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Antimutagenic and Anticytotoxic Activity of *Carica Papaya* Leaf Extracts

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ABSTRACT

Carica papaya, a member of the family Caricaceae, is commonly known for its food and nutritional value throughout the world. During last few decades considerable progress has been achieved regarding the biological activity and medicinal application of papaya and now it is considered as valuable neutraceutical fruit plant. The present study was designed to determine the antimutagenic and anticytotoxic activities of different fractions (Aqueous, Chloroform, Ethanol and Ethyl acetate extracts) of *Carica papaya* leaves. Antimutagenic activity was determined by the *Allium cepa* root chromosomal assay using Cyclophosphamide monohydrate as the mutagenic agent and anticytotoxic activity of the extracts was evaluated by determination of mitotic index. All the four fractions displayed significant antimutagenic and anticytotoxic activities against cyclophosphamide-induced chromosomal aberrations and alltogether, the results of our study lend pharmacological credence to the anti-cancerous and ethnomedical use of this plant in traditional system of medicine and these results could be used to develop antimutagenic compounds for cancer therapy.

Keywords: *Carica papaya*, antimutagenicity, anticytotoxicity, ethyl acetate, aqueous, ethanol, chloroform fractions.

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INTRODUCTION

Carica papaya L. is a member of the Caricaceae family[1]. It is a dicotyledonous, polygamous and diploid, giant herbaceous plant which originated in Central America and is now grown in tropical areas world-wide for its large, sweet, melon-like fruits[2]. The papaya plant has a hollow, green or purple stem and has long-petioled leaves. Both leaves and stems contain large amounts of white, milky latex. The papaya fruit is globally consumed either in its fresh form or in the form of juices, jams and crystallized dry fruit[3]. The ripe fruit is said to be a rich source of vitamin A, C and calcium[4].

Various parts of *Carica papaya* Linn. (CP) have been traditionally used as ethnomedicine for a number of disorders. There are many commercial products derived from the different parts of the *Carica papaya* plant, the most prominent being papain and chymopapain, which is produced from the latex of the young fruit, stem and the leaves. *C. papaya* leaves have been used in folk medicine for centuries as remedy for various disorders. Recent studies have shown its beneficial effect as an anti-inflammatory agent[5], for its wound healing properties[6], and as an antioxidant[7]. A toxicity study (acute, subacute, and chronic toxicity) conducted on Sprague Dawley rats administered with *Carica papaya* leaves juice revealed that it was safe for oral consumption[8] and administration of *Carica papaya* leaves juice in dengue fever and dengue haemorrhagic fever induced rapid increase in platelet count in patients[9]. Also the free radical scavenging (antioxidant) activities of ethanolic extracts of *Carica papaya* leaves is used in malaria therapy in Southwestern Nigeria[10]. A study showed that papaya latex formulated in the Carbopol gel is effective in the treatment of burns[11] and *Carica papaya* latex also promotes wound healing in diabetic wistar rats[12]. There have been anecdotes of patients with advanced cancers achieving remission following consumption of tea extract made from CP leaves.

The use of antimutagens and anticarcinogens in everyday life is the most effective procedure for preventing human cancer and genetic diseases. There are several ways in which the action of mutagens can be reduced or prevented. Chemicals which act to interfere with DNA repair or with mutagen metabolism can be effective antimutagens[13]. Cyclophosphamide, also known as cytophosphane, is a cytotoxic drug. It is an alkylating agent of the nitrogen mustard type[14]; specifically, the oxazaphosphorine group[15]. An alkylating agent adds an alkyl group to DNA. It attaches the alkyl group to the guanine base of DNA, at the number 7 nitrogen atom of the imidazole ring. This interferes with DNA replication by forming intrastrand and interstrand DNA

crosslinks. The drug thus blocks DNA duplication completely, resulting in cell death or incompletely, causing mutation[16].

The antioxidant, anti-inflammatory, anti-sickling, wound healing, and immunomodulatory potential of leaves extract of *Carica papaya* along with the potential to treat dengue, malaria and cancer has been previously reported[17]. In the continuous effort to evaluate potential medicinal values of the leaves of *C. papaya*, the present research was conducted to investigate anticytotoxic and antimutagenic activities of ethanolic, ethyl acetate, chloroform and aqueous extracts of *C. papaya*.

The present study is designed to investigate the action of CP leaves extract against cyclophosphamide induced chromosomal aberrations in *Allium cepa* root meristem cells. Among different plant assays, the *A. cepa* test is an easy, fast and very sensitive assay to detect environmental genotoxicity/antigenotoxicity of chemicals or natural plant products.

This assay is related to the study of effect of chemicals at the genetic level which includes both microscopic and macroscopic parameters. The *Allium cepa* root chromosomal aberration assay has also been adopted by the International Program on Plant Biossays (IPPB) for the evaluation of the environmental pollutants[18]. This assay has also been used to monitor the antigenotoxic nature of various plants and plant products. Different parameters of *Allium cepa* such as root shape, growth, mitotic index can be used to estimate the cytotoxicity while and chromosomal aberrations can be used to estimate the mutagenicity[19].

MATERIALS AND METHOD

Chemicals:

Acetic acid, Acetone, Aceto-orcein, L-Ascorbic acid, Cedar wood oil, Chloroform, Cyclophosphamide monohydrate, Ethanol, Ethyl acetate, Hydrochloric acid (HCl) and Orcein.

Equipment:

Equipment used were Grinder, Weighing balance, Homogenizer, Rocker 300 Vacuum pump, REMI 2MLH Magnetic Stirrer, REMI R-8C Centrifuge, Water bath, Glass slides, Coverslips and Labomed CXL Mono Optical Microscope.

Plant source:

Carica papaya leaves were taken from a fruit bearing papaya plant at Mount Carmel College, Vasanth Nagar, Bangalore, Karnataka. Young and healthy leaves were used for the study.

Acetone powder preparation

Procured CP leaves were deveined, ground and depigmented using ice cold acetone. It was then filtered and dried in a vacuum pump. The resulting acetone powder was stored in an air tight plastic container and used further for the preparation of different fractions.

Preparation of 0.5 % extracts using different solvents

50 mL of respective solvents- chloroform, distilled water, ethanol and ethyl acetate, were added to 250 mg of the acetone powder and each extract was placed on a magnetic stirrer at 120 rpm at low temperature for 3 hours. Each extract was then centrifuged at 3000 rpm for 10 min. Supernatant was collected. 0.5 % extracts of supernatant were prepared with water and used for further analysis.

Anticytotoxic activity of *Carica papaya* extracts against cyclophosphamide induced cytotoxicity.

Onion bulbs of same size were purchased from a local market and sun dried for a day until the outer scales were easily removable. During the de-scaling process, the older roots from the primordial root ring were carefully removed without harming the root ring. The bulbs were then thoroughly rinsed and thereafter suspended in tap water (negative control), 0.5mM ascorbic acid (standard control), 0.5mM Cyclophosphamide (positive control) and different 0.5% extracts (ethanol, ethyl acetate, chloroform and aqueous) and incubated for 48hrs.

Anticytotoxic effects of fractions were evaluated by pre-growing the *A. cepa* in the medium containing the individual fractions, ascorbic acid and water for 48h and then transferred to cyclophosphamide medium for another 24 hours (post-treatment). The root length and root form were assessed for each extract. A temporary squash was prepared of the root tips and viewed under high power of a light microscope. On each slide, total cells under study and total dividing cells were scored. The data were used for statistical analysis of Mitotic Index (MI) and mitotic inhibition (MH).

Mitotic index (MI) = number of dividing cells divided by total counted cells in the treatment × 100.

Mitotic inhibition (MH) = mitotic index of control - mitotic index of treatment × 100.

MH corresponds to reduction in MI and thus serves as a direct indicator of cytotoxicity.

***Allium cepa* Chromosomal Assay for the evaluation of Antimutagenic activity of *Carica papaya* extracts against Cyclophosphamide induced mutagenicity.**

The *Allium cepa* assay used in this study was with few modifications of the previously reported method[20]. Onion bulbs were subjected to the same treatment as described earlier and *Allium cepa* root squash was prepared for microscopic examination. The slides were viewed under oil

immersion (100X). On each slide, total cells under study and abnormal/aberrant dividing cells were scored and the data was used for statistical analysis of frequency of aberrant cells/ Frequency of aberrations (FOA).

Frequency of aberrations (FOA) = number of aberrant cells in the treatment divided by total counted cells in the treatment × 100.

RESULTS AND DISCUSSION

Anticytotoxic activity of *Carica papaya* extracts against cyclophosphamide induced cytotoxicity.

The cytotoxic level of a test compound can be determined based on the increase or decrease in the mitotic index (MI), which can be used as a parameter of cytotoxicity in studies of environmental biomonitoring[21]. The cytotoxic level can be determined by the decreased rate of MI. Therefore MI was reported to be a good indicator to assess the cytotoxicity of chemicals in cells[22].

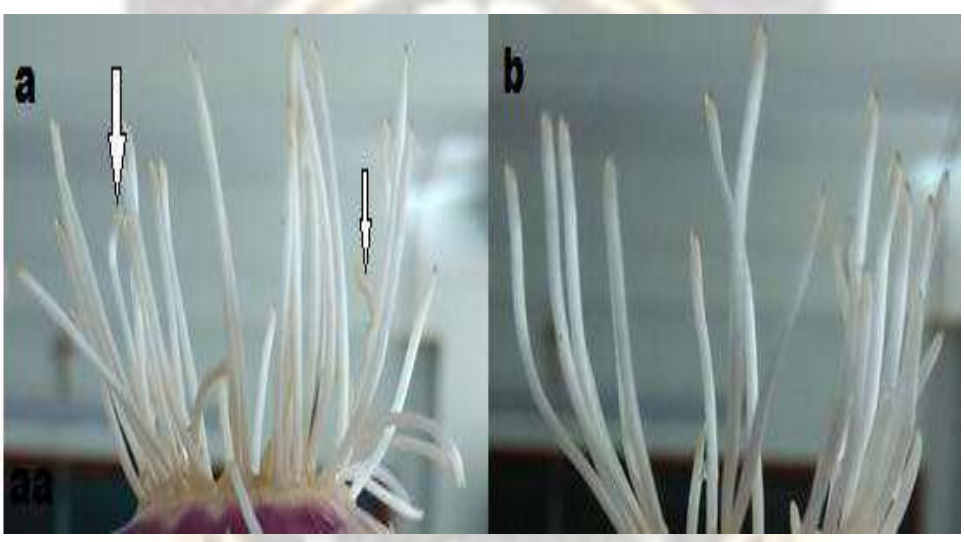

Figure 1: Effect of cyclophosphamide on root form- (a) Bending of root tips and roots with increased opacity seen in onions grown in 0.5mM cyclophosphamide solution (b) relatively less bent tips, with slight discoloration seen in onions pre-grown in extracts and post treated with cyclophosphamide.

Table 1 represents the effects of leaves extracts of *Carica papaya* on root growth of *Allium cepa*. Negative control (tap water) gave the highest root growth compared to plant extracts. A relatively low level of root growth inhibition was seen among all the tested extracts (order of growth inhibition: aqueous<ethanol<ethyl acetate<chloroform), however root growth inhibition was highest with 0.5mM cyclophosphamide, indicating its cytotoxic effect. Also variations in root form were also seen in the positive control, which were significantly reduced in test as shown in figure 1.

Table 1: Effect of CP extracts and Cyclophosphamide on Root form, Root length, Cell division, Mitotic index and Mitotic inhibition observed in *Allium cepa* root cells.

	Root form	Root length (in cm)		Mean TCC	Mean of dividing Cells	Mitotic Index	Mitotic Inhibition
		Largest	Smallest				
Negative control	Normal	2.8	0.2	183 ± 0.07	20 ± 0.14	9.1	0
Standard Control	Normal	2.0	0.1 - 0.2	157 ± 0.13	18 ± 0.05	8.7	0.4
Positive Control	Bent root tips , Thicker, Brighter roots	2.2	0.3	148 ± 0.03	19 ± 0.09	7.7	1.4
Aqueous	Normal	2.7	0.1 - 0.2	207 ± 0.06	22 ± 0.12	9.4	- 0.3
Chloroform	Normal	1.8	0.1 - 0.2	147 ± 0.15	18 ± 0.14	8.1	0.7
Ethanol	Normal	2.15 - 2.2	0.1 - 0.2	162 ± 0.07	19 ± 0.06	8.5	0.6
Ethyl -Acetate	Normal	2.05 - 2.1	0.1 - 0.2	142 ± 0.17	17 ± 0.13	8.4	0.7
Tap water +CPH	Bent, Thicker	2.4	0.1	218 ± 0.09	24 ± 0.04	9.0	0.1
Ascorbic acid +CPH	Very slight bending	2.0	0.1 - 0.2	205 ± 0.05	23 ± 0.07	8.9	0.2
Aqueous +CPH	Slight bending	2.1	0.1 - 0.2	238 ±0.04	25 ± 0.10	9.2	- 0.4
Chloroform + CPH	Very slight bending	1.9	0.1	158 ± 0.09	19 ± 0.04	8.3	0.8
Ethanol +CPH	Very slight bending	2.1	0.1	173 ± 0.12	20 ± 0.05	8.6	0.5
Ethylacetate +CPH	Slight bending	2.0	0.1 - 0.2	167± 0.10	20±0.3	8.3	0.8

CPH: Cyclophosphamide, TCC: Total counted cells.

The table 1 also represents the effect of all the fractions of CP, ascorbic acid and CPH on cell division and mitotic index. The number of dividing cells and mitotic index in the control group were higher compared to treated groups. *Allium cepa* roots pre grown with the fractions of CP and ascorbic acid on treatment with cyclophosphamide had negative effects on the reduction of mitotic index which was more pronounced with aqueous fraction followed by ethanolic fractions and ethyl acetate. Chloroform extract however caused acute reduction of MI. These findings show that aqueous, ethanolic and ethyl acetate fractions have significant anticytotoxic effects while the chloroform extract fails to exhibit potent anticytotoxicity. The reduction of MI value lower than control suggests the level of cytotoxicity that substance inflicts on meristematic cells. Conversely it was seen that though the extracts did not cause any cytotoxicity, they did not cause any significant increase in the MI, which means the extracts do not have any cytoproliferative activity. It is important to assess this property because vigorous proliferation of cells is considered dangerous to organisms, since this situation might results in malignant or tumor cells formation[22].

Antimutagenic activity of *Carica papaya* extracts against Cyclophosphamide induced mutagenicity.

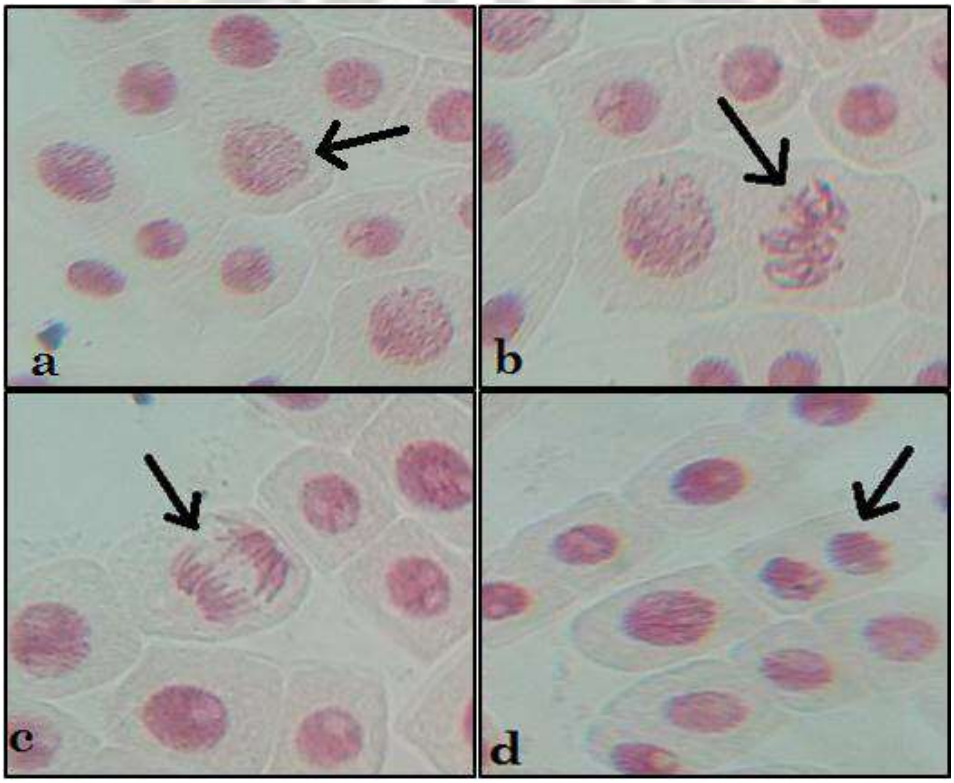

Figure 2: Dividing cells of *A. cepa* treated with plant extracts, negative and standard controls test - Normal prophase, metaphase, anaphase, and telophase are represented by (a), (b), (c) and (d), respectively.(Magnification 100X)

Chromosomal aberrations (CAs) are characterized by change in either total number of chromosomes or in chromosomal structure which occur as a result of the exposure of chemical treatment. To evaluate the different chromosomal abnormalities, several types of CAs are considered in different stages of cell cycle- Prophase, metaphase, anaphase and telophase[23]. *Allium cepa* chromosome assay was employed as a preliminary test to investigate the antimutagenic potential of four extracts of CP leaves, namely aqueous, chloroform, ethanol extract and ethyl acetate extract. On treatment with CP extracts, negative and standard controls the *Allium cepa* meristematic cells showed normal cell division without any kind of aberration as shown in figure 2, whereas treatment with cyclophosphamide caused various chromosomal aberrations in the *A. cepa* root cells.

Table 2: Chromosomal Aberrations (CAs) analysed by treatment with Controls, CP extracts, Cyclophosphamide and combination of the three in *Allium cepa* root cells.

Solution composition	Mean TCC	TAC	FOA
Negative control	183 ± 0.07	Nil	Nil
Standard Control	157 ± 0.13	Nil	Nil
Positive Control	148 ± 0.03	16	10.8 %
Aqueous	207 ± 0.06	Nil	Nil
Chloroform	147 ± 0.15	Nil	Nil
Ethanol	162 ± 0.07	Nil	Nil
Ethyl -Acetate	142 ± 0.17	Nil	Nil
Tap water+ CPH	218 ± 0.09	13	5.9 %
Ascorbic acid +CPH	205 ± 0.05	4	1.9 %
Aqueous +CPH	238 ±0.04	1	0.4 %
Chloroform +CPH	158 ± 0.09	3	1.8 %
Ethanol +CPH	173 ± 0.12	1	0.5 %
Ethylacetate +CPH	167± 0.10	2	1.1

TCC: Total counted cells, TAC: Total aberrant cells, FOA: Frequency of aberrations.

Figure 3 represents the cyclophosphamide induced aberrations: vagrant metaphase, anaphasic bridge, fragmented chromosomes, sticky prophase, nucleolar vacuolization and chromosome laggard. Pre-growing of *A. cepa* bulbs with extracts followed by treatment with cyclophosphamide caused over 90% reductions in FOA in the root cells as shown in table 2. This shows that all the fractions have the ability to protect *A. cepa* meristematic cells from cyclophosphamide induced mutagenesis, confirming the potent antimutagenic property of *C. papaya* extracts. The order of mutation prevention potential was found to be: aqueous>ethanol>ethyl acetate>chloroform.

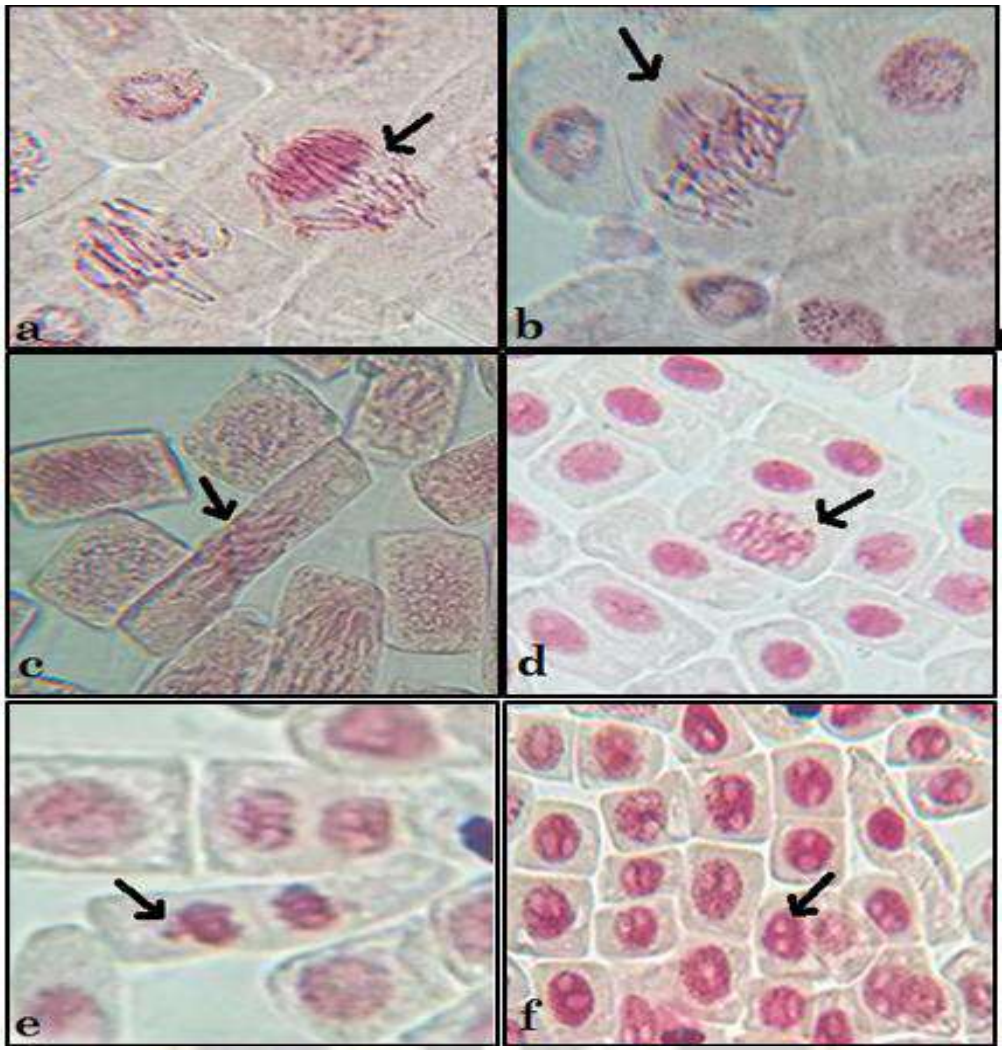

Figure 3: Dividing cells of *A. cepa* treated with cyclophosphamide (positive control): (a) Anaphasic bridges (b) Vagrant metaphase (c) Fragmented chromosomes (d) Sticky prophase (e) Laggard chromosome (f) Nuclear vacuolization. (Magnification 100X)

Mutagens are known to prompt gene mutations or alteration in cells and thus, may affect somatic and germinal cells. Improper or un-repair oxidation of DNA deoxyribose sugar and a nitrogenous base of ROS lead to double strand break, which contributes to chromosomal aberrations[24,25]. Induction of vagrant metaphase has been shown to lead to the formation of daughter cells with unequal sized or irregularly shaped nuclei at interphase because of unequal numbers of chromosomes in the daughter cells[26]. Formation of bridges also could be attributed to chromosome breaks, stickiness and breakage/reunion of broken ends[27]. The presence of stickiness has been interpreted to be due depolymerization of DNA[28], DNA condensation[29] or physical adhesion of chromosomal proteins[30]. Lagging chromosomes occur as the direct effect of mutagen on spindle apparatus and mostly cause somatic instability. These lagging chromosomes or fragments could

sometimes disappear in the cytoplasm and thus be lost giving rise to chromosome deletion which is always lethal to humans[31]. High frequency of all the above mentioned aberrations and more were present in cyclophosphamide treated *A. cepa* roots (FOA=10.8), However a steep reduction in FOA (FOA= 0.4%) contributed by the CP extracts show that these extracts have the potency to suppress the mutagen and thus could be a promising antimutagen.

CONCLUSION

In the continuous effort to evaluate potential medicinal values of the leaves of CP, the present research was conducted to investigate antimutagenic and anticytotoxic activities of ethanolic, ethyl acetate, chloroform and aqueous extracts of *C. papaya*. Mitotic index was used as an indicator to assess the cytotoxic level, whereas chromosomal aberrations were used to test mutagenicity of chemicals in cells. On evaluation of the mitotic index, mitotic inhibition and frequency of chromosomal aberration in *Allium cepa* root cells, treated with the mutagen, CP extracts and combination of both it was seen that the extracts did not exhibit any significant cytotoxic or cytoproliferative effect. However aqueous, ethanolic and ethyl acetate fractions prevented cyclophosphamide induced cytotoxicity in *Allium cepa* root cells. Assessment of frequency of aberration showed that all the fractions had the ability to protect *A. cepa* meristematic cells from cyclophosphamide induced mutagenesis, the order of aberration prevention being: aqueous>ethanol>ethyl acetate>chloroform, thus confirming the antimutagenic potential of the plant. Many cancer drugs available in markets and used in chemotherapies are cytotoxic to cells which explain their suppressiveness against cancer cells. Unfortunately, their cytotoxic capability might also affect the neighbouring normal cells which were obvious through many side effects encountered by patients undergoing chemotherapy such as nausea, fatigue, hair loss, and etc. Concerning this issue, it is very crucial to investigate potential antimutagens with less cytotoxic activity to normal cells. All in all, the results showed that extracts of *C. papaya* have the potency to suppress the mutagen and thus could be a promising antimutagen with less cytotoxic to normal cells.

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