IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 2 Ver. II (Mar.- Apr.2016), PP 41-44
www.iosrjournals.org

Genotoxic Effect of Garlic Extract on Root Tips of Allium Cepa L.

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***Abstract**: The experiment was carried out to study the inhibition of the root growth and cytotoxic effects of garlic aqueous extract on onion bulbs (Allium cepa L.). The onion bulbs were treated with different concentrations of garlic aqueous extract (0, 5, 10, 15, 20 mg / l) for 72 hours in plastic laboratory tubes. The results indicated that the mitotic index and root growth rate of onion were considerably decreased in comparison to the control. We found that the cytotoxic effect of garlic extracts depends on the concentration and the exposure time. The EC50 value of garlic extract was 5 mg/l after 72 hours and the mitotic index value was (9%) at the same concentration (5 mg/l). The chromosomal aberrations were found to be increased as the concentration of the garlic extract increased when compared to control. The observed chromosomal abnormalities were chromosomal bridge, nuclear lesion, sticky chromosome and abnormal metaphase. We conclude that garlic extract have a genotoxic effect on the root tip cells of onion bulbs.*

***Key words:** Garlic extract, Allium cepa, genotoxic, mitotic index, aberrations.*

I. Introduction

Garlic (*Allium sativum*) plant has many medical properties, it has been used in the treatment of many diseases, since before the time of the Sumerian civilization (2600-2100 BC), when it was already widely cultivated in India , Arabia and China (1,2). Garlic is composed of mainly of water (60-70g/100g fresh weight) and the most significant components, medicinally, is the organosulfur-containing compounds (3). Garlic contain many active compounds which possess the anticancer agents present in the garlic induces suppression of cell cycle proliferation, modification in DNA repair mechanism, upregulation of antioxidant defences (4). When garlic bulbs are crushed, alliinase rapidly lyses the cytosolic alliin to form allicin (diallyl thiosulfinate) which is the dominant sulfur compound in crushed garlic (5).

Several individual compounds have been isolated from garlic and two major groups of compounds that show active anticancer effects have been identified. One group is the lipid-soluble allyl sulfur compounds such as diallyl disulfide (DADS) and diallyl trisulfide (DATS), and the other one is the water-soluble compounds c-glutamyl S-allylcysteine group such as S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC) (6). Other studies showed that S-allyl mercaptocysteine stops the growth of breast cancer cells, erythroleukemia (Sigounas et al., 1997) and colon cancer cells (7). S-allyl mercaptocysteine prevented colon cancer cell growth by 71%, disrupting cellular microtubules that form the cytoskeleton and the mitotic spindle in cells, thus disrupting cell division. In addition, S-allyl mercaptocysteine induced cell suicide (apoptosis) in the colon cancer cells, by activating apoptosis signalling pathway enzymes, including caspase that ultimately kills the cells (7).

Prager-Khoutorsky et al (8) found that the effects of allicin on cell polarization, migration, and mitosis are similar to the effects of microtubule-depolymerizing drugs such as nocodazole. The treatment of cultured fibroblasts with micromolar doses of allicin results in microtubule depolymerization in cells within minutes of its application, without disrupting the actin cytoskeleton or inducing direct cytotoxic effects.

II. Materials And Methods

Healthy equal sized onion bulbs were chosen and allowed to germinate in plastic container containing distilled water for 24 hours at 22 °C until the root- length reached about (0.3-0.4 cm). The onion bulbs divided in to two groups. The first group was treated with different concentrations (5, 10, 15, 20 mg / l) of the aqueous garlic extract for 72 h. The second group of onion bulbs placed in distilled water and served as negative control. After 72 h, the roots length were measured using a millimeter ruler. The inhibition percentage of roots growth in relation to the control for each extract was determined and the result used to calculate the EC_{50} [(9)]. Five roots per onion were cut, washed with distilled water and fixed directly in 3:1 alcohol: acetic acid for 24 h. The roots were hydrolyzed in 1N HCl at 60 °C for 5 min, then stained by acetocarmine stain. About 2 to 3 mm terminal root tips were cut off using a sharp blade , then placed on a clean slide with acetocarmine drop. The slide was covered with coverslip on the root tip and was squashed by applying uniform pressure. The slides were examined under the light microscope. Photomicrographs were taken with Sony digital camera (China made), directly from eyepiece of microscope.

The number of cells at dividing phase, abnormal cells and chromosomal aberrations were recorded in each concentration and mitotic index (MI) was calculated using formula (10): MI = Total number cells in division/Total number of cells observed X 100

III. Result

This study determined the cytotoxic effects of garlic aqueous extract on root growth used detecting of root length, MI and chromosome aberrations.

EC50 was defined as the value that reduces the control group root length by half. The control group change rate (%) was accepted 100%, and the extract concentrations were compared to this group. EC_{50} value of root length were detected at 5 mg/l, where the root length reduced from 3.78 ± 0.19 cm (100%) at control group to 1.8 ± 0.16 (48 %) at 5 mg/l of extract concentration after 72 hours (table 1). The root length reduced to 0.6 ± 0.082 cm (16 %) and 0.38 ± 0.083 cm (10 %) at 10 and 15 mg/l of extract respectively in compared to control after 72 hours.

Table 2 shows the cytological effects of garlic aqueous extract on root tip cells of *A. cepa*. Exposure of garlic aqueous extract inhibited the mitotic index in a concentration-dependent manner when compared to the mitotic index of 17.30 % in the control group. The lowest Mitotic Index (MI) value of 7.8 % was recorded for 15 mg/L treated with garli*c* aqueous extract. The mitotic index for *garlic extract* decreased significantly ($p < 0.05$) at 15 mg/ L.

The mitotic indexes were 7.8 % as compared to mitotic index at 5mg/L and 10 mg/L which were 9 % and 8.7 % respectively. This may indicate that *garlic* extract exerted a genotoxic effect at 15 mg/L. The mitotic indexes in treated cells were lower compared to the distilled water (negative control) which was17.30 %.

Chromosome aberrations were observed in stages of mitosis. Figure 1 that showed the types of chromosome aberrations induced by garlic extract. At 5 and 10 mg/l concentrations nuclear lesion, sticky chromosomes, laggard chromosome and chromosome bridges were the most common chromosome aberrations observed.

Nuclear lesion was observed at all the concentration of extract except in control and it was found in all cells at 15 mg/l concentration.

IV. Discussion

Cytogenetic tests are desirable for identifying the damaging effects of substances known in various concentrations under different exposure times for evaluation and influence on living organisms **(** 11**).**The *Allium cepa* test has been used to evaluate the genotoxic potential of medicinal plants (12; 13; 14), because this test uses a model that is adequately sensitive to detect innumerous substances that cause chromosomal alterations.

The study was performed using the plant cell test (*Allium* test) . This test was previously used for mutagenicity and cytotoxicity determination (15; 16; 17; 18; 19).The effects of garlic aqueous extract on the root growth of *Allium cepa* revealed dose dependent increase of the inhibitory effect. The root growth inhibition test is proved to be useful tool for detection of the concentrations used for cytotoxicity and genotoxicity evaluation of different chemical compounds (20,21). EC50 determination is also widely used as a first step in cytogenetic studies on medicinal plants extracts (22, 23, 24).

In the present study the observed decrease in mitotic index values combined with the significant decrease in root lengths. In control, the root length was 3.78 ± 0.19 cm and mitotic index was 17.30 %, then the root length decreased with increasing of mitotic index and reached 0.38 ± 0.083 cm, when mitotic index was 7.8 % at 15 mg/l concentration. Changes in duration of mitotic phases are also accepted as an indicator for cytotoxic influence (25).Chromosomal aberrations (CA) are characterized by change in either a chromosomal structure or a total number of chromosomes, which occur spontaneously due to the exposure of physical or chemical mutagens (26). In the present study chromosomal aberrations were observed, as chromosomal bridge, nuclear lesion and sticky chromosome. Occurrence of chromosomal bridge may be due to stickness or formation of dicentric chromosome caused by breakage and reunion (27). Chromosomal bridge mainly arises due to the non disjunction of sticky chromosome or breakage and reunion during separation at anaphase (28). Nuclear lesion was observed in all the concentration of effluent except in control and the percentage of the nuclear lesion was found to be increased as the garlic extract concentration increased. Similar results were observed by (29 and 26) in *Allium cepa* root cells treated by petroleum hydrocarbons contaminated water.

Garlic extracts have a strong antibacterial and antifungal effect , and allicin was assumed to be the main component responsible for the inhibition of fungal growth (30, 31, 32). (33) evaluated the garlic's cytotoxic effect on human gingival fibrobalsts (HGFs). The garlic extract induced DNA damage in lymphocytes cultures (34). Higher concentration of garlic extract has been shown to be clastogenic in mice which was appreciably reduced at lower concentration (35). The results obtained from this study reveals, that the garlic extract having genotoxic efficacy by inhibition of cell divisions in *allium cepa* root tip cells.

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Table (1): Inhibitory effect of different garlic extract concentrations (mg/l) on *Allium* cepa root growth.

Concentration (mg/l)	Mean root length (±S.D.) at time (hour):			Change rate (100 %) at 72 hr.
	24	48	72	
Control	1.46 ± 0.11	2.04 ± 0.21	3.78 ± 0.19	100
5	1.33 ± 0.095	1.48 ± 0.083	1.8 ± 0.16	48
10	0.5 ± 0.12	0.54 ± 0.13	0.6 ± 0.082	16
15	0.33 ± 0.096	0.3 ± 0.081	0.38 ± 0.083	10

Table (2): Effect of different garlic extract concentrations (mg/l) on Mitotic index of the examined root tip cells of *Allium cepa*.

Concentration (mg/l)	Total cells examined	Interphase	Prophase	Metaphase	Anaphase	Telophase	Mitotic Index (%)
Control	800	682	60	30	16	12	17.30
5	800	734	28	18	10	10	9.00
10	800	736	40	8	8	8	8.7
15	800	742	32	10	10	6	7.8

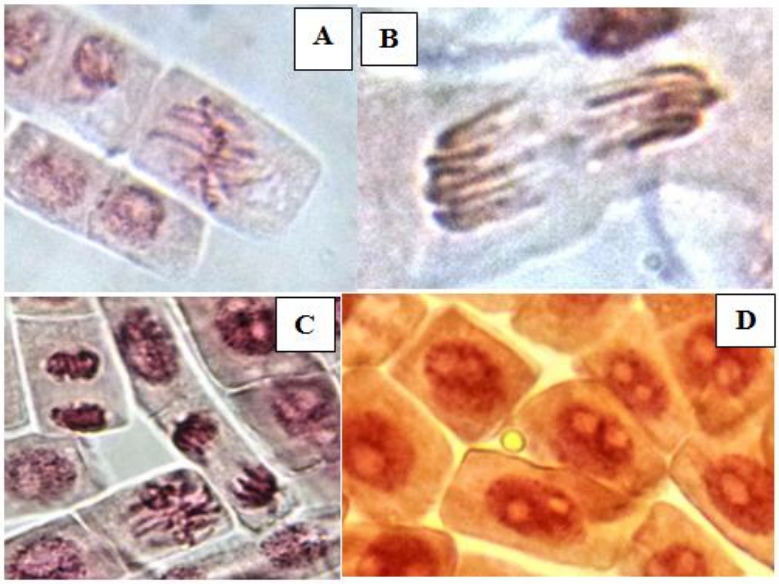

Figure (1): Different types of aberration induced by garlic extract in *Allium cepa* root tips: (A) Metaphase, (B) Chromosome bridge, (C) Sticky chromosome and (D) Nuclear lesion