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RESEARCH ARTICLE

Anticancer Activity of *Zea mays* Leaf Extracts on Oxidative Stress-induced Hep2 Cells

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Abstract

Cancer is one of the leading causes of death in humans. It is believed that plants can provide potential bioactive compounds for the development of "new leads" to combat cancer and other diseases. The present study focuses on the ability of the different extracts (aqueous, methanol, and chloroform) of the leaves of *Zea mays* in influencing the process of apoptosis induced by hydrogen peroxide (H_2O_2) in Hep2 (laryngeal carcinoma) cells. Various apoptosis-related parameters, such as cell viability, morphological changes, nuclear changes, and apoptotic index were characterized. sulforhodamine B and MTT assays were used to quantify the extent of cell death in the group exposed to H_2O_2, plant extracts, and their combination. Treatment with H_2O_2 caused cytotoxicity in cancer cells. The administration of leaf extract also caused an increase in the death of cancer cells. Oxidatively stressed cancer cells co-treated with all the *Z. mays* leaf extracts (except the chloroform extract) demonstrated cytotoxicity on a par with the H_2O_2-treated groups. This indicated that the aqueous and methanol leaf extracts did not influence the cytotoxic action of H_2O_2 in the cancer cells. Thus, various apoptosis-related events in Hep2 cells exposed to leaf extract throw light on the potential anticancer activity of the *Z. mays* leaves. The maximum activity was exerted by the methanolic extract followed by the aqueous and chloroform extracts.

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1. Introduction

Chemoprevention, a relatively new and promising strategy to prevent cancer, is defined as the use of natural dietary compounds and/or synthetic substances to block, inhibit, reverse, or retard the process of carcinogenesis. Various cancer chemopreventative agents can encourage apoptosis in premalignant and malignant cells *in vivo* and/or *in vitro*, which is conceivably another anticancer mechanism [1].

Thus, the search for natural products represents an area of great interest in which the plant kingdom has been documented as the most important source to provide many antioxidant and cancer chemopreventative agents with novel structures and unique mechanisms of action. With this backdrop, the present study was formulated to analyze the anticancer potential of *Zea mays* leaves. *Z. mays* is commonly known as *makkacholam* or maize. It belongs to the family Gramineae. Our preliminary studies with three different extracts (aqueous, chloroform, and methanol) revealed that it possesses strong antioxidant properties.

2. Materials and methods

2.1. Preparation of plant extracts

Z. mays seeds were procured from Tamil Nadu Agriculture University (TNAU) in Coimbatore district, Tamil Nadu. They were grown within the university campus in pots. Plant growth was observed at 5, 10, 15, 20, 25 and 30 days after sowing. The leaves on the 10th day of growth were found to have maximum antioxidant activity [2]. Hence, 10-day-old plants were selected for the present study. The plantlets were uprooted and washed thoroughly with running tap water. Then the leaves were blotted dry between folds of filter paper to remove excess water.

2.2. Aqueous extract

The leaves were homogenized in water (1 g/mL) using a micropestle in a microfuge tube. They were centrifuged at low speed to clarify the extract. The supernatant corresponding to the concentration of 20 mg/20 μL was used for the assay.

2.3. Methanolic/chloroform extract

Z. mays leaves (1.0 g) were homogenized in approximately 1 mL of the solvent (methanol/chloroform). The supernatant was collected and dried at 60°C, well protected from light. The residue obtained after drying the chloroform and methanol extracts was weighed and dissolved in a known amount of dimethylsulfoxide (DMSO) to yield a concentration of 20 mg/5 μL. DMSO was maintained at a minimum level to avoid DMSO-induced events, if any. The extract preparation took around 2 hours.

2.4. Hep2 cell line culture

The cell line was procured from the National Centre for Cell Science, Pune, India. The cells were maintained in a CO_2 incubator with 5% CO_2 and 95% humidity, and supplemented with Dulbecco's modified Eagle's medium (DMEM) and 10% fetal bovine serum (FBS). Penicillin and streptomycin (PAA) were also added to the medium to 1× final concentration from a 100 × stock.

Once the cells had attained confluent growth, the cells were trypsinized using Trypsin-EDTA (PAA) and the number of cells needed for carrying out various assays was seeded into sterile six-well and 96-well plates. In each well of the six-well plates, a clean, dry, sterile coverslip was placed before the cells were seeded. Then the plates were incubated in a CO_2 incubator with 5% CO_2 and 95% humidity.

Hydrogen peroxide (H_2O_2) at a concentration of 200μM was used as an oxidant. The concentration of plant extract used was 20 mg. The cells were treated with the oxidant, both in the presence and absence of the leaf extracts. The cells were exposed to H_2O_2 for 1 hour at 37°C. The time points were arrived at by conducting a time-related response analysis of each cell type.

After treatment, the coverslips from the six-well plates were removed and placed on a glass slide and sealed with Vaseline. These slides were used for various staining techniques; however in 96-well plates, the medium was removed and replaced with fresh medium. These were used for checking the viability status.

2.5. Parameter analysis

The apoptotic events that occurred after the oxidant treatment were analyzed using various parameters [viability assays and Giemsa, propidium iodide (PI), acridine orange/ethidium bromide (AO/EtBr), 4,6-diamino-2-phenylindol dihydro chloride (DAPI), and EtBr staining], as described below.

2.6. MTT assay

A total of $10^6/10^7$ cells per Eppendorf were seeded into 96-well plates and exposed to H_2O_2/plant extracts for 1 hour. The cytotoxicity of the drugs was assessed by the MTT assay according to the procedure used by Igarashi and Miyazawa [3]. MTT (50 μL) was added to the treated wells. The plate was incubated at 37°C for 3 hours with mild shaking. The cells were re-suspended in 200 μL of 2-propanol containing 0.04N HCl overnight in the dark. The absorbance was read at 650 nm, in a microtiter plate reader (Anthos, Germany).

2.7. Sulforhodamine B assay

Sulforhodamine B (SRB) binds to basic amino acid residues in TCA-fixed cells to provide a sensitive index of cellular protein content that is linear over a range of cell densities [4]. The cell survival was measured at 492 nm.

2.8. Giemsa staining

The incubated cells were spread on the microscopic slides with a drop of diluted Giemsa stain. The slides were mounted with cover slips and observed under a phase contrast microscope (Nikon, Japan) for morphological changes as described by Chih et al [5]. The number of cells showing apoptotic morphological changes was counted in

each experimental group per 100 cells in 10 different fields and the experiment was repeated five times.

2.9. PI staining

PI staining was employed to discriminate apoptotic cells from normal cells, which reflects the nuclear changes during apoptosis using the protocol developed by Sarker et al [6]. After incubation with H_2O_2 and/or plant extracts, the cells were permeabilized with a mixture of acetone and methanol (1:1) at −20°C for 10 minutes. Then, 10 μL of 5 μg/mL PI was added to each slide, spread with a cover slip and incubated at 37°C for 30 minutes in the dark. The apoptotic cells were detected using the green filter of a fluorescence microscope (Nikon, Japan).

2.10. EtBr staining

The treated cells were incubated for 5 minutes with 10 μL of EtBr (50 μg/mL) and spread by placing a cover slip over it. The apoptotic cells were scored by counting the cells with condensed chromatin and fragmented nuclei under a fluorescence microscope (Nikon, Japan) using UV 2A filter at 400× magnification. The ratios of apoptotic cells to normal cells were calculated in each staining method [7].

2.11. DAPI staining

The apoptotic ratios of the oxidant-treated cells with or without the leaf extract to the untreated cells were calculated by DAPI staining [8]. The cells were then transferred to slides and were immediately fixed with 3% paraformaldehyde and permeabilized with 0.2% Triton X-100 in phosphate-buffered saline (PBS) for 10 minutes at room temperature. They were then incubated with 5 μg/mL of DAPI. The apoptotic nuclei were observed under a fluorescence microscope (Motic, Germany) using a DAPI filter.

2.12. AO/EtBr staining

AO (0.1 mg/mL) and EtBr (0.1 mg/mL) were used to label nuclear DNA in Hep2 cells. Both solutions were prepared in PBS buffer (pH 7.4). PBS buffer was used to preserve normal physiological activity for unicellular cells. For cell staining, Hep2 cell samples (100 μL) were stained with AO/EtBr (5 μL) and observed under a fluorescence microscope (Nikon, Japan) with a B-2A filter [9].

2.13. Statistical analysis

Statistical significance was determined by a two-way analysis of variance ($p < 0.01$) using Agress statistical software (version 3.01).

3. Results and discussion

3.1. Cell viability assays

Cell viability was assessed by MTT assay, which is a colorimetric assay for measuring the activity of cellular enzymes

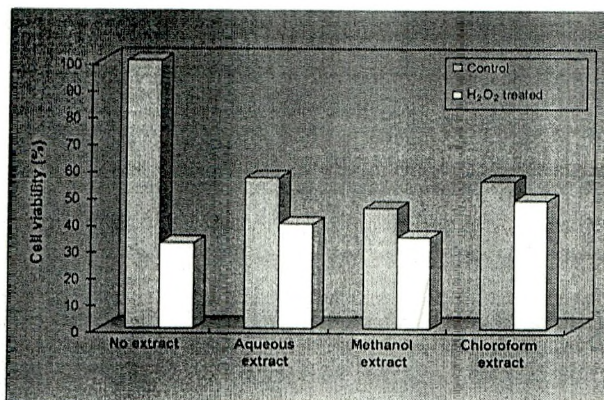

Figure 1 Effect of *Zea mays* leaf extracts on the viability of Hep2 cells subjected to oxidative stress as determined by the MTT assay. The values of the negative (untreated) control group were fixed as 100% viability and the percentage viabilities in the other groups were calculated relative to this.

that reduce the tetrazolium dye, MTT, to its insoluble formazan, giving a purple colour. Fig. 1 shows the results of evaluation of cytotoxicity of all three extracts of *Z. mays* at a concentration of 20 mg/mL and an exposure time of 1 hour. These data showed that the treatment with H_2O_2 exhibited cytotoxicity in the cancer cells. The administration of leaf extracts also caused increased death of cancer cells. Oxidatively stressed cancer cells co-treated with the *Z. mays* leaf extracts (apart from the chloroform extract) showed cytotoxicity on a par with H_2O_2-treated groups. This indicates that the aqueous and methanol leaf extracts did not influence the cytotoxic action of H_2O_2 in the cancer cells. Using the MTT assay, many researchers found time- and dose-dependent inhibition in different cells with different agents.

Inhibition of cell proliferation by sesquiterpene lactones (tomentosin and inuviscolide) from *Inula viscosa* (Compositae) leaves against human melanoma cell lines was reported by Rozenblat et al [10].

An SRB assay was also performed to assess cell survival. The results of this assay showed a similar trend to that

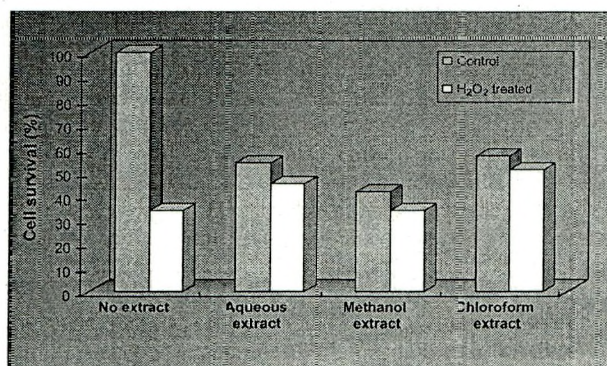

Figure 2 Effect of *Zea mays* leaf extracts on the viability of Hep2 cells subjected to oxidative stress as determined by the SRB assay. The values of the negative (untreated) control group were fixed as 100% viability and the percentage viabilities in the other groups were calculated relative to this.

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Table 1 Effect of *Zea mays* leaf extracts on the morphological changes in Hep2 cells subjected to oxidative stress as determined by Giemsa staining.

Sample	No. of apoptotic cells/100 cells		Apoptotic ratio	
	Control	H_2O_2 treated	Control	H_2O_2 treated
No extract	9 ± 1	$52 \pm 2^{*}$	0.10	1.10
Aqueous extract	$45 \pm 2^{*}$	$60 \pm 1^{*,\dagger,\ddagger}$	0.81	1.50
Methanol extract	$63 \pm 3^{*}$	$68 \pm 2^{*,\dagger,\ddagger}$	1.70	2.13
Chloroform extract	$40 \pm 2^{*}$	$54 \pm 2^{*,\ddagger}$	0.67	1.17

The values are means ± SD of triplicates. CD value = 4.7.
* Statistically significant ($p < 0.01$) compared with untreated control group.
† Statistically significant ($p < 0.01$) compared with oxidant treated group.
‡ Statistically significant ($p < 0.01$) compared with respective plant extract treated group.

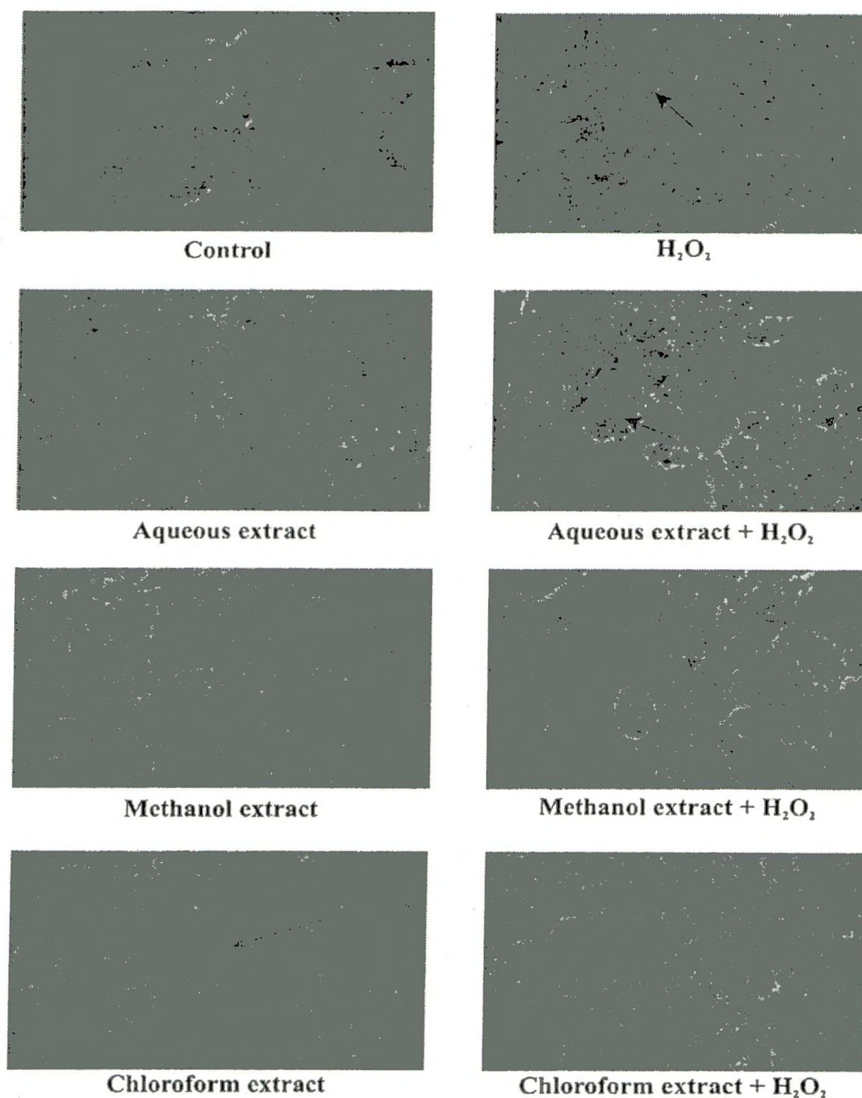

Figure 3 Apoptotic Hep2 cells showing membrane blebbing when stained with Giemsa exposed to 200 μM of H_2O_2 at 100× magnification. The number of shrunken cells was found to increase in the groups co-treated with the leaf extracts. The apoptotic cells with blebbing, cell shrinkage, nuclear fragmentation, and margination of the nucleus are indicated by arrows.

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Table 2 Effect of *Zea mays* leaf extracts on nuclear changes in Hep2 cells subjected to oxidative stress as determined by PI staining.

Sample	No. of apoptotic cells/100 cells		Apoptotic ratio	
	Control	H_2O_2 treated	Control	H_2O_2 treated
No extract	8 ± 3	51 ± 2*	0.09	1.04
Aqueous extract	48 ± 2*	59 ± 2*,†,‡	0.92	1.44
Methanol extract	61 ± 1*	73 ± 1*,†,‡	1.56	2.70
Chloroform extract	34 ± 2*	49 ± 1*,‡	0.52	0.96

The values are means ± SD of triplicates. CD value = 4.46.
* Statistically significant ($p < 0.01$) compared with the untreated control group.
† Statistically significant ($p < 0.01$) compared with the oxidant-treated group.
‡ Statistically significant ($p < 0.01$) compared with the respective plant extract-treated group.

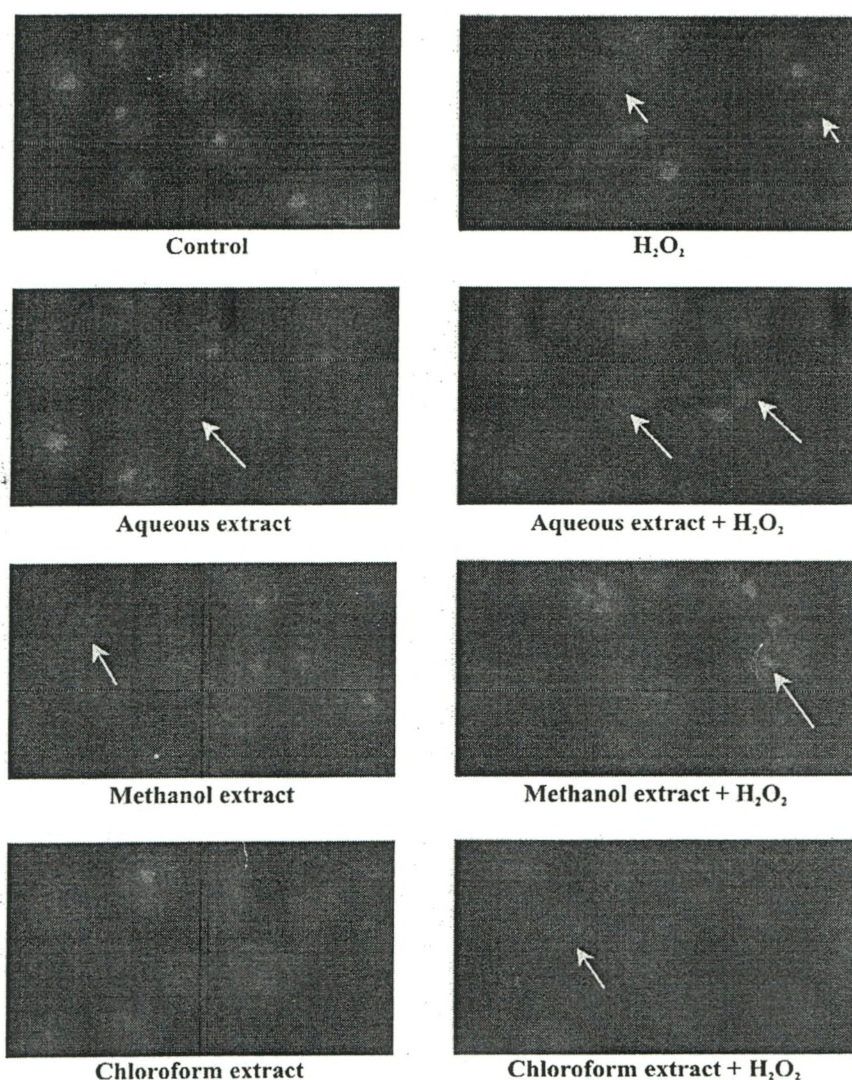

Figure 4 Photographic record of the apoptosing cells in each treatment group stained by PI. A greater number of apoptotic cells were observed in the leaf extract-treated groups exposed to oxidative stress. The apoptotic cells with blebbing, cell shrinkage, nuclear fragmentation, and margination of the nucleus are indicated by arrows.

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Table 3 Effect of *Zea mays* leaf extracts on nuclear changes in Hep2 cells subjected to oxidative stress as determined by EtBr staining.

Sample	No. of apoptotic cells/100 cells		Apoptotic ratio	
	Control	H_2O_2 treated	Control	H_2O_2 treated
No extract	5 ± 3	48 ± 2*	0.05	0.92
Aqueous extract	47 ± 2*	55 ± 2*,†,‡	0.89	1.22
Methanol extract	61 ± 2*	70 ± 1*,†,‡	1.56	2.33
Chloroform extract	35 ± 2*	50 ± 2*,‡	0.54	1.00

The values are means ± SD of triplicates. CD value = 4.9.
* Statistically significant ($p < 0.01$) compared with the untreated control group.
† Statistically significant ($p < 0.01$) compared with the oxidant-treated group.
‡ Statistically significant ($p < 0.01$) compared with the respective plant extract-treated group.

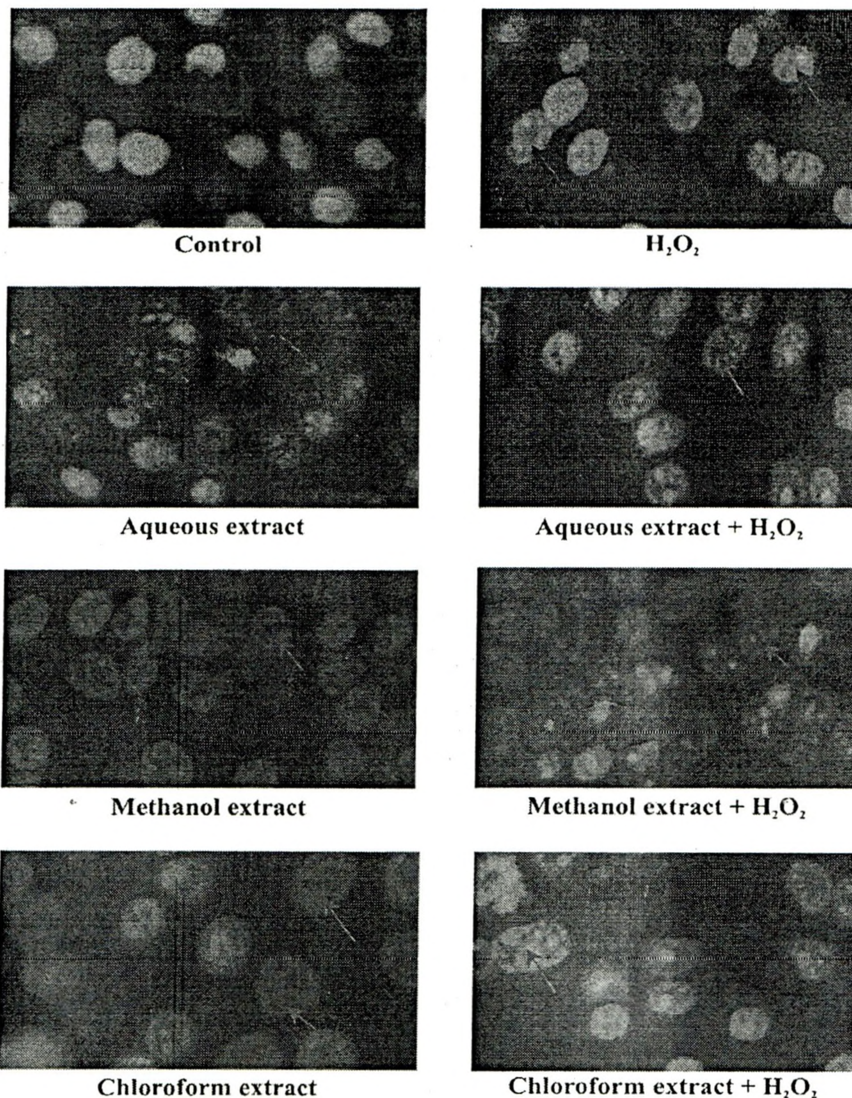

Figure 5 Apoptotic Hep2 cells showing membrane and nuclear changes when stained with ethidium bromide exposed to 200 μM of H_2O_2 at 100× magnification. These pictures indicate that the *Zea mays* leaves were highly efficient at inducing apoptosis in cancer cells, both in the presence and absence of H_2O_2. The apoptotic cells with blebbing, cell shrinkage, nuclear fragmentation, and margination of the nucleus are indicated by arrows.

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Table 4 Effect of *Zea mays* leaf extracts on nuclear changes in Hep2 cells subjected to oxidative stress as determined by DAPI staining.

Sample	No. of apoptotic cells/100 cells		Apoptotic ratio	
	Control	H_2O_2 treated	Control	H_2O_2 treated
No extract	6 ± 2	68 ± 2*	0.06	2.13
Aqueous extract	47 ± 2*	56 ± 1*,†,‡	0.89	1.27
Methanol extract	50 ± 3*	58 ± 3*,†,‡	1.00	1.38
Chloroform extract	43 ± 3*	53 ± 1*,†,‡	0.75	1.13

The values are means ± SD of triplicates. CD value = 5.4.
* Statistically significant ($p < 0.01$) compared with the untreated control group.
† Statistically significant ($p < 0.01$) compared with the oxidant-treated group.
‡ Statistically significant ($p < 0.01$) compared with the respective plant extract-treated group.

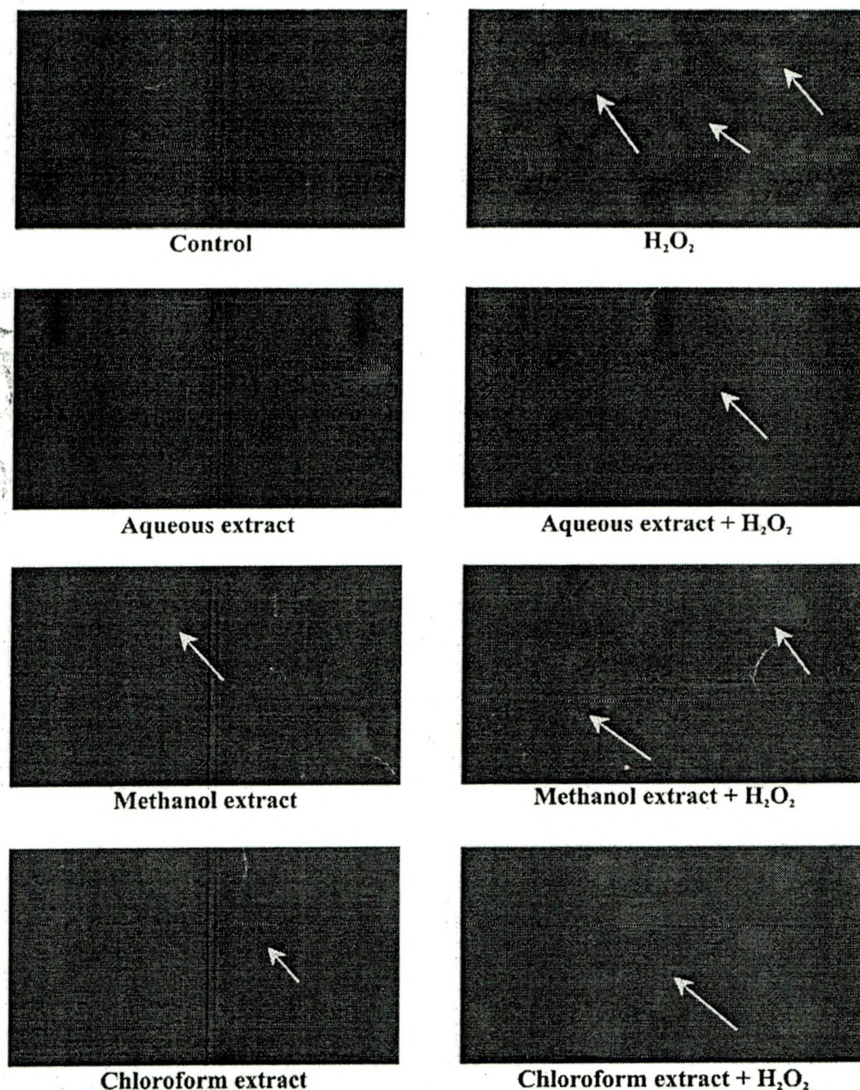

Figure 6 Apoptotic shrunken Hep2 cells with nuclear changes when stained with DAPI exposed to H_2O_2. The most apoptotic cells were found in the leaf extract-treated groups. The apoptotic cells with blebbing, cell shrinkage, nuclear fragmentation, and margination of the nucleus are indicated by arrows.

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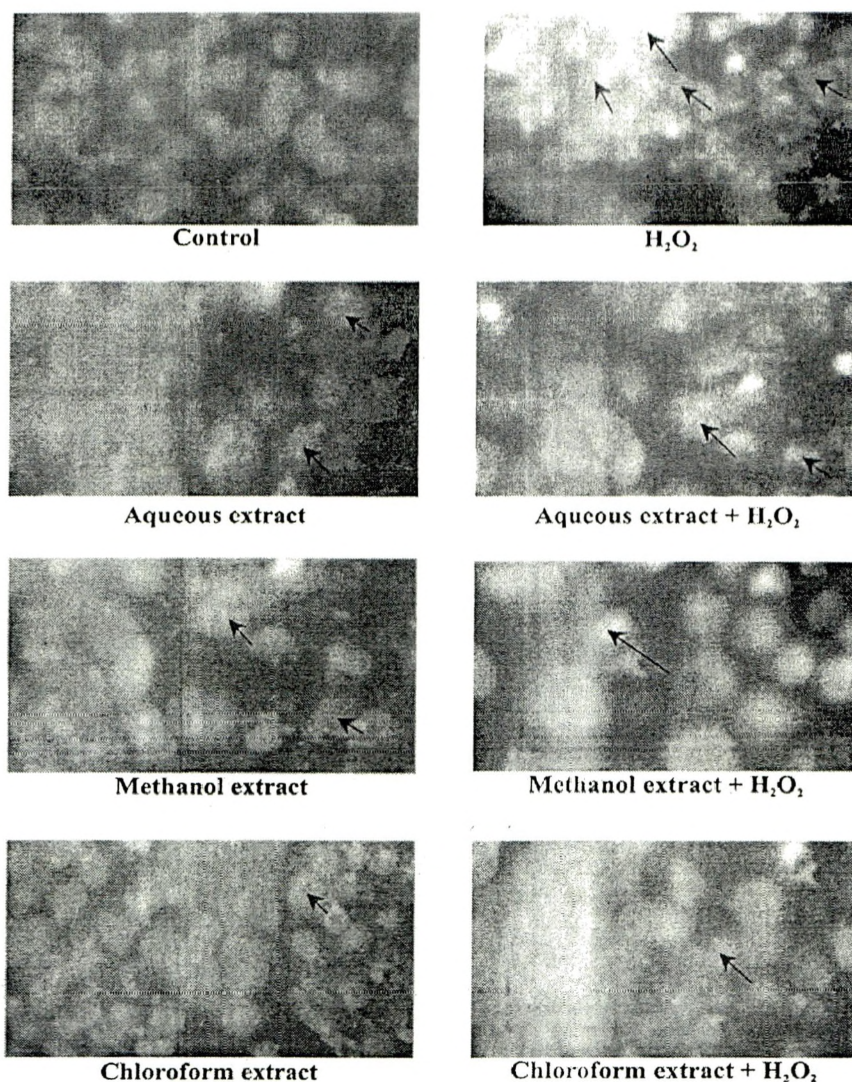

Figure 7 Hep2 cells showing red fluorescence indicated that they are undergoing apoptosis with acridine orange/ethidium bromide stain and normal cells showed green fluorescence. This staining method clearly indicates the anticancer effect of *Zea mays* leaf extracts. The apoptotic cells with blebbing, cell shrinkage, nuclear fragmentation, and margination of the nucleus are indicated by arrows.

observed in the MTT assay, and are presented in Fig. 2. An extract of the rhizomes of *Iris tectorum* Maxim exhibited a very high cytotoxicity against MCF-7 (breast cancer) and C32 (melanoma) cell lines [11].

3.2. Membrane morphological characteristics

Morphological changes, including plasma membrane blebbing, changes to the cell membrane, such as loss of membrane asymmetry and attachment and cell shrinkage, are the early stages of apoptosis that can be analyzed by Giemsa staining. The ratios of cells that undergo apoptosis to normal cells, when exposed to H_2O_2 in both the presence and absence of *Z. mays* leaf extracts, are presented in Table 1.

The results revealed that the occurrence of morphologically altered cells was greatest in the H_2O_2-treated group. Treatment with the leaf extracts also exerted an apoptosis-inducing effect in the cancer cells (Fig. 3). The number of apoptotic cells increased further upon co-exposure of the leaf extract with H_2O_2. The methanolic extract of *Z. mays* leaves was found to be very active against Hep2 cells. A crude ethanolic extract of the plant *Lycopodium clavatum* considerably inhibited the growth of HeLa cells through the induction of apoptosis via caspase-3 activation, as determined by morphological changes [12].

4. Nuclear changes

Apoptosis is a form of cell death that allows for the elimination of damaged or unwanted cells without damaging the organism. The most obvious characteristics of this form of cell death are cytoplasmic and nuclear condensation, followed by internucleosomal DNA cleavage, membrane blebbing, and finally cell fragmentation [13].

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Table 5 Effect of *Zea mays* leaf extracts on nuclear changes in Hep2 cells subjected to oxidative stress as determined by AO/EtBr staining.

Sample	No. of apoptotic cells/ 100 cells		Apoptotic ratio	
	Control	H_2O_2 treated	Control	H_2O_2 treated
No extract	8 ± 2	$54 \pm 3^{*}$	0.09	1.17
Aqueous extract	$47 \pm 3^{*}$	$65 \pm 1^{*,\dagger,\ddagger}$	0.89	1.85
Methanol extract	$64 \pm 1^{*}$	$72 \pm 1^{*,\dagger,\ddagger}$	1.78	2.58
Chloroform extract	$39 \pm 1^{*}$	$54 \pm 3^{*,\ddagger}$	0.64	1.17

The values are means ± SD of triplicates. CD value = 4.98.
* Statistically significant ($p < 0.01$) compared with the untreated control group.
† Statistically significant ($p < 0.01$) compared with the oxidant-treated group.
‡ Statistically significant ($p < 0.01$) compared with the respective plant extract-treated group.

Nuclear changes were observed in Hep2 cells exposed to H_2O_2 in both the presence and absence of the *Z. mays* leaf extracts using various staining techniques (PI, EtBr, DAPI, and AO/EtBr).

The data obtained with PI staining and the apoptotic indexes calculated are presented in Table 2. As shown in Fig. 4, control cells exhibited intact nuclei, but cells treated with 200 μM of H_2O_2 showed significant nuclear fragmentation. The apoptotic ratio of cells treated with oxidant was 1.04, whereas the treated cells co-exposed to leaf extracts (water, methanol, and chloroform) were 1.44, 2.70, and 0.96, which indicates that the *Z. mays* leaf extracts did not influence the cytotoxic action of H_2O_2 in the cancer cells. The effect of an ethanolic extract of *Tremella mesenterica* on the induction of apoptosis in human lung carcinoma A549 epithelial cells was also assessed by PI staining [14].

In addition to the observation of nuclear fragmentation, the anticancer potential of leaf extracts was confirmed by EtBr staining in each treatment group and the results are given in Table 3. The nuclear changes observed are shown in Fig. 5.

H_2O_2-treated cells co-treated with leaf extract exhibited morphological changes typical of apoptosis, including cell shrinkage, plasma membrane blebbing, chromatin condensation, and nuclear fragmentation, compared with the control cells with prominent rounded nuclei and defined plasma membrane contours. The leaf extracts, by themselves, also caused an induction of apoptosis in Hep2 cells. The aqueous and chloroform extracts also exhibited considerable apoptotic effects in the cancer cells.

Two phytochemicals, resveratrol and citroflavon-3-ol, and four plant extracts (grape seed polyphenols, olive oil extract, bearberry, and *Echinacea*) examined for their effect on cell viability by the EtBr assay provided evidence for strong protection against oxidative stress in H_2O_2 stress-induced U937 cells [15].

DAPI staining is used to observe the nuclear contents in shrunken cells. DAPI has been used to study pamidronate, anti-proliferative, apoptotic, and anti-migratory effects in hepatocellular carcinoma cells [16]. The extent of nuclear changes observed during H_2O_2-induced apoptosis after DAPI staining in the different treatment groups of Hep2 cells are presented in Table 4.

As is deducible from the values listed in Table 4, a trend similar to that of EtBr was noted with DAPI staining. The cells with altered nuclear changes were found to be greatest in the H_2O_2-treated group, which shows the apoptotic ratio of 0.92. The extracts of *Z. mays* leaves were also highly efficient in inducing apoptosis in cancer cells, both in the presence and in the absence of H_2O_2 (Fig. 6). Human breast cancer cells treated with the extracts of *Astrodaucus persicus* also showed potential decrease in the cell proliferation by staining with DAPI. [17] The results with Giemsa and the nuclear stains were further affirmed by AO/EtBr staining (Fig. 7), as is evident from the data obtained (Table 5).

AO stain is membrane permeable and marks the nuclei green, and EtBr, which binds to DNA, is mainly taken up by cells when membrane integrity is lost and stains the nuclei red. Since AO intercalates in the DNA but only interacts with the RNA, viable cells do not uptake EtBr and these cells exhibit green nuclei. However, EtBr is taken up by dying cells, which turn red [18].

Treatment with a combination of AO/EtBr has been used as a reliable index of cellular degeneration [19]. Sanguinarine, a benzophenanthridine alkaloid derived from the root of *Sanguinaria canadensis*, induced apoptosis in human cancer cells, which was assessed by AO/EtBr staining [20].

Phytochemical screening of the leaves identified phenolics and flavonoids as the major antioxidant components in the leaves of *Z. mays* [21]. The cancer-protective effects of flavonoids and phenolics have been attributed to a wide variety of mechanisms, including free radical scavenging, modifying enzymes that activate or detoxify carcinogens [22].

5. Conclusion

The aim of anticancer agents is to trigger the apoptosis signaling system in these cancer cells while disturbing their proliferation. Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory, and anticancer activities. Therefore, many plants have been examined to identify new and effective antioxidants and anticancer compounds, as well as to elucidate the mechanisms of cancer prevention and apoptosis. Our

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findings suggest that the *Z. mays* leaf extracts most likely have anticancer properties. The qualitative analysis revealed the presence of phenolics and flavonoids in *Z. mays* leaves, which may contribute to their medicinal properties.

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References

1. Hail Jr N, Cortes M, Drake EN, Spallholz JE. Cancer chemoprevention: a radical perspective. *Free Radic Biol Med.* 2008; 45:97–110.
2. Kiruthika B, Padma PR. Screening of antioxidant properties of *Zea mays* leaves at different time periods of growth. *J Pharm Res.* 2012;5:4034–4037.
3. Igarashi M, Miyazawa T. The growth inhibitory effect of conjugated linoleic acid on a human hepatoma cell line, HepG2, is induced by a change in fatty acid metabolism, but not the facilitation of lipid peroxidation in the cells. *Biochem Biophys Acta.* 2001;1530:162–171.
4. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst.* 1990;82:1107–1112.
5. Chih HW, Chiu HF, Tang KS, Chang FR, Wu YC. Bullatacin a potent antitumor annonaceous acetogenin, inhibits proliferation of human hepatocarcinoma cell line 2.2.15 by apoptosis induction. *Life Sci.* 2001;69:1321–1331.
6. Sarker KP, Obara S, Nakata M, Kitajima I, Maruyama I. Anandamide induces apoptosis of PC-12 cells: involvement of superoxide and caspase-3. *FEBS Lett.* 2000;472:39–44.
7. Mercille S, Massie B. Induction of apoptosis in nutrient-deprived cultures of hybridoma and myeloma cells. *Biotechnol Bioeng.* 1994;44:1140–1154.
8. Rashmi R, Santhosh Kumar TR, Karunagaran D. Human colon cancer cells differ in their sensitivity to curcumin-induced apoptosis and heat shock protects them by inhibiting the release of apoptosis-inducing factor and caspases. *FEBS Lett.* 2003;538:19–24.
9. Parks DR, Bryan VM, Oi VT, Herzenberg LA. Antigen specific identification and cloning of hybridomas with a fluorescence activated cell sorter. *Proc Natl Acad Sci USA.* 1979;76:1962–1966.
10. Rozenblat S, Grossman S, Bergman M, Gottlieb H, Cohen Y, Dovrat S. Induction of G2/M arrest and apoptosis by sesquiterpene lactones in human melanoma cell lines. *Biochem Pharmacol.* 2008;75:369–382.
11. Fang R, Houghton PJ, Hylands PJ. Cytotoxic effects of compounds from *Iris tectorum* on human cancer cell lines. *J Ethnopharmacol.* 2008;118:257–263.
12. Mandal SK, Biswas R, Bhattacharyya SS, Paul S, Dutta S, Pathak S, et al. Lycopodine from *Lycopodium clavatum* extract inhibits proliferation of HeLa cells through induction of apoptosis via caspase-3 activation. *Eur J Pharmacol.* 2010;626: 115–122.
13. Fritzer-Szekeres M, Salamon A, Grusch M, Horvath Z, Hochtl T, Steinbrugger R, et al. Trimidox, an inhibitor of ribonucleotide reductase, synergistically enhances the inhibition of colony formation by Ara-C in HL-60 human promyelocytic leukemia cells. *Biochem Pharmacol.* 2002;64:481–485.
14. Chen NY, Lai HH, Hsu TH, Lin FY, Chen JZ, Lo HC. Induction of apoptosis in human lung carcinoma A549 epithelial cells with an ethanol extract of *Tremella mesenterica*. *Biosci Biotechnol Biochem.* 2008;72:1283–1289.
15. Carpenter R, O'Callaghan YC, O'Grady MN, Kerry JP, O'Brien NM. Modulatory effects of resveratol, citroflavan-3-01 and plant derived extracts on oxidative stress in U937 cells. *J Med Food.* 2006;9:187–195.
16. Wada A, Fukui K, Sawai Y, Imanaka K, Kiso S, Tamura S, et al. Pamidronate induced anti-proliferative, apoptotic, and anti-migratory effects in hepatocellular carcinoma. *J Hepatol.* 2006;44:1[illegible]–150.
17. Abdolmohammadi MH, Fouladdel SH, Shafiee A, Amin GH, Ghaffari SM, Azizi E. Anticancer effects and cell cycle analysis on human breast cancer T47D cells treated with extracts of *Astrodaucus persicus* (Boiss.) Drude in comparison to doxorubicin. *Daru.* 2008;16:112–118.
18. Giral H, Villa Bellosta R, Catalan J, Sorribas V. Cytotoxicity of peroxisome proliferator-activated receptor α and γ agonists in renal proximal tubular cell lines. *Toxicol In Vitro.* 2007;21: 1066–107[illegible].
19. Campos-de-Paz M, Pereira LO, Bicalho LS, Dorea JG, Pocas-Fonseca M, Santos Mde F. Interaction of bracken-fern extract with vitamin C in human submandibular gland and oral epithelium cell lines. *Mutat Res.* 2008;652:158–163.
20. Han MH, Yoo YH, Choi YH. Sanguinarine-induced apoptosis in human leukemia U937 cells via Bcl-2 downregulation and caspase-3 activation. *Chemotherapy.* 2008;54:157–165.
21. Kiruthika B, Padma PR. Protection of precision cut-goat liver slices by *Zea mays* leaf extracts from hydrogen peroxide induced oxidative stress *in vitro*. *Int J Pharm Bio Sci.* 2012;3: 201–2[illegible]0.
22. Moon YJ, Wang X, Morris ME. Dietary flavonoids: effects on xenobiotic and carcinogen metabolism. *Toxicol In Vitro.* 2006; 20:1[illegible]–2[illegible].

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