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# Mitotic Inhibitor Activity of Glucosinolytes in *Brassica juncea.*

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**ABSTRACT**

Colchicine inhibits microtubule polymerization by binding to tubulin, one of the main constituent microtubules. The mitosis inhibiting activity of colchicine has been of great use in the study of cellular genetic. The aim of this study is to investigate the mitotic inhibitor activity of mustard oil (*brassica juncea*), in vivo study. A treatment with mustard oil *brassica juncea* caused no divided cells in the metaphase indexes compared to the colchicines 22%.

**Keywords**: Glucosinolytes*, Brassica juncea,* Colchicine, Mitotic.

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## INTRODUCTION

The mustard oil (brassica juncea), show an anti-oxidant activity in vivo study [1]. This activity suggests that they might be chemotherapeutic agents against cancer cells with multi drug resistance phenotypes [2]. So for more investigation activity of mustard oil (brassica juncea) on metastasis of chromosomes, we are carrying out this experiment.

Eventually cells need to duplicate. There is two main methods of replication, namely Mitosis and Meiosis. The cell capable of undergoing division Passes through cell cycle. The events surrounding eukaryotic DNA replication and cell division (mitosis) are coordinated to produce the cell cycle [3].

Mitosis is the simple duplication of a cell and all of its parts. It duplicates its DNA and the two new cells (daughter cells) have the same pieces and genetic code , that resemble the parental cell in terms of chromosomal number [3]. Two identical copies come from one original. Start with one; get two that are the same. This maintains constant number of chromosomes in each cell of successive generation.

### The Phases of Mitosis

**Prophase: A.** The chromatin condenses into chromosomes by dehydrating and coiling. The chromosomes consist of two identical sister chromatids, joined together by a centromere. For the first time, they can be seen with alight microscope. **B.** The nucleolus and nuclear envelope disappear, and the centriole (animal cells only) divides into two centrosomes, which move apart, creating the spindle. This eventually fills the whole of the cell and is made of the protein tubulin which forms spindle fibresa form of microtubule.

**Metaphase:** The chromosomes are moved to the equator of the cell, and the centromeres are attached to the spindle fibres, so that the sister chromatids line up in the centre of the cell.

**Anaphase:** The centromeres of each chromosome divide and are pulled apart by the contraction of the spindle fibres, thus moving the chromosomes (as they must now be called) to opposite poles of the cell.

**Telophase: 1.** After the chromosomes reach the poles, the spindle disappears and the chromosomes return to their functional chromatin state by rehydrating and uncoiling.

A new nuclear envelope begins to form around the chromosomes at each end of the cell; each with its own nucleolus. With mitosis now over, cytokinesis can begin.

**Cytokinesis:** Following mitosis, cytokinesis completes the process of cell division. The two cells formed are roughly equal in size (paclitaxel, docetaxel) promote polymerization and stabilize microtubules [4].

Cancer is believed to become the main cause of death in world wide [5]. Antimitotic agents constitute a major class of cytotoxic drugs of the current anticancer chemotherapeutic agents that act by preventing polymerization or depolymerization of microtubules (motor of mitotic stage of cell cycle) by binding to its tubulin subunits [6-8]. Even when the current antitumor therapeutic strategy comprises multiple points of intervention, cytotoxic drugs remain a mainstay in cancer chemotherapy for the next future [9].

Antimitotic agents that act on microtubules can be classified in two categories according to their mechanism of actions. *Vinca* alkaloids and colchicine (andseveral other molecules) inhibit microtubule polymerization; in contrast taxanes (paclitaxel, docetaxel) promote polymerization and stabilize microtubules. Colchicine is a medication It was used originally to treat rheumatic complaints especially gout. colchicine inhibit microtubule polymerization by binding to tubulin, one of the main constituents of microtubules. Availability of tubulin is essential to mitosis, and therefore colchicine effectively functions as a "mitotic poison" or spindle poison [10].

## EXEPERIMANTAL

The following solutions were prepared according to Yaseen1990 [11] in this experimental include:

**Colchicine Solution**

Colchicine solution was prepared by dissolving one tablet 0.5mg of crystallized colchicine in 5ml distilled water. The solution was filtered and, then it was stored at 4°C. for four days as a maximum**.**

**Hypotonic Solutionof (KCl)**

The hypotonic solution was prepared by dissolving 1.1175gmof KCl powder in 200 ml of distilled water, the stock solution was stored at 4°C. until used for cytogenetic study.

**Fixative Solution**

It was employed for cytogenetic studies; freshly prepare mixture of absolute methanol with glacial acetic acid in the ratio 3:1(V/V) for cytogenetic study.

**Experimental Design of Antimitotic Study**

Rats were divided in to 2 group (5 rats in each) depended onprotocol of Allen *et al*., 1977[12] as following:

- **First group:** Rats were injected intraperitoneally with colchicines dissolved in distilled water at dose of 0.5 mg /ml before two hours from procedure.
- **Second group:** Rats were treated orally with mustard oil (*brassica juncea*) by 0.5ml at dose of 112 mg / kg before six hours from procedure.

**Methods of Bone Marrow Cells Harvesting**

The protocol of Allen *et al*., 1977 [12] was done to study as follow:

- Skin and muscle tissues were removed from both femurs immediately after sacrifice of rat. Both epicondyle tips were removed with scissors, and the marrow expelled, using a syringe with a 24-gauge needle with 5ml of warm sterile PBS until the bone being clear, into a centrifuge tube. The suspension was mixed well to assure dissociation of the cells.

- The cell suspension was treated with 0.1ml colchicin at 37°C. for 20 minutes.

- The cell were centrifuged at 1500 rpm for 10 minutes and the supernatant was discarded.

- Five ml of hypotonic solution (warmed KCl) at 37ºC was added to the cell pellet and the suspension mixed thoroughly. The cells were incubated at 37ºC in a water bath for 30 minutes.

- The cells were centrifuged at 1500 rpm for 10 minutes and the supernatant was discarded.

- Freezer-chilled freshly prepared fixative methanol: glacial acetic acid (3:1) was added drop-wise, with initial mixing, to give a total volume of 5ml. The cells were gently resuspended and then refrigerated at 4ºC for 1 hour.

- The cells were centrifuged at 1500 rpm for 10 minutes and the supernatant was discarded.

- Five ml of fresh fixative was added, cells resuspended and centrifuged at 1500 rpm for 10 minutes. Two other consecutive washes with the fixative were made. 1 ml of the fixative was added to the cells after the last wash.

**Slide Preparation and Staining for Antimitotic Test**

The cells were resuspended and then dropped from a height of about 0.5 meter, using a Pasteur pipette onto wetslides, chilled, grease-free slides and allowed to dry at room temperature. The slides were

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stained with freshly prepared Giemsa stain (1 volume Giemsa stain stock and 4 volume Sorenson's buffer) for 2 minutes, then washed with Sorenson's buffer and left to dry at room temperature. Microscopic examination under (100X) objective lens was performed to determine the mitotic index MI% [11].

**Mitotic Index Analysis (MI)**

The MI was determined as a ratio of the mitotic cells to the cells in 1000 calculated cells [11].

$$\mathbf{MI\%} = \frac{\text{Number of metaphaseal dividing cell}}{\text{Total Number of 1000 cell}} \times \mathbf{100}$$

**RESULTS and DISCUSSION**

In the present study when treated rats with mustard oil (*brassica juncea*) shown no divided cells at metaphase. (See Table 1) In the group of Colchicine shown that the number of cell divided is (220)22%, and the number of cell not divided is (780) 78%. A treatment with mustard oil (*brassica juncea*) caused mitotic inhibiter (See Figure 2) as compared to the Colchicine 22% (See Figure 1).

Observation was made using bright field and photographs were taken with a100X oil objective lens. The results indicated of glucosinolates with its dose causes reduction mitotic index (MI) in rat bone marrow cells, this may be related to the protein required for mitosis which were not produced at the same quantities, or the code was not reached the cell to induce it to proliferate [13] or due to defect occurred in the mitotic spindle composition during cell division[14].

**Table 1: Effect of Colchicine and mustard oil (*brassica juncea*) on chromosomal cells.**

| Material / Cell division | Colchicine/cell | Mustard oil/cell |
|---|---|---|
| The cell which not divided | 780 | 1000 |
| The cell which divided | 220 | 0 |
| The percentage of cell not division | 78% | 100% |
| The percentage of cell division | 22% | 0% |

$$\mathbf{MI\%} = \frac{\text{Number of metaphaseal dividing cell}}{\text{Total Number of 1000 cell}} \times \mathbf{100}$$

**First group:** Treated with mustard oil (*brassica juncea*).

$$\mathbf{MI} = \frac{0}{1000} \text{x}100$$

**MI= 0%**

**Second group:** Treated with Colchicine.

$$\mathbf{MI} = \frac{220}{1000} \text{x}100$$

**MI = 22%**

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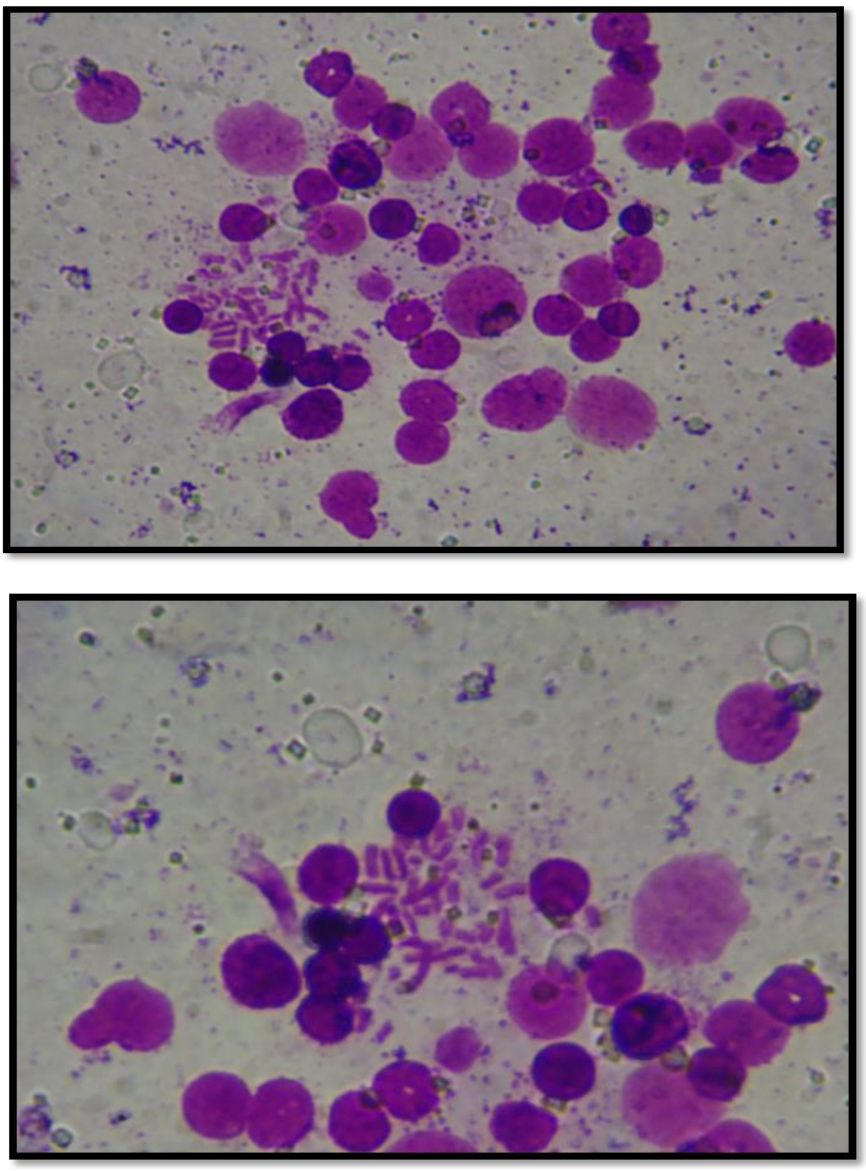
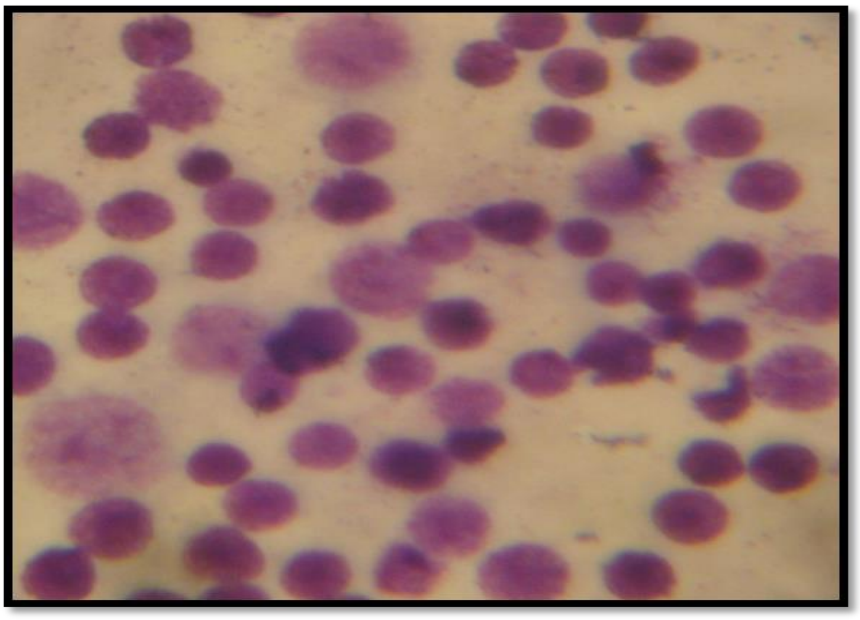


Figure 1: Chromosomal division by using the colchicine (100X oil objective lens).



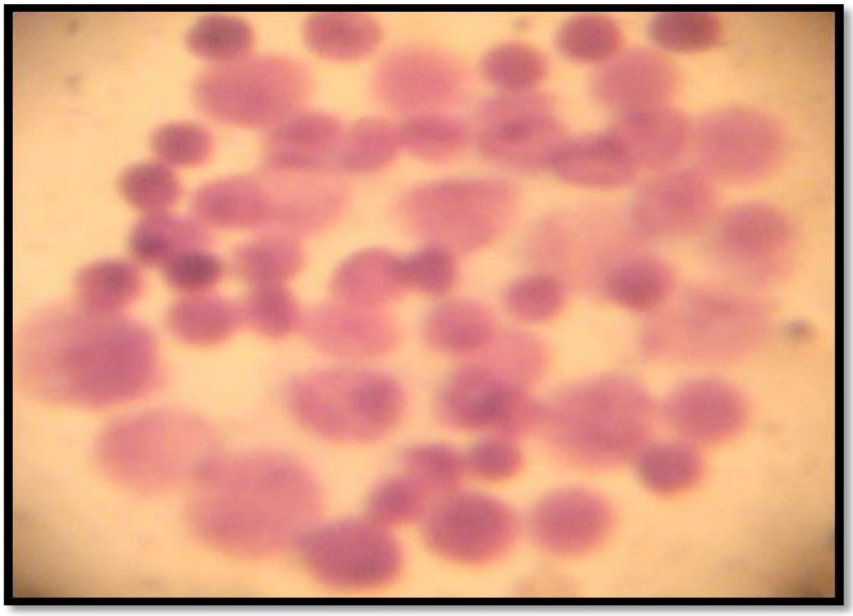


**Figure 2: Chromosomal division by using the mustard oil (*brassica juncea*) (100X oil objective lens).**

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