Free Radical Scavenging Activity and Cytotoxic Effect of Anisaldehyde on Human Cancer Cell Lines

C. Arulvasu*, S.Sivaranjani, M. Revathi, M. Hemavathi
*Department of Zoology, Life Science Building, University of Madras, Guindy Campus,
Chennai-600 025, India
E-mail: carulvasu@gmail.com

Abstract-**The present investigation was an attempt to evaluate the antioxidant and anticancer property of Anisaldehyde *in vitro.* Anisaldehyde is an organic compound that consists of a benzene ring substituted with an aldehyde and a methyl group. It is very effective as a carminative (to relieve gas pains). In this study, free radical scavenging activity was evaluated using DPPH (α,α-diphenyl-β-crylhydrazyl), Iron chelating, Reducing power assay and the cytotoxicity was assessed by MTT method using human cancer cell lines. The lowest DPPH free radical scavenging activity of Anisaldehyde obtained was 20% at 400 µg/ml and the highest DPPH free radical scavenging activity obtained was 21% at 800µg/ml concentrations.Maximum chelation of metal ions obtained was 27% at 1000 µg/ml and the reductive capability was high in 23% at 1000 µg/ml. The Anti-cytotoxic activity was evaluated against various cancer cell lines such as human breast adrenocarcinoma cell line (MCF 7), liver adrenocarcinoma (HepG2) and epidermoid carcinoma (ME 180). The IC_{50} values of 400,600 and 800µg/ml at MCF 7, HepG2 and ME 180 cell lines respectively. In this result the Anisaldehyde showed a superior effect on MCF 7 compared to other cell lines. Therefore, the Anisaldehyde proved to be a potent antioxidant and anticancer property against human cancer cell lines.**

Key words: DPPH; MTT; MCF-7; Hep G2; ME 180; Apoptosis.

I. INTRODUCTION

All living organisms contain complex system of antioxidant enzymes. Antioxidants in biological system have multiple functions, including defending against oxidative damage and participating in the major signaling pathways of cells. One major action of antioxidants in cells is to prevent damage caused by the reactive oxygen species, which includes hydrogen peroxide (H_2O_2), the superoxide anion (O^{-2}), and free radicals such as hydroxyl radical ($^{\cdot}OH$). These molecules are unstable and highly reactive, can damage cells by chain reaction and by lipid peroxidation or formation of DNA adducts that could cause cancer-promoting mutations or cell death. Free radical mediated modification of DNA, proteins, lipids and small cellular molecules are associated with a number of pathological processes, together with atherosclerosis, arthritis, diabetes, catarctogenesis, cancer, muscular dystrophy, pulmonary dysfunction, inflammatory disorders, ischemia reperfusion tissue damage, and neurological disorders, such as Alzheimer's disease [1].

The consumption of fruits and vegetables containing antioxidants has been found to offer protection against these diseases. Dietary antioxidants can augment cellular defenses and help to prevent oxidative damage to cellular components [2] besides antioxidant playing an important role in physiological system. Antioxidants have been used in the food industry to prolong the shelf life of foods, especially those rich in polyunsaturated fats. These components in food are readily oxidized by molecular oxygen and are major causes of quality deterioration, nutritional losses, off-flavor development and discoloration. Moreover, the addition of several synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) are commercially available and currently used. However, these antioxidants have been restricted for use in foods as they are suspected to be carcinogenic. Some toxicological studies have also implicated the use of these synthetic antioxidants in promoting the development of cancerous cells in rats. These findings, together with consumer's interests in natural food additives, have reinforced the efforts for the development of alternative antioxidants of natural origin [3]. In particular, interest has intensified in the class of organic compounds present in normal diet and in many folk medicines still in use. Organic compound derived from natural products have been implicated as beneficial agent in a multitude diseases states, most commonly cancer [4].

Anisaldehyde or anisic aldehyde is an organic compound that consists of a benzene ring substituted with an aldehyde and a methyl group. It is a clear colorless liquid with a strong aroma in nature, Star anise was considered as antibacterial, antifungal and expectorant which has been used to relieve bronchitis and has even

been induced in cough mixtures to relieve dry cough as well as a flavoring. It is believed to be a particular effective stomachic that strengthens stomach function and help to relieve colic flatulence, nausea and vomiting. Star anise is regarded as an antispasmodic for the gastrointestinal tract and helps to relieve abdominal pain and general gastrointestinal distress. Anise also contains compounds that are estrogenic (anethol, similar to estrogen female hormone) and promotes menstruation, facilitating childhood, increasing libido in women and oddly enough people have reported androgenic (male hormone) effect as well. Star anise oil is used in perfumery and soaps, improves memory, gets rid of oily skin, calm cough, increases milk production for nursing mothers and serve as a natural antacid. Commercially, it is very popular as a fragrance and a flavoring. It is very effective as a carminative (to relieve gas pains). Therefore, we focused on Anisaldehyde compound, which had been reported to contain various biological activities. Hence, the present study was carried out to evaluate the antioxidant capacities of Anisaldehyde and also its impact of cytotoxicity against human cancer cell lines.

II. MATERIALS AND METHODS

A. Chemicals

Anisaldehyde compound (4-Methoxybenzaldehyde) was purchased from sigma chemical company, st. Louis, Mo, USA., 2, 2-diphenyl-1- picrylhydrazyl (DPPH), Dulbecco's Modified Eagle Medium (DMEM), Trypsin-EDTA, Fetal Bovine Serum (FBS), 3-(4, 5- dimethylthiazol-2yl-2, 5-diphenyltertrazolium bromide (MTT) and antibiotic solution were purchased from Hi Media Laboratories, Mumbai, India.

B.Free radical scavenging assay

a. DPPH radical Scavenging Assay

DPPH assay was performed according to the method of [6] with a slight modification in the concentration and volume. Briefly, reaction mixture contain 200 µl of different concentration (200, 400, 600, 800 & 1000 µg/ml corresponding to 1.5, 3, 4.5, 6 & 7.5 mM/ml) of Anisaldehyde. Finally 2 ml of DPPH (0.1 mM) in methanolic solution was added to each tube and incubated in dark for 30 min at 37ºC. The absorbance was measured at 517 nm against a blank and positive control of Gallic acid. The radical scavenging activity was expressed as an inhibition percentage as follows;

$$\text{Percentage (\%) of radical scavenging activity} = [A_{\text{Contorl}} - A_{\text{Sample}} / A_{\text{Control}}] \times 100$$

b. Ion- chelating assay

The chelating activity of ferrous ions by anisaldehyde and standard was evaluated following the method of [7], with slight modification. Briefly, 600 µl of anisaldehyde was taken in different concentration and mixed with 3.5 ml of methanol and then the mixture was reacted against ferrous chloride (2 mM, 0.1 ml) and ferrozine (1 mM, 0.2 ml) for 10 min at room temperature. The absorbance was measured at 562 nm. The % of inhibition of ferrozine-Fe^{2+}complex formation was determined using following formula,

$$\text{Percentage (\%) of Inhibition} = [1 - AS/AC] \times 100$$

c. Reducing power assay

Reductive power of anisaldehyde was determined by the method of [8]. Briefly, the Different concentrations (2, 4, 6, 8 and 10 µl) of anisaldehyde were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1 % potassium ferric cyanide. The mixture was incubated at 50ºC for 20 min. A volume of 2.5 ml 10 % trichloroacidic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. Aliquot of supernatant (1 ml) was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (0.1 %), the absorbance was recorded at 700 nm. Increase in absorbance indicated increased reducing activity. BHA was used as the standard.

B. Effects on different cancer cell lines

a. Cell lines

Cancer cell lines MCF 7, Hep G2 and ME 180 were obtained from National Centre for Cell Science (NCCS), Pune, India. Cells were maintained as a monolayer in cell culture flask at 37°C under 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) and supplemented with 10% Fetal Bovine Serum (FBS), 100 µg/ml penicillin and streptomycin.

b. Cytotoxicity assay

The cytotoxic effects of different cancer cell lines were evaluated by MTT (3- (4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide) method as described by [9].In order to detect cytotoxicity, the cancer cells were plated in 96 well plates and incubated for 24 h, then replaced with fresh medium. The control well received serum free medium and treated wells received 2–10 µl/ml of anisaldehyde compound containing medium (corresponding concentration is 200-1000 µg/ml). The cultures were again incubated as above. After 48 h 100 µl

of 0.5 mg/ml MTT solution was added to each well and the cultures were further incubated for 4 h. Then 100 µl of 20% SDS in 50% dimethylformamide (DMF) was added and the formed crystals were dissolved gently by pipetting 2 to 3 times. A micro plate reader was employed in measuring the absorbance at 570 nm. Growth inhibition rate was calculated as percentage.

c. Morphological Studies

General morphological structure of the cells was examined to determine the effect of anisaldehyde on MCF-7 cell line, for which the cells were cultured in 100 mm dishes. After the treatment with Anisaldehyde (48 h incubation), the cells were photographed under inverted light microscope (Nikon, Sclipse TS100) at 40X.

d. DNA Fragmentation assay

DNA Fragmentation was performed by the method of [10] Cancer cells (3×10^5 cells/ml) were plated per well in 6 well plates and the cells were maintained as mentioned above. The control plates received 0.01% DMSO containing medium and treatment plates received 2-10 µl of anisaldehyde compound containing medium. After 48 h of incubation, the DNA was extracted from the cell lysate described as follow. The cells were washed with PBS and added 0.5 ml of lysis buffer, transferred to a microfuge tube and incubated for 1 h at 37°C. To this 4 µl of protienase K was added and the tubes were incubated at 50°C of phenol: chloroform: isoamyl alcohol was added, mixed and centrifuged at 10, 000 rpm for 10 mins to separate the DNA containing upper aqueous phase. Phenol-chloroform extraction was repeated twice, followed by chloroform extraction. To the resulting a aqueous phase, 2 volumes of ice-cold absolute ethanol and $1/10^{th}$ volumes of 3 M sodium acetate were added and incubated for 30mins on ice to precipitate DNA. DNA was pelleted centrifuging at 13, 000 rpm for 10mins at 4 °C, the supematant was aspirated and the pellet was washed with 1 ml of 70% ethanol. After repeating the above centrifugation step and the pellet was allowed to dry at room temperature and resuspended in 50 µl of TE buffer. The DNA was quantified by UV-visible spectroscopy and 10 µg of DNA was electrophoresed in a 1.2% agarose gel containing ethidium bromide, gel was examined under UV trans illuminator (Bio-Rad) and photographed.

III. RESULTS

A. DPPH radical scavenging

In the present study, the lowest DPPH free radical scavenging activity in Anisaldehyde obtained was 20% at 200, 400, 600 µg/ml and highest scavenging activity was 21% at 800 and 1000 µg/ml concentrations respectively. The IC_{50} value was considered as the rate of inhibition of 21% at 800 and 1000 µg/ml (Fig.1).

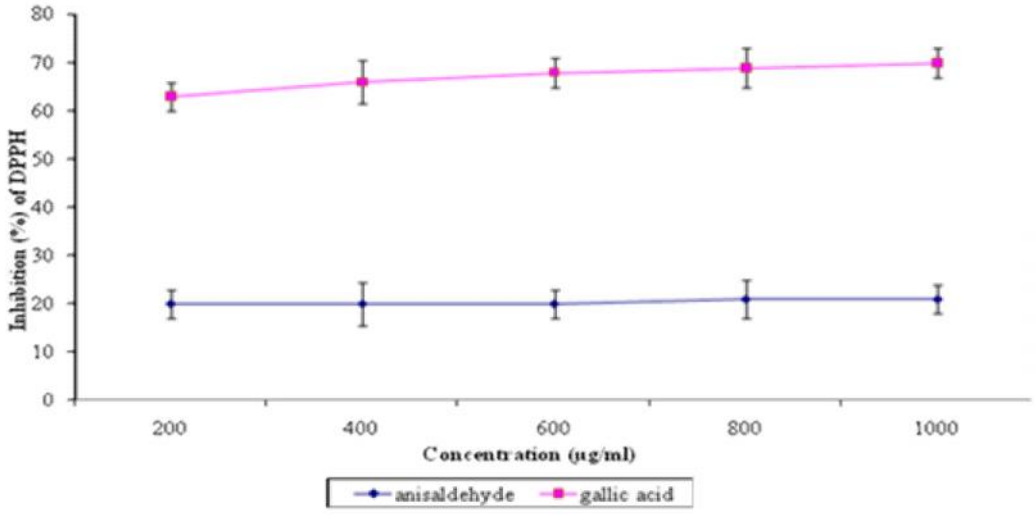

Figure 1. Free radical scavenging effect of Anisaldehyde on DPPH assay

B. Chelating Assay

In the present study, the chelating effect of Anisaldehyde compound on ferrous ions slightly increased as the concentration increases from 200-1000 µg/ml. Anisaldehyde in 1000 µg/ml concentration had a maximum (IC_{50}) chelating effect on the ferrous ions at 27%. The chelating effect of Anisaldehyde was 6%, 8%, 13% and 19% at the concentrations of 200, 400, 600 and 800 µg/ml respectively (Fig. 2).

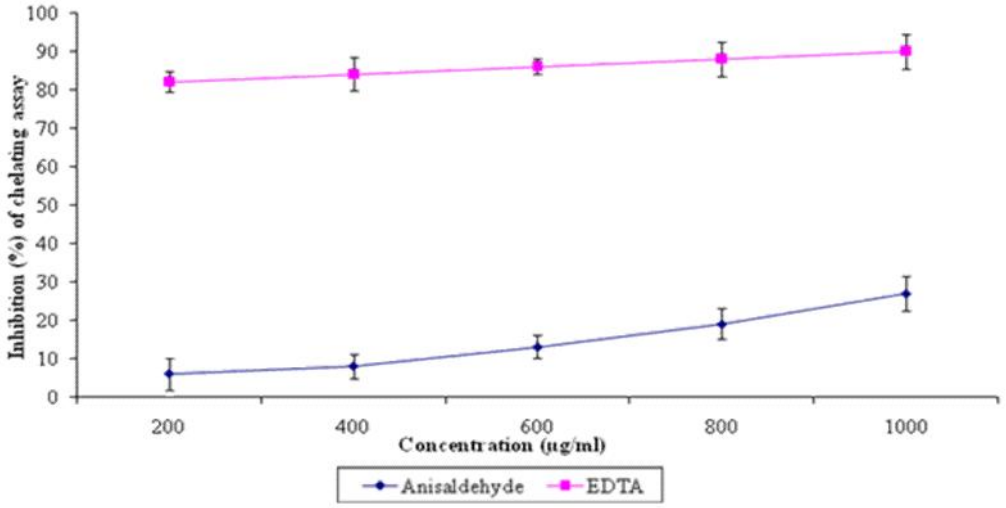

Figure 2. Free radical scavenging effect of Anisaldehyde on Chelating assay

C. Reducing Power Assay

In the present study, the reducing power of Anisaldehyde slightly increased with increase in the concentration. Anisaldehyde compound had a maximum (IC_{50}) reducing power at 23% in 1000 µg/ml but it was very much lower when compared to the standard BHA. Reducing ability of Anisaldehyde compound obtained was 14%, 18%, 21% and 22% at 200, 400, 600 and 800 µg/ml respectively (Fig. 3).

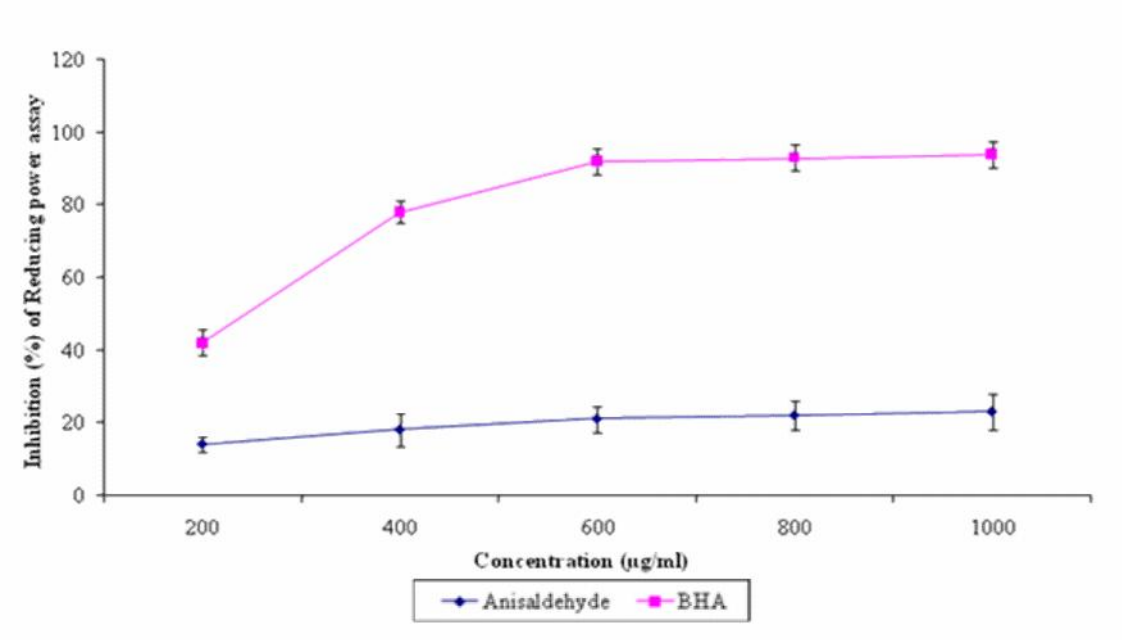

Figure 3. Free radical scavenging effect of Anisaldehyde on sreducing power assay

D. Cytotoxicity assessment by MTT Assay

Cell viability was measured by using MTT assay. The cells (MCF 7, Hep G2 and ME 180) were incubated for 48 h with different concentrations of Anisaldehyde. The Cell viability was decreased in the concentration ranging from 200-1000 µg/ml of Anisaldehyde compound. In the present study 50% (IC_{50}) viability was obtained at the concentrations of 400, 600 and 800 µg/ml on MCF 7, Hep G2 and ME 180 respectively. In the MTT assay, MCF 7 cells were more susceptible to the Anisaldehyde compound when compared to other cell lines (Fig. 4, 5&6). Thus the MCF 7 cell lines were taken for the further experiments.

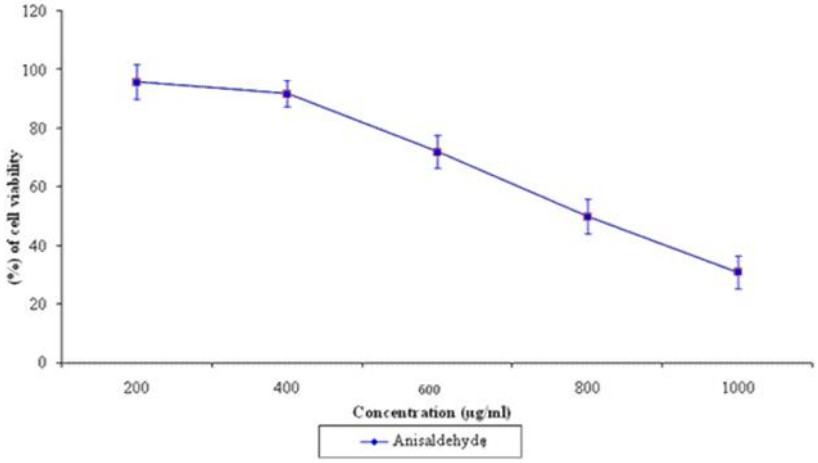

Figure 4. Effect of different concentration of Anisaldehyde on the growth of MCF 7 cells at 48 h incubation

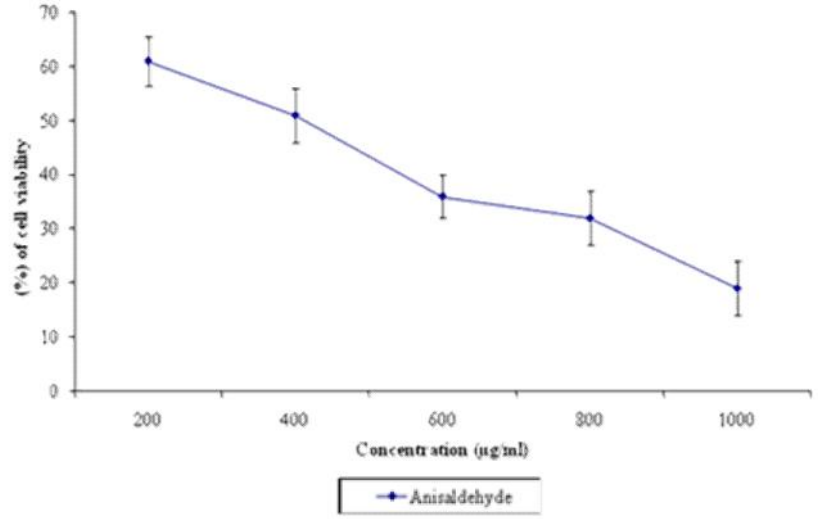

Figure 5. Effect of different concentration of Anisaldehyde on the growth of Hep G2 cells at 48 h incubation

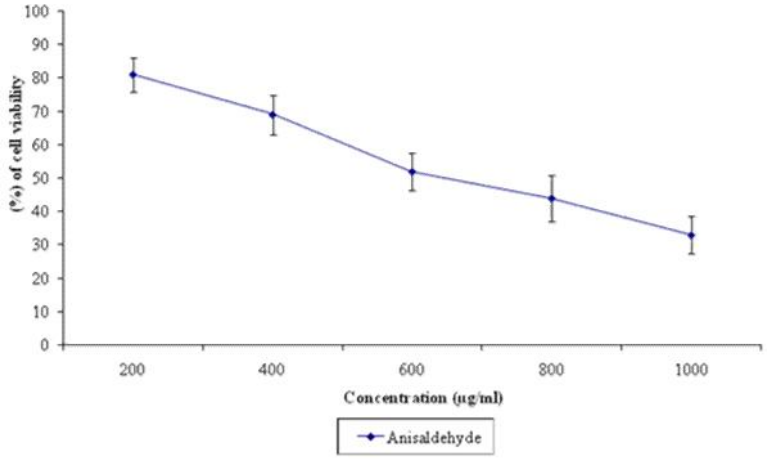

Figure 6. Effect of different concentration of Anisaldehyde on the growth of ME 180 cells at 48 h incubation

E. Morphological Examination

The cancer cells MCF 7 were trypsinized and treated with the Anisaldehyde at the concentration of 400 µg/ml at different time interval (24 and 48 h). The control cells did not show any morphological changes and treated cells were in irregular confluent aggregates with round and polygonal cell morphology. In treated MCF 7 cells destruction of monolayer was observed. The treated polygonal cells after 48 h of incubation begun to shrink and became spherical in shape (Fig. 7). The cell shrinkage increased progressively in dose and time dependent manner. This shrinkage may be due to the growth inhibitory effect of Anisaldehyde compound.

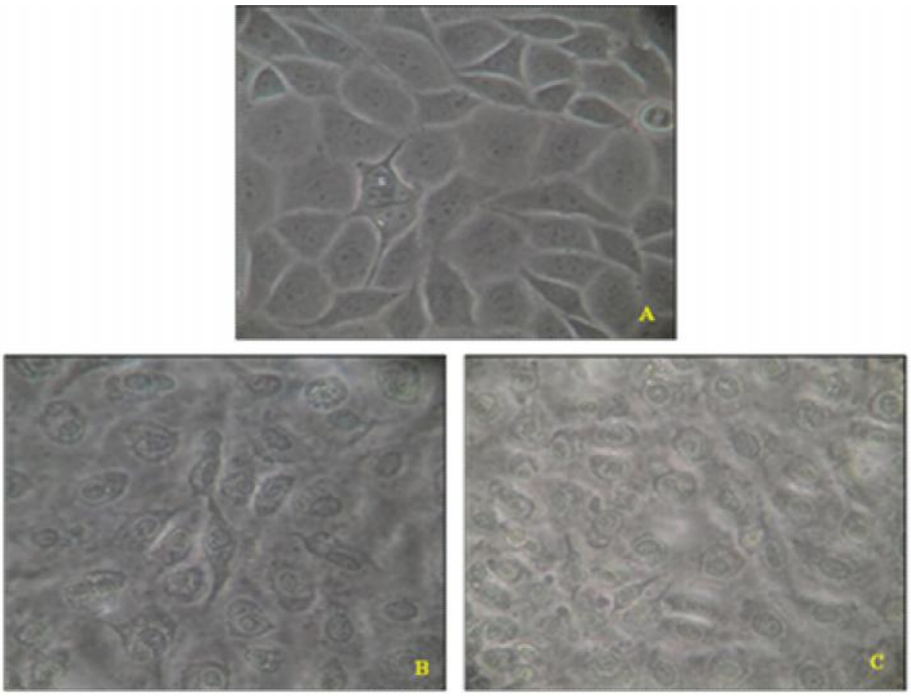

Figure 7. Morphological changes of MCF 7 cell line after 48 h incubation of Anisaldehyde treatment; A: Control cells; B: Cells treated with 400 µg/ml at 24 h incubation; C: Cells treated with 400 µg/ml at 48 h incubation

F. DNA Fragmentation

In this study MCF 7 cells were treated with Anisaldehyde compound for 48 h. Figure 8 represent the Anisaldehyde compound induced DNA fragmentation of MCF 7 cells. In case of control, the cells have intact DNA whereas in Anisaldehyde compound treated cells showed multi-tracked bands of DNA. DNA fragmentation was observed at concentrations of 400 µg/ml at 48 h.

Figure 8. DNA fragmentation analysis of Anisaldehyde compound on MCF 7 cells; C: Control cells; A: Cells treated with 400 µg/ml at 48 h incubation

IV. DISCUSSION

Cancer is a growing health problem around the world. Natural products have long been used to prevent and treat many diseases, including cancer and thus they are good candidates for the development of anti-cancer drugs [11]. Discovery of active compounds from natural products with the potential for use as chemotherapy and/or chemotherapeutic agents are of great interest for cancer treatment. Chemotherapy constitutes an essential treatment option for breast cancer although the overall therapeutic results are not completely satisfactory and new advances or needed [12]. Anisaldehyde contain a number of biodynamic compounds of therapeutic value. These compounds are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammations [13]. Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, Cancer, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, and aging and neoplastic diseases [14].They are also involved in autoimmune disorders like rheumatoid arthritis etc. Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation [15]. Therefore, the importance of search for natural antioxidants has increased in the recent years and so many researchers focused the same [16]. Hence The present studies is an attempt to find the efficacy of the formulated drug anisaldehyde compound, tested from antioxidant and free radical mediated anticancer activity, which specifically target breast cancer cells without causing adverse effect on normal breast epithelial cells. The DPPH radical has been widely used to test the potential of compounds as free radical scavengers of hydrogen donors and to investigate the antioxidant activity of plant extracts. The DPPH radical scavenging activity of Anisaldehyde compound was constant at all concentration because of having no more free/less OH group. Decreased concentration of DPPH radical due to the scavenging ability of the soluble constituents in the Anisaldehyde compound and the standard Gallic acid as a reference compound presented the highest activity at all concentrations. This shows that Anisaldehyde compound possess hydrogen donating capabilities and acts as an antioxidant. A possible explanation of the free radical scavenging activity is the neutralization of DPPH free radical by the antioxidant components of crude extract / fractions, either by transfer of hydrogen or of an electron [17]. The antioxidant and anticancer activities of organic extract from *Platycodon grandiflorum*. These organic extract have that the lipid peroxidation and free radial scavenging assay on the fractions from the silica gel column and TLC suggest that the antioxidant active and probably phenolic compound has a high activity. The same author studied the antioxidant and anticancer activities of organic extracts from *P. grandiflorum*. Their MTT assay revealed that *P. grandiflorum* also contains a strong polyacetylenic anticancer compound which exhibited cytotoxicity on the three human cancer cell lines HT 29, HRT 18 and Hep G2 [18]. Antioxidants inhibit interaction between metal and lipid through formation of insoluble metal complexes with ferrous ion[19]. The iron-chelating capacity test measures the ability of antioxidants to compete with ferrozine in chelating ferrous ion [20]. Figure 3 shows the ion chelating effect of Anisaldehyde compound and compared with reference compound of EDTA. Ferrozine can quantitatively from complexes with Fe^{2+}. However, in the presence of chelating agents, the complex formation is decreased. Therefore, measurement of the rate of colour reduction helps to estimate the chelating of the samples. The transition metal ion, Fe^{2+} possess the ability to move single electrons by virutue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive

radicals [21]. The chelating capacity of the Anisaldehyde compound increased with increase in the concentration. The reducing capacity of a compound Fe^{3+}/ferricyanide complex to the ferrous form may serve as a significant indicator of its antioxidant capacity [22]. The existence of reductions are the key of the reducing power, which exhibit their antioxidants activities through the action of breaking the free radical chain by donating a hydrogen atom [23]. The reduction of the Fe^{3+}/ferricyanide complex to the ferrous form occurs due to the presence of resultants in the solution. The reductive capabilities of the Anisaldehyde compound were compared with Butylated Hydroxyl Anisole (BHA) as a standard compound for the reduction of the Fe^{3+}- Fe^{2+} transformation in the presence of the Anisaldehyde compound. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [24]. The result showed that the reducing power of the Anisaldehyde compound slightly increased with increase in the concentration. These finding shows Anisaldehyde compounds possess minimum reducing capabilities and acts as an antioxidant. Similar antioxidant activity has been reported to be related to reducing power by some investigators [25].

The use of MTT test assesses the cell metabolism based on the ability of mitochondrial succinate-dehydrogenase to convert the yellow compound MTT to a blue formazan dye. The amount of dye produced is proportional to the number of live and metabolically active cells indeed, the anticancer activities of the *P. grandiflorum* organic **e**xtracts and its fractions were investigated using a MTT assay on three human cancer cell lines, HT 29, HRT 18 and Hep G2. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells [26] and cytotoxic activity of eudesmane sesquiterpene glucosides from lychee seed. Likewise in this study, the Anisaldehyde compound was studied for its probable antitumor activity against human cancer cell line (MCF 7, Hep G2 and ME 180). The Anisaldehyde compound had high growth inhibitory activity against MCF 7 cells. MTT assay is a colorimetric method effectively to screen the anti-proliferation of drugs. In our present investigation the cancer cells were incubated for 48 h, as the cytotoxic effects of the drug on cancer cell lines were time and dose dependent. The result of MTT assay shows MCF 7, Hep G2 and ME 180 cell lines were concordant with the data of performed viability test for them. The Cell viability was decreased in the concentration ranging from of Anisaldehyde compound. In the present study 50% viability was obtained at the concentrations of 400, 600 and 800 µg/ml of MCF 7, Hep G2 and ME 180 respectively. Therefore, the Anisaldehyde compound showed potential antitumor activity against MCF 7, when compare to other cell lines.

Apoptosis is an important way to maintain cellular homeostasis between cell division and cell death [27] thus, induction of apoptosis in cancer cells is one of the useful strategies for anticancer drug development [28]. In this respect, many studies were performed for screening of apoptosis inducing components from plants [29],[30] carried over a work on antioxidant and anticancer activities of high pressure-assisted extract of longan (*Dimocarpas longan*) fruit pericarp. The results indicated that HPEL possessed abundant phenolic content and exhibited good antioxidant and anticancer activities compared to that synthetic ones. In the present study, Anisaldehyde compound showed significant cytotoxic activity and induced apoptosis on MCF 7 cells. In the electrophoretic DNA fragments were obtained at 400 µg/ml concentrations on MCF 7 cell line. Based on the results obtained in the present study, we can conclude that the Anisaldehyde compound did exhibit considerable cytotoxic effect against human breast cancer cells. Therefore, more studies for final application of this compound could be important in the field of natural antitumor investigation. DNA fragment analysis is a typical assay to find out the drug induced apoptosis cell death. In general, fragmentation of chromatin to units of single or multiple nucleosomes that form the nucleosomal DNA ladder in agarose gel is an established hallmark of programmed cell death or apoptosis, was induced by activation of caspase cassette and finally it leads to activation of DNase enzyme which results in DNA fragmentation and is a useful marker for screening compounds for subsequent development as possible anticancer agents. The treatment of Anisaldehyde compound induced cell selective apoptosis in the cell lines Viz. human breast (MCF 7), Liver (Hep G2) and Cervical (ME 180) cancer cell lines.

V. REFERENCES

[1] W.Andlauer and P.Fürst. Cereal Foods World, Antioxidative power of phytochemicals with special reference to cereals,43: pp. 356-359,1998.
[2] A..M.Aboul-Enein,F.K. El-Baz,G.S. El-Baroty, A.M Youssef and H.H Abd El-Baky, J.Med.Sc, Antioxidant activity of algal extracts on lipid peroxidation,3: pp. 87-98,2003.
[3] M.S Blois, Nature Antioxidant determination by the use of a stable free radical,26: 1199-1200. 1958.
[4] C.D Bortner, N.B Oldenburg, J.A. Cidlowski,Trends cell Biol,The role of DNA fragmentation in apoptosis. 5, pp. 21-26, 1995.

[5] M.David pineiro,Elena martin, Natalia, Matilde Salinas, M Victor and Gonzaler Experimental cell Research Calpain inhibition stimulates caspase – dependent apoptosis indused by taxol in NIH3T3 cells.313:pp. 369-379, 2007.
[6] T.C.P.Dinis, V.M.C. Madeir, C.M. Almeida, Arch Biochem Biophys,Action of phenolic derivatives (acetoaminophen, salicylate, and 5- aminosalicylate) as inhibitors of membrane lipid peroxidation and as perxyl radical scavengers. 315: pp. 161-169,1994.
[7] M.Elmastas and I. Glucin , J.Iran, chem.soc,Radical scavenging activity and antioxidant capacity of bay extracts. 3: pp. 258-266,2006.
[8] I. Frlich.,and P. Riederer,Drug Research, Free radical mechanisms in dementia of Alzheimer type and the potential for anti-oxidative treatment.45: pp. 443–449,1995.
[9] G.Galati and S.M.Y. Teng ,Drug metabol drug,Interact Cancer chemoprevention and apoptosis mechanisms induced by dietary polyphenolics. 17: pp. 311-349,2000.
[10] B. Halliwel and J.M.C. Gutteridge,oxford university press,Free radicals in biology and medicine. 3rd edition.Oxford, 1989.
[11] W. Hu and J.J. Kavanagh. Lancet Oncology Anticancer therapy targeting the apoptotic pathway.. 4: 721,2003.
[12] H.L. Huang and B.G Wang,Journal of Agriculture Food Chemistry, Antioxidant capacity and lipophilic content of seaweeds collected from the Qingdao coastline.52: pp. 4993–4997,2004.
[13] C.L. Hsu., W. Chen., Y..M. Weng and C.Y. Tseng, Food Chemistry, Chemical composition, physical properties, and antioxidant activities of yam flours as affected by differentdrying methods.. 83: pp. 85-92,2004.
[14] G.K. Jayaprakasha, A. Tamil Selvi and K.K.. Sakariah,Food Res ,Antibacterial and antioxidant activities of grape (Vitis vinifera) seed extracts,Int. 36: pp. 117-122,2003.
[15] Ji-young Lee, Woo-Ik Hwang, Seung-Taik Limm,. J. Ethanopharmacol, Antioxidant and anticancer activities of organic extracts from Platycodon grandiflorum A. De Candolle roots, 93(2-3):409-415,2004.
[16] S.H. Kaufmann and M.O. Hengartner,Trends in cell biology, Programed cell death: alive and well in the new millennium,11: 526-534. 2001.
[17] M.Luisa pannoa,Francesca Giordanoa, Fabrizia mastroiannib, Catia morettib, Elvira Brunellic, Grazia palmab M, Michele pellegrinob, Saveria Aquilab, Antonella Migliettad, Loredana mauroa, Daniela Bonotigliob and Sebastiano Ando. Evidence that low dose of toxal enhance the functional transactivatory properties of p^{53} on p^{21} was promoter in MCF-7 breast cancer cell FEBS. 580: 2371-2380,2006.
[18] T. Mosmann J. Immuno,Rapid colorimetric assay of cellular growth and survival: A application to proliferation and cytotoxicity assays,Meth. 65: 55-63,1983.
[19] S.Meir and J.Kanner,J. Agric. Food Chem,Determination and involvement of aqueous reducing compounds in oxidative defense system of various senescing leaves, 43: 5030-5034,1995.
[20] M. Oyaizu Nature Studies on product of browning reaction prepared from glucose amine. . 44: 307-315,1986.
[21] K. Nagendra Prasad, Jing Hao, Chun Yi., Dandan Zhang., Shengxiang Qiu., Yueming Jiang., Mingwei Zhang and Feng Chen, Journal of Biomedicine and Biotechnology.. Antioxidant and Anticancer Activities of Wampee (Clausena lansium (Lour.) Skeels) Peel, pp- 6, 2009.
[22] H. Roy and Burdon, Elsevier Science b. V. Netherlands, Free Radical Damage and Its Control,1994.P: 125.
[23] A.V.Schally and A. Nagy, Eur J Endocrinol, Cancer chemotherapy based on targeting of cytotoxic peptide conjugates to their receptor on tumors,141: 1- 14,1999.
[24] J.Selvin and A.P. Lipton, J. Mar. Sci. Tech, Biopotential of Ulva fasciata and Hypnea musciformis collected from the peninsular coast of India. 12: 1-6,2004.
[25] K. Shimada, K.Fujikawa K.Yahara and T.Nakamura,Journal of Agricultural and Food Chemistry, Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion40: 945–948,1992.
[26] S.A.Smith- Warner, P.J.Elmer T.M.Tharp, L.Fosdick, B.Randall, M.Gross, J.Wood and J.D. Potter, Epidemiol. Biomar. Increasing vegetable and fruit intake: randomized intervention and monitoring in an at- risk population,Cancer Prev. 9(3): 307-317,2000.
[27] M.L. Tan,S.F, Suaiman, N.Najimuddin, M.R. Smian and T.S. Tengku Muhammad,Journal of Ethanopharmacology Methonalic extract of Pereskia bleo (kunth) DC. (Cactaceae) induces apoptosis in breast carcinoma, T47-d cell line,96: 287-294,2005.
[28] R.E. Xing, S Liu, Z.Y. Guo, H.H. Yu, P.B.Wang. C.P.Li, Z.E.Li and P.C.Li Bioorg. Med.Chem Antioxidant activity of differently regioselective chitosan sulfates *in vitro*,13(4): 1387-1392,2005.
[29] A.Yildrim and A.Mavi,J. Argic food chem.,Comparison of antioxidant and antimicrobial activity of tilia (Tilia argentea deset ex DC) (*Salvia triloba*) and (*Lamellia sinensis*) extracts, 48: 5030-5034,2004.

[30] G.Yen and H.Chen, J. Agric,Antioxidant activity of various tea extract in relation to their antimutagenicity Food. Chem. 43: 7-32,1995.