaxseed extract at different concentration and treatment times.

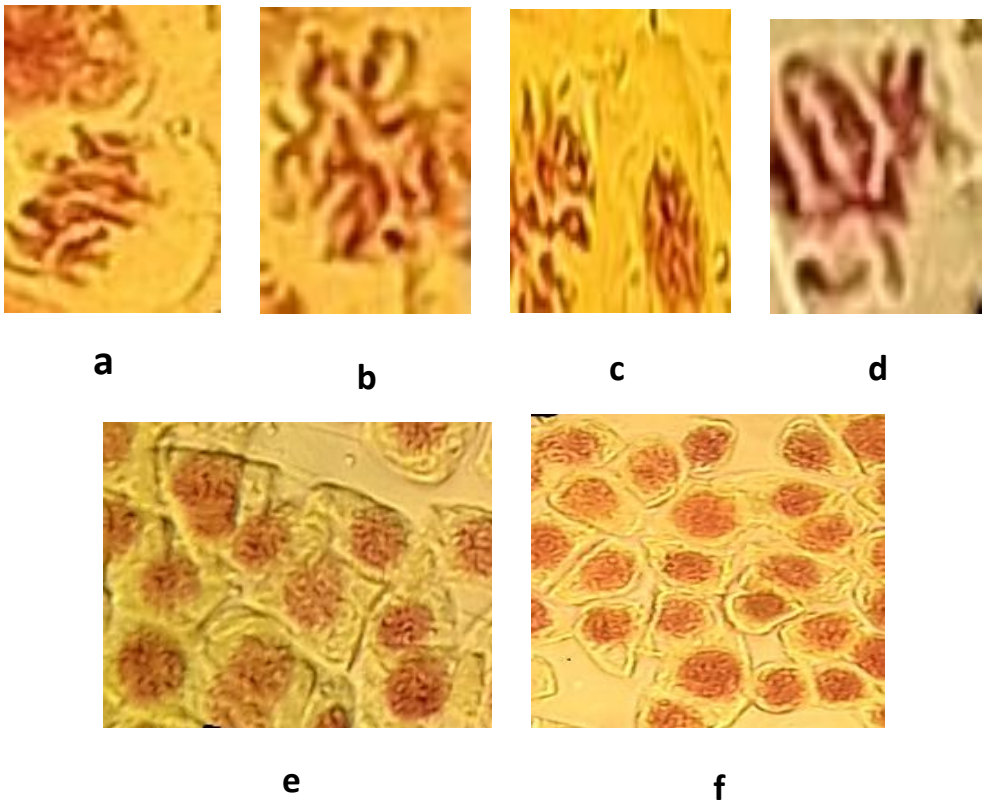

Figure 5. types of Mitotic abnormalities in *Allium cepa* L. root tip cells treated with flaxseed extract. a, b, c and d cells arrested in metaphase. e and f cells arrested in interphase

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