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INHIBITORY EFFECT OF 17-BETA ESTRADIOL ON HEPATOCELLULAR CARCINOMA CELL LINE

KEYWORDS: 17-betaestradiol, hepatocellular carcinoma, chemoprevention

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ABSTRACT *Hepatocellular carcinoma is one of the major malignant diseases in the world today. Phytoestrogens are a group of natural compound with estrogen-like activity.The aim of the present study was to analyse the apoptotic and antiprolifrative effect of 17-beta estradiol in the hepatocellular carcinoma PLC/PRF5 cell line. Materials and Methods: Cells were treated with various concentration of E2 and the MTT assay was used. Furthermore, Cells were treated with single dose of E2 (25µM) and flow cytometry assay was performed.Results: E2 inhibited the growth of liver cancer cells and induced apoptosis significantly with a time- and dose-dependent manner.Discussion: Our finding clearly indicated that E2 has a significant inhibitory effect and induces apoptosis with a dose- and time-dependent manner.Conclusion : E2 can significantly inhibit the growth of HCC cells and plays a significant role in apoptosis of this cell line .*

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the major malignant diseases in the world today and has increased in numbers year by year. The disease is a common malignancy in developing countries and the fifth most common cancer in men and the seventh in women and also the third most common cause of cancer-related deaths worldwide (1).The geographical prevalence of HCC isn't uniform in different regions of the world .More than 700,000 new cases are diagnosed each year throughout the world and also unfortunately more than 600,000 deaths are attributed to HCC each year. The distribution of the disease is related to distribution of HBV and HCV infection, with the highest rates of HCC in regions with high infection, the major risk factors of HCC(2-5).

The incidence of HCC in Asian countries is lower than that of the developing countries (6-9).Major risk factors of HCC including ; chronic infections of hepatitis B virus (HBV) and hepatitis C virus (HCV), cirrhosis, alcohol consumption , dietary digestions of aflatoxin (Aflatoxin is a mycotoxin that commonlycontaminates corn, soybeans, and peanuts), obesity , diabetes mellitus, iron overload and tobacco smoking(10).Other risk factors include male gender, advanced age(11) , hereditary hemochromatosis ,environmental toxins,oral contraceptive. It should be noted that, HCC can also occur without known risk factors(12,13).

Phytoestrogens are a group of natural compound with estrogen-like activity and similar structure of estradiol,biologicall active phenolic compounds that structurally mimic the mammalian estrogen 17-betaestradiol(E2). Phytoestrogens, originating from various plant sources include fruits , vegetables, legumes,soy beans, whole rye and flax seeds and whole grains.Infact, phytoestrogens are phenolic non-steroidal plant derived compounds possessing estrogen like activity, the structure of which is similar to that of 17-β-estradiol (fig.1) (14). This group is divided into three sub groups: isoflavones, lignans,and coumestans which the lignans are usually broken down to enterolactone and enterodiol before absorbtion (15).

Phytoestrogen are hydrolysed in the gastrointestinal tract with the help of bacteria before entering the blood stream and also lignans secoisolariciresinol and matairesinol are changed to mammalian lignansenterolactone and enterodiol by human gut bacteria (16).Generally , phytoestrogens are ingested as conjugates and then reconjugated in the liver and intestinal epithelium by glucuronosyl and sulphotransferases(17).

Fig.1 about here

Phytoestrogens are known to act as agonists or antagonists of E2(18).They exert weak estrogenic activity in some tissues and antiestrogenic activity in others.Really they exert dual actions (both inhibitory and stimulatory effects) depending on their concentration (19-21) and have both tumorigenic and antitumorigenic effects. Because of antiprolifratory effect, they protect against some cancer such as, uterine, breast , prostate, lung and colon cancer(22-25). Therefore, there is an ever growing interest in treatment with these compounds.High consumption of phytoestrogens is associated with low incidence of cancer risk.The incidence of breast cancer in Asian populations that consume large amount of compound rich in phytoestrogen is lower than Western populations with low intake of these compounds (26-29).Many epidemiologic studies indicated an association between high consumption of foods rich in phytoestrogens and low incidence of prostate cancer(30). Similar epidemiologic studies demonstrated that high intake of soy product (main source of phytoestrogen) reduces colorectal cancer risk(31-36) and also increasing intake of soy foods is associated with a reduced risk of lung cancer.

It has been shown that phytoestrogens have anti-metastatic and anti-angiogenic effects and play a role in cell cycle arrest. Furthermore, phytoestrogens have significant inhibition on distant metastases of breast, prostate, bladder, and pancreatic cancer in animal models (37).17-β-estradiol can act as a carcinogenic agent and is able to induce complete neoplastic transformation of human breast epithelial cells (38-44).On the other hand,studies of breast cancer

have demonstrated that estrogen is a mammary-gland carcinogen(45).It is also known that renal carcinoma can be induced by exposure to estrogens (46) .There is a good correlation between hormonal activity and carcinogenicity of estrogen in the hamster kidney (47-49).Recent data indicates that estrogens decrease tumor progression in HCC in vivo by reducing tumor cell invasion, arresting cell cycle progression, and promoting apoptosis(50) and also estrogen and the estrogen-like compounds may induce anti-proliferative and apoptotic effects in Hep3B cells(51).

Since there is few studies for E2 effect on HCC and we can't find any data about effect of E2 on PLC/PRF5 cell line (in vitro),therefore ,this study was designed to investigative the apoptotic and anti-proliferative effects of E2 on the human hepatocellular PLC/PRF5 cell line.

MATERIALS AND METHODS

Human hepatocellular carcinoma cells (PLC/PRF5) were purchased from the National Cell Bank of Iran-Pasteur Institute. E2, DMEM (Dulbecco minimal essential medium) and MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide) were purchased from Sigma (Sigma, St. Louis, MO) . All other chemicals were obtained from the best sources available.

CELL CULTURE

The cells were cultured in DMEM with pH 7.2–7.4 (Sigma) containing 1% sodium pyruvate (sigma), 3.7 mg/ml sodium bicarbonate (Sigma), 10% fetal bovine serum (FBS) (sigma) and 1% antibiotics which include 10,000 units/ml penicillin G sodium(sigma), 10,000 ug/ml streptomycin sulfate and 25 ug/ml amphotericin B (sigma) at 37°C in 5% CO_2 to promote attachment.When cells became >80% confluent, 5×10^5 cells were seeded into 24-well plates (Becton-Dickinson) for 24 h in DMEM culture medium before they were incubated with certain concentrations of E2 (1, 5, 10, 25, 50, 75, and 100 μM/lit) , which was dissolved in dimethyl sulfoxide (DMSO); DMSO was present at 0.01–0.3% in the medium based on IC50 index, at different times (24, 48 and 72 h). The control cells were treated with DMSO only. Photography was done for cultures before and after treatment with E2 at different times using inverted microscope (Nikon, TE 2000-U, Japan) .

DETERMINATION OF IC50 VALUE BY MTT ASSAY

The effect of E2 on cellular proliferation was assessed by MTT assay, according to standard protocols. After 24, 48 and 72 h of the treatment, the IC50 value for E2 in PLC/PRF5 groups were determined. The MTT assay was commonly used to assess cell proliferation and viability by measuring the reduction of yellow MTT by mitochondrial dehydrogenases in viable cells. Briefly, 5×10^5 Cells were counted and placed into each well of a 24-well micro plate and were treated with various drug concentrations (1, 5, 10, 25, 50, 75, and 100 μM/lit) of E2 for 24 ,48 and 72 h and the MTT survival assay was then carried out for the evaluation of the cell viability with different drug concentrations. The cells were measured spectrophotometerically at 570 nm. All experiments were repeated three times, with at least three measurements (triplicates).

DETERMINATION OF CELL VIABILITY BY MTT ASSAY

To determine the effect of E2 , the cells were seeded in triplicate in 24-well plates and treated with E2 at concentration of 25 μM in different period times (24, 48 and 72 h).The cell viability was estimated by a colorimetric assay based on the conversion of tetrazolium dye (MTT) to a blue formazan product . The absorbance of the cell lysates in DMSO solution was read at 570 nm by a microplate reader (Bio-Rad Hercules, CA).

DETERMINATION OF APOPTOTIC CELLS BY FLOW CYTOMETRY ASSAY

The cells were seeded in 24-well plates. After 24 h, the medium was changed and medium contains E2 (25μM) was added. After 24 ,48 and 72 h of incubation, all the adherent cells were collected with 0.05% trypsin, washed with cold phosphate-buffered saline (PBS) and resuspended in Binding buffer (1x). After addition of AnnexinV-FITC and propidium iodide (PI, Becton-Dickinson, San Diego, CA), analysis was carried out according to the manufacturer's protocol (BMS500F1/100CE AnnexinV-FITC, eBiscience, USA). Finally the apoptotic cells were counted by FACScan flow cytometry (Becton Dickinson, Heidelberg, Germany). All experiments were processed independently three times. A minimum of 5×10^5 cell/ml were analyzed for each sample.

RESULTS

RESULT OF DETERMINATION OF IC50 BY MTT ASSAY

Cell vitality in the human hepatocellular carcinoma cell line, were analysed by using the MTT assay as described previously. The result of MTT assay indicated that, E2 inhibits the growth of liver cancer cells significantly in all treatment groups except the control group ($P < 0.001$) (fig.2). The IC50s value for PLC/PRF5 cells were 25 μM of E2 at different time periods. According to Fig.2, the percentage of living cells in treatment groups (24, 48 and 72h) at a concentration of 25 μM were 51%, 48%, 45 %, respectively ($P < 0.001$). The effect of E2 was dose- and time-dependent. This experiment was repeated three times for each group.

Fig .2 about here

RESULT OF DETERMINATION OF CELL VIABILITY BY MTT ASSAY

The cell vitality in the cells treated with E2 at concentration of 25 μM in different time periods were analysed by using the MTT assay. The amounts of reduced MTT in the all groups treated with E2 were significantly lower than that of the control group ($P < 0.001$). The percentage of living cells in treatment groups at different time periods (24, 48 and 72h) were 53, 49 and 46%, respectively at a concentration of 25 μM of E2. This experiment was repeated three times for each group (fig.3).

Fig. 3 about here

RESULT OF DETERMINATION OF APOPTOTIC CELLS BY FLOW CYTOMETRY ASSAY

The cells were treated with 25 μM concentration ofE2 for different time periods (24, 48 and 72h). Flow cytometry was performed to observe the apoptotic cells which had been visualized using Annexin V-FITC and/or propidium iodide staining. Flow cytometric analysis indicated that E2 at 25 μM concentration induces apoptosis in hepatocellular cancer cells in a time – dependent manner ($P < 0.001$). The amount of apoptotic cells was significantly increased in all three groups (Fig.4). Percentage of apoptotic cells at different time periods (24,48 and 72 hours) were 28, 36 and 62%, respectively. Apoptotic effects were not observed in DMSO group.

Fig. 4 about here

DISCUSSION

Hepatocellular carcinoma represents approximately 4% of

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all new cancer cases diagnosed worldwide.Main risk factors of HCC including; HBV, HCV. Other risk factors include cirrhosis, alcohol consumption, dietary digestions of aflatoxin (Aflatoxin is a mycotoxin that commonly contaminates corn, soybeans, and peanuts), obesity, diabetes mellitus, iron overload and tobacco smoking(52).Phytoestrogens display pharmacologic properties with both tumorigenic and anti-tumorigenic effects. Epidemiologic evidence have indicated that diet and nutrition rich in phytoestrogen can influence cancer development and high intake of phytoestrogens reduce various cancers(53).

Our data clearly shown that E2 has a significant inhibitory effect on the growth of liver cancer cells and induces apoptosis in this cell line with a dose- and time-dependent manner. Similar results have obtained in other tissues by other investigators which reported that 17β-estradiol at a concentration of 1 nM significantly increase apoptosis ofMDA-MB-231 breast cancer cells(54).Besides, dietary phytoestrogens play a protective role against prostate and colon cancer and the formation of polyps(55,56). Furthermore,several experimental studies have reported that phytoestrogens reduce colorectal cancer development (57) and also similar study has shown that dietary phytoestrogens are associated with a reduction in colorectal cancer risk(58).Over the past decades, epidemiological studies have indicated that consumption of diets rich in phytoestrogens areassociated with low risk of breast cancer(59).On the other hand, it has been demonstrated that phytoestrogen promotes cell death of human osteosarcoma cells U2OS by induction of apoptosis (60).

Many studies have reported a protective role of phytoestrogens against lung cancer and also reported that phytoestrogens reduce the risk of ovarian cancer and human cervical carcinoma HeLa cells through an apoptotic pathway.(61)There are many researches which reported that phytoestrogens inhibit prostate cancer-cell growth during the promotional phase of the disease(62).

It has been reported that 17β-estradiol significantly inhibit the growth ofER-positive esophageal squamous carcinoma cell line (ES-25C) and the proliferation of the ER-positive KSE-1 esophageal squamous carcinoma cell line (63,64).Extensive researches have demonstrated that estrogen inhibits the proliferation, migration, and invasion of renal cell carcinoma and increasesrenal cell carcinoma apoptosis. All reports mentioned above confirm our finding but many studies have reported that phytoestrogen have stimulatory and prolifratory effects(65).Wu et al have demonstrated that steroid hormone has stimulatory effect on human gastric carcinoma cell line NUGC-3(26). On the other hand, it has been indicated that low concentrations of 17β-estradiol stimulate cell growth of MKN28, KATO III and MKN45 human gastric cancer cell lines (66)and also high phytoestrogen intake promote growth of estrogen-sensitive tumors.

Furthermore, it has been shown that 17β-estradiol administration exerts a growth-inhibitory effect on ER-positive cell lines (HGC-27,AGS) (67-69).In vitro study has demonstrated that 17β-estradiol increases both the invasion and proliferation of ovarian cancer cell.

It should be noted that phytoestrogens act through different mechanisms and pathways.They exert anticancer activity by two mechanisms including anti-estrogenic mechanism which is due to structural similarity with estradiol and anti-aromatase activity. They act through competing with endogenous estrogens in binding to estrogen receptors by which inactivate enzyme involved in biosynthesis of estrogen from androgens such as aromatase (59,70).Many studies have reported that phytoestrogens exert antiproliferative effects by inhibition of tyrosine kinase activity, DNAtopoisomerase II and angiogenesis(71). Other studies have reported that estrogen act through four molecular pathways: ligand-independent, ligand-dependent,cell-surface (nongenomic) signaling and DNA binding-independent(72). Phytoestrogens reduce cancer risk by binding to estrogen receptors (ER) or interacting with enzymes involved in sex steroid biosynthesis and metabolism (73). Other mechanism of phytoestrogens action reported by many studies include prevention of oxidation, induction of detoxification enzymes, antiproliferation, induction of cell cycle arrest and apoptosis (74-76).Many investigators have reported that phytoestrogens exert antitumorogenic activity on colorectal cancer by regulation of ERβexpression(77).

and also epidemiological and pathological data have reported that thyroid cancer is an estrogen-dependent disease and phytoestrogen consumption is associated with reduced risk of thyroid cancer(78).

In summary, based on data from many investigators, as mentioned above,17-beta estradiol is a plant-derived estrogen-like compound with estrogenic activity and biphasic effects that acts through estrogen receptor probably by epigenetic pathways which need more researches. We did not perform enzyme activity assay related to methylation and histone modifications and also enzyme immunoassay related to protein levels but we will perform in next researches. It is important to note that, E2 had an apoptotic effect based on our data while other studies have reported that its effect is biphasic (inhibitory and stimulatort effects).

CONCLUSION

Our findings suggest that, E2 may be a potent antiestrogenic compound and can effectively inhibit growth and induce apoptosis in PLC/PRF5 HCC cells. In future studies, the mechanisms and pathways of antiestrogenic effects of E2 on PLC/PRF5 should be evaluated.

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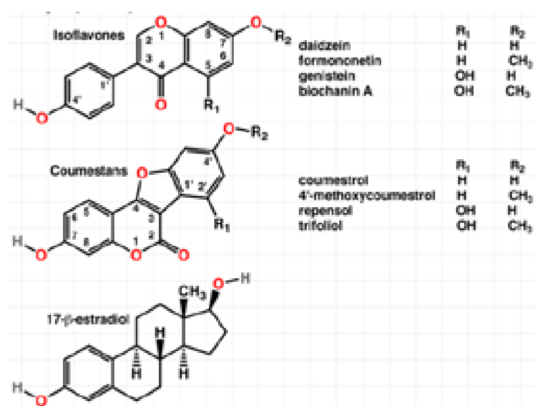


Fig.1 Chemical structure of phytoestrogens (top two structures) and the structure of estradiol (estrogen produced within the body - the bottom structure)

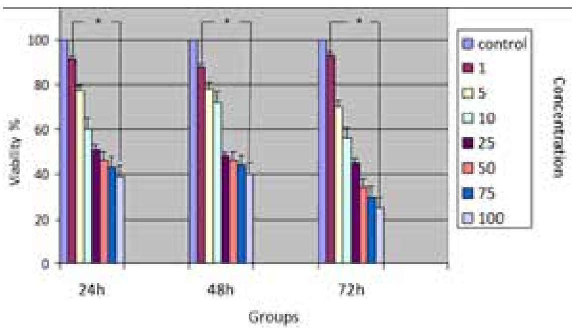


Fig.2. Effect of E2 on PLC/PRF5 human hepatocellular carcinoma cell proliferation. Cells were seeded in 24-well plates and allowed to attach overnight and then were treated with E2 (1,5,10,25,50,75,100µm/lit) for 24 , 48 and 72 hours. Cell survival was determined by the MTT assay . Data are presented as mean ± standard error of the mean (SEM) from atleast three different experiments. . Asterisks (*) indicate significant differences between treated cells and the control group. *P < 0.001 as compared to the control.

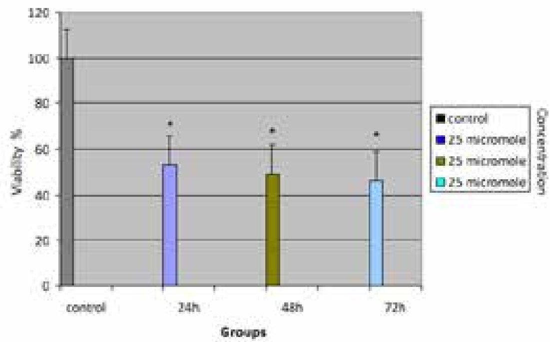
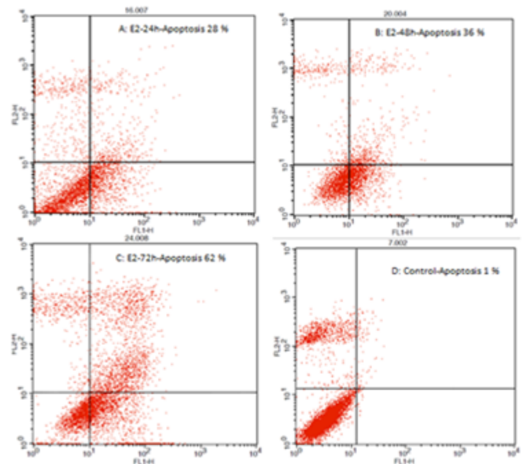


Fig. 3.Effect of E2 at a concentration of 25 µM on cell viability of PLC/PRF5 cells. The effect of E2 on the viability of PLC/PRF5 cells was determined by MTT assay at different time periods(24, 48 and 72h). Mean values from the three experiments ± standard error of mean (SEM) are shown. Asterisks (*) indicate significant differences between treated cells and the control group (*P< 0.001)



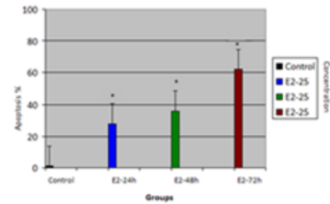


Fig.4. Effects of E2 on PLC/PRF5 cell apoptosis. The Cells were treated with E2 (25 µM) for 24,48 and 72 h and the apoptosis-inducing effect of E2 was investigated by flow cytometric analysis of PLC/PRF5 cells stained with Annexin V and propidium iodide. Results were obtained from three independent experiments and were expressed as mean ± standard error of mean (S.E.M). P <0.001 , n=3. (A) 24 h. (B) 48 h . (C) 72 h .

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REFERENCE | 1.Michael C Kew . Hepatocellular carcinoma: epidemiology and risk factors. Journal of Hepatocellular Carcinoma. 2014:1 115–125 || 2.Roniel Cabrera . Hepatocellular carcinoma: current trends in worldwide epidemiology, risk factors,diagnosis, and therapeutics. Hepatic Medicine: Evidence and Research 2012:4 19–37 || 3. Surveillance Research Program, National Cancer Institute. Fast stats: | an interactive tool for access to SEER cancer statistics. November 10, 2011.Available from: http://seer.cancer.gov/faststats. Accessed August 15,2011. || 4. World Health Organization. Hepatitis B fact sheet. August 21, 2008.Available from: http://www.who.int/mediacentre/factsheets/fs204/en/.Accessed February 20, 2012. || 5. World Health Organization. Facts and figures: hepatitis C. May 25, 2010.Available from: http://www.euro.who.int/en/what-we-do/health-topics/communicable-diseases/hepatitis/facts-and-figures/hepatitis-c. Accessed February 20, 2012. || 6. Yoshizawa H. Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. Oncology. 2002;62(1):8–17. || 7. Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005.J ClinOncol. 2009;27(9):1485–1491. || 8. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med. 1999;340(10):745–750. || 9. Howlader N, Noone AM, Krapcho M, et al, editors; National Cancer Institute. SEER cancer statistics review, 1975–2008. November 10,2011. Available from: http://seer.cancer.gov/csr/1975_2008/. AccessedFebruary 20, 2012. || 10. Jing Gao, Li Xie1,Wan-Shui Yang, et al . Risk Factors of Hepatocellular Carcinoma - Current Status and Perspectives. Asian Pacific J Cancer Prev.2012; 13, 743-752 || 11. Sarbah SA, Gramlich T, Younoszai A, et al. Risk factors for hepatocellular carcinoma in patients with cirrhosis. Dig Dis Sci 2004; 49:850-3 || 12. Davila, JA, Morgan, RO, Shaib, Y, et al. Hepatitis C infection and the increasing incidence of hepatocellularcarcinoma: A population-based study. Gastroenterology 2004; 127:1372 || 13. Bralet, MP, Regimbeau, JM, Pineau, P, etal.Hepatocellular carcinoma occurring in nonfibrotic liver:Epidemiologic and histopathologic analysis of 80 French cases. Hepatology 2000; 32:200-212 || 14. Stefan O. Mueller, Stephanie Simon, Kun Chae, et al . Phytoestrogens and Their Human Metabolites Show Distinct Agonistic and Antagonistic Properties on Estrogen Receptor a (ERa) and ERb in Human Cells. TOXICOLOGICAL SCIENCES. 2004; 80: 14–25 || 15. Ashish Kumar Sharma, Vipin Agarwal1,Bhupendra Chauhan1,et al. ROLE OF PHYTOESTROGEN IN TREATMENT OF CANCER. International Journal of Pharma Research and Development. Publication Ref No.: IJPRD.2010;2 (9) :22-32 || 16. Borriello SP, Setchell KDR, Axelson M, Lawson AM. Production and metabolism oflignans by human faecal flora. J.Appl.Bacterial.1985; 58:37-43. || 17. Archana Sharma, Jyoti, NeerupmaDhiman, ShikhaSaxena. HEALTH BENEFITS OF PHYTOESTROGENS. International Journal of Advanced Research .2014; 2 (4):1024-1030 || 18. Delia M. Brownson, Nicolas G. Azios, Brie K. Fuqua,et al. Flavonoid Effects Relevant to Cancer. International Research Conference on Food, Nutrition & Cancer J. Nutr.2002;132: 3482–3489 || 19. Kyselova, Z. Toxicological aspects of the use of phenolic compounds in disease prevention.Interdiscip. Toxicol.2011; 4: 173–183. ||| 20. Patisaul, H.B.; Jefferson, W. The pros and cons of phytoestrogens. Front. Neuroendocrinol.2010; 31: 400–419. || 21. Ziegler, R.G. Phytoestrogens and breast cancer. Am. J. Clin. Nutr.2004; 79: 183–184. || 22. Breithofer, A., Graumann, K., Scicchitano, M. S., Karathanasis, S. K.,Butt, T. R. &Jungbauer, A. Regulation of human estrogen receptor by phytoestrogens in yeast and human cells. J. Steroid Biochem. Mol. Biol. 1998;67: 421–429. || 23. Setchell, K. D. & Cassidy, A. Dietary isoflavones: biological effects and relevance to human health. J. Nutr. 199;129: 758–767. || 24. Tikkanen, M. J. &Adlercreutz, H. Dietary soy-derived isoflavone phytoestrogens. Could they have a role in coronary heart disease prevention? Biochem. Pharmacol.2000; 60: 1–5. ||| 25. Wiseman, H. The therapeutic potential of phytoestrogens. Exp. Opin.Investig.Drugs.2000; 9: 1829–1840. || 26. LitalKeinan-Boker, Yvonne T van Der Schouw, Diederick E Grobbee,et al. Dietary phytoestrogens and breast cancer risk. DIETARY LIGNANS AND BREAST CANCER RISK Am J ClinNutr.2004;79:282–8. || 27. Messina MJ, Persky V, Setchell KDS, Barnes R. Soy intake and cancer risk: a review of the in vitro and in vivo data. Nutr Cancer.1994;21: 113–31. || 28. Adlercreutz H, Mousavi Y, Clark J, et al. Dietary phytoestrogens and cancer: in vitro and in vivo studies. J Steroid BiochemMol Biol. 1992;41:331–7. || 29. Adlercreutz H, Mousavi Y, Höckerstedt K. Diet and breast cancer.ActaOncol 1992;31:175–81. || 30. SwapnaKolukula1, and Robert J. Anderson1. Phytoestrogens and their Potential Roles in Prostate Cancer Prevention and Treatment. Citation: Kolukula S, Anderson RJ (2011) Phytoestrogens and their Potential | Roles in Prostate Cancer Prevention and Treatment. J Cancer SciTher S1. | doi:10.4172/1948-5956.S1-002 || 31. MichelleCotterchio, Beatrice A. Boucher, Michael Manno,. Dietary Phytoestrogen Intake Is Associated with Reduced Colorectal Cancer Risk. J Nutr.2006 ; 136(12): 3046–3053 || 32. Le Marchand L, Hankin JH, Wilkens LR, Kolonel LN, Englyst HN, Lyu LC. Dietary fiber andcolorectal cancer risk. Epidemiology 1997;8:658–65. || 33. Hoshiyama Y, Sekine T, Sasaba T. A case-control study of colorectal cancer and its relation to diet,cigarettes, and alcohol consumption in Saitama Prefecture, Japan. Tohoku J Exp Med 1993;171:153–65. || 34. Hu J, Liu Y, Yu YK, Zhao TZ, Liu SD, Wang QQ. Diet and cancer of the colon and rectum: a casecontrolstudy in China. Int J Epidemiol 1991;20:362–7. || 35. Hirayama T. Contribution of a long-term prospective cohort study to the issue of nutrition and cancerwith special reference to the role of alcohol drinking. ProgClinBiol Res 1990;346:179–87. || 36. Tajima K, Tominaga S. Dietary habits and gastrointestinal cancers: a comparative case-control studyof stomach and large intestine cancers in Nagoya, Japan. Jpn J Cancer Res 1985;76:705–16. || 37. Mandeep K. Virk-Baker1, Tim R. Nagy1, and Stephen Barnes2,3. Role of phytoestrogens in cancer therapy.Planta Med. 2010 ; 76(11): 1132–1142. || 38. J. Russo(1) and Irma H. Russo . THE ROLE OF ESTROGEN IN THE INITATION OF BREST CANCER. J Steroid BiochemMol Biol. 2006 ; 102(1-5): 89–96. || 39. Dorgan JF, Longcope C, Stephenson HE Jr. Falk RTet al. Relation of prediagnostic serum estrogen and androgen levels to breastcancer risk. Cancer Epidemiol. Biomarkers Prev 1996;5:533–539. || 40. Thomas HV, Key TJ, Allen DS, et al.A prospective studyof endogenous serum hormone concentrations and breast cancer risk in postmenopausal women onthe island of Guernsey. Br. J. Cancer 1997;76:401–405. || 41. Hankinson SE, Willett WC, Manson JE,et al. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. J. Natl. Cancer Inst 1998;90:1292–1299. || 42. Toniolo PG, Levitz M, Zeleniuch-Jacquotte A, et al. A prospective study of endogenous estrogens and breast cancer in postmenopausal women. J.Natl. Cancer Inst 1995;87:190–197. || 43. Helzlsouer KJ, Alberg AJ, Bush TL, et al. A prospective studyof endogenous hormones and breast cancer. Cancer Detect. Prev 1994;18:79–85. || 44. Endogenous Hormones and Breast Cancer Collaborative Group. Endogenous sex hormones and breastcancer in postmenopausal women: reanalysis of nine prospective studies. J. Natl. Cancer Inst2002;94:606–616. || 45. James D. Yager and Nancy E. Davidson . Estrogen Carcinogenesis in Breast Cancer. Thenew england journal of medicine . N Engl J Med 2006;354:270-82. || 46. Aleksandra Fucic, MarijaGamulin, ZeljkoFerencic, et al. Environmental exposure to xenoestrogens and oestrogen related cancers: reproductive system, breast, lung, kidney, pancreas, and brain. Brussels, Belgium. 2010 ; 15: 201-221. | 47. Li, J. J., Li, S. A., Klicka, J. K., et al. Relative carcinogenic activity of various synthetic and natural estrogens in the hamster kidney.Cancer Res. 1983; 4: 5200-5204. || 48. Li. J. J., and Li, S. A. Estrogen-induced tumorigcnesis in the Syrian hamster: roles for hormonal and carcinogenic activities. Arch. Toxicol.1984; 55: 110-118. || 49. Li, J. J. and Li, S. A. Estrogen carcinogenesis in hamster tissues: role of metabolism. Fed. Proc.1987; 46: 1858-1863. || 50. Xu H, Wei Y, Zhang Y, et al. (2012) Oestrogenattenuates tumour progression in hepatocellular carcinoma. J Pathol.2012;228 (2): 216-229. || 51.Erh-Jung Huang, Cheng-Chung Wu, Hsien-Ping Huang, et al. Apoptotic and anti-proliferative effects of 17 -estradiol and 17 -estradiol-like compounds in the Hep3B cell line. Molecular and Cellular Biochemistry . 2006; 290:1-7 . || 52.Nordenstedt H, White DL, El-Serag HB. The changing pattern of epidemiology in hepatocellular carcinoma.GastroenterologicaItaliana .2010 ;42(3):206-14. | 53. Sarah M. Mense, Tom K. Hei, Ramesh K, et al. Phytoestrogens and Breast Cancer Prevention: Possible Mechanisms of Action. Environ Health Perspect.2008;116:426–433 || 54.TALITHA T. RAJAH, KEVIN J. PEINE . NGA DU, CHRISTINA A. SERRET, et al. Physiological Concentrations of Genistein and 17 -Estradiol Inhibit MDA-MB-231 Breast Cancer Cell Growth by Increasing BAX/BCL-2 and Reducing pERK1/2. Anticancer Research April.2012 ; 32 (4): 1181-1191 || 55.ParaskeviMoutsatsou. The spectrum of phytoestrogens in nature: our knowledge is expanding. HORMONES 2007, 6(3):173-193 || 56.Adlercreutz H.Phyto-estrogens and cancer. The Lancet Oncol.2002;3: 364-373. || 57. Michele Barone and Alfredo Di Leo. Estrogen Receptor beta in Colorectal Cancer Prevention: Do we have Conclusive Proof?.Genetic Syndromes & Gene Therapy.2013, 4:11-19. || 58. Michelle Cotterchio, Beatrice A. Boucher, Michael Manno. Dietary Phytoestrogen Intake Is Associated with Reduced Colorectal Cancer Risk1. J. Nutr.2006; 136: 3046–3053 || 59.ShikhaSaxena, Jyoti, Archana Sharma. SOYBEAN SEEDS- AN APPROACH TO TREATMENT OF BREAST CANCER.WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES.2008; 3 (8): 1972-1982. || 60.Xiaosheng Lu, Jinming Zhao, Tingyang Li, et al. 5,7-dihydroxy-4′-methoxyisoflavone induces apoptosis by inhibiting the ERK and Akt pathways in human osteosarcoma cells. Informa.Posted online on November 3, 2014. (doi:10.3109/03008207.2014.984064) || 61.SanjeevShukla and Sanjay Gupta. Apigenin: A Promising Molecule for Cancer Prevention. Pharm Res. 2010 ; 27(6): 962–978. || 62. Dr. Herman.Phytoestrogens: Epidemiology and a Possible Role in Cancer Protection .Environ Health Perspect.1995; 103(7):103-1 12 || 63.Huiqi Yang, Olga A Sukocheva, Damian J Hussey. Estrogen, male dominance and esophageal adenocarcinoma: Is there a link?. World J Gastroenterol.2012 ;18(5): 393-400 || 64.Ueo H, Matsuoka H, Sugimachi K, et al. Inhibitory effects of estrogen on the growth of a human esophageal carcinoma cell line. Cancer Res 1990; 50: 7212-7215. || 65. Yi-Ting Huang, Tai-Lung Cha, Guang-Huan Sun. Estrogen Inhibits Renal Cell Carcinoma Cell Progression through Estrogen Receptor- Activation. plos. Published: February 27, 2013 DOI: 10.1371/journal.pone.0056667 || 66. Myung-Jin Kim, Sung-Il Cho, Kun-Ok Lee1, et al. Effects of 17 -Estradiol and Estrogen Receptor Antagonists on the Proliferation of Gastric Cancer Cell Lines. J Gastric Cancer 2013;13(3):172-178 . || 67. Myung-Jin Kim, Sung-Il Cho, Kun-Ok Lee1, et al. Effects of 17 -Estradiol and Estrogen Receptor Antagonists on the Proliferation of Gastric Cancer Cell Lines. J Gastric Cancer 2013;13(3):172-178 || 68.Messa C, Russo F, Pricci M, Di Leo A. Epidermal growth factor and 17beta-estradiol effects on proliferation of a human gastric cancer cell line (AGS). Scand J Gastroenterol 2000;35:753-758. || 69. Messa C, Pricci M, Linsalata M, et al. Inhibitory effect of 17beta-estradiol on growth and the polyamine metabolism of a human gastric carcinoma cell line (HGC-27). Scand J Gastroenterol 1999;34:79-84. || 70. Loukovaara, M.; Carson, M.; Palotie, A.; et al. Regulation of sexhormone-binding globulin production by isoflavonoids and patterns of isoflavonoidconjugation in HepG2 cell cultures. Steroids.1995; 60: 656-661. || 71. MARCELLO MAGGIOLINI, DANIELA BONOFIGLIO, STEFANIA MARSICO, et al. Estrogen ReceptorMediates the Proliferative but Not the Cytotoxic Dose-Dependent Effects of Two Major Phytoestrogens on Human Breast Cancer Cells . MolPharmacol.2001; 60:595–602. || 72. Julie M. Hall, John F. Couse and Kenneth S. Korach .The Multifaceted Mechanisms of Estradiol and Estrogen Receptor Signaling. J. Biol. Chem. 2001, 276:36869-36872 || 73. Cross Heide S., Eniko° Ka´ llay, Daniel Lechner, WaltraudGerdenitsch, HermanAdlercreutz, H and H. James Armbrecht Phytoestrogens and Vitamin D Metabolism:A New Concept for the Prevention and Therapy of Colorectal, Prostate, and MammaryCarcinomas 2004 American Society for Nutritional Sciences. || 74.Birt DF, Hendrich S, Wang W. Dietary agents in cancer prevention: flavonoids andisoflavonoids. PharmacolTher. 2001; 90(2-3):157-77. || 75. Chrzan BG, Bradford PG. Phytoestrogens activate estrogen receptor 1 andestrogenic responses in human breast and bone cancer cell lines. MolNutr Food Res . 2007;51: 171–177. || 76. Murata IC, Itoigawa M, Nakao K, et al. Inductionof apoptosis by isoflavonoids from the leaves of Millettiataiwaniana, in humanleukemia HL-60 cells.Planta Med 2006;72 (5): 424–429. || 77. Barbara Pampaloni, Gaia Palmini, Carmelo Mavilia, et al. In vitro effects of polyphenols on colorectal cancer cells. World J GastrointestOncol2014 ; 6(8): 28–9-300 || 78. JOANNA WIETRZYK, GRZEGORZ GRYNKIEWICZ and ADAM OPOLSKI .Phytoestrogens in Cancer Prevention and Therapy –Mechanisms of their Biological Activity. ANTICANCER RESEARCH.2005; 25: 2357-2366 |