

Bcl2 Gene Expression Profile on Administration of Novel Active Compound from Soursop Leaves (SF-1603) as a New Molecular Target in Liver Cancer Therapy

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Abstract: Most cases of liver cancer present in advanced stages so the prognosis remains poor. Apoptotic dysregulation of liver cancer cells by BCL-2 gene expression is linked to tumor progression and resistance to treatment. Soursop plant compound is believed will be able to induce apoptosis by interfering Bcl2 gene expression. The objectives of the study was to explore the role of novel active compound isolated from soursop leaves againts Bcl2 gene expression in order to find new molecular target for liver cancer therapy. This study was in vitro experimental to assess active compound (SF-1603) effects on Bcl2 mRNA expression. Treatment groups were treated with SF-1603 dosage of 0,5xIC₅₀, IC₅₀ and 2xIC₅₀. Observations were assessed in hours 0, 24, 48, and 72. The results showed that Bcl-2 gene optimum expressions were achieved with 2xIC₅₀ dose at hour 24. There were strong correlation between Bcl-2 gene expression with apoptosis level (r=0,558). This evidence indicates that administration of SF-1603 promote expression of Bcl-2 gene to produce a peak signal that activate the apoptosis. It was concluded that SF-1603 affect Bcl2 gene expression as liver cancer therapy molecular target on HepG2 cell line culture.

1 INTRODUCTION

As an effort to reduce mortality, various studies to find effective treatments for liver cancer are still being developed. Curative approaches such as surgery and transplantation can only be done on a limited basis due to various causes such as lack of donors and considering its effects on liver function. Therapy recommendations based on the classification of the Barcelona Clinic Liver Cancer (BCLC) for liver cancer with advanced stages are a systemic chemotherapy. Curative approaches such as resection and transplantation can only be limited to patients with early stage liver cancer. Chemotherapy is still the best choice for patients with advanced liver cancer, however until now the effectiveness of chemotherapy in liver cancer patients is often considered relatively ineffective. This therapeutic option for patients with liver cancer is still very limited which is also one of the reasons for the prognosis of liver cancer remain poor. (Ho et

al., 2009, Huynh et al., 2010, Lencioni et al., 2008, Robotin et al., 2009, Park et al., 2006, Ghassan and Abou-alfa, 2004, Wirth et al., 2005, Sherman et al., Bruix and Sherman, 2005)

Systemic chemotherapy has been shown repeatedly to provide no benefit in improving survival, regardless of whether it is given as a single agent or as a combination chemotherapy part. Systemic chemotherapy using existing chemotherapy agents is generally stated to be relatively ineffective for liver cancer. Liver cancer is conciders resistant to chemotherapy because of the high mutation load and also the mechanism of drug resistance. The mechanism of resistance that arises relates to the administration of low-dose chemotherapy because it considers liver dysfunction and is also carried out to reduce toxicity (Park et al., 2006, Wirth et al., 2005, Ghassan and Abou-alfa, 2004)

Although many chemotherapeutic agents have been tested, the role of systemic chemotherapy for liver cancer remains unclear. New therapeutic

strategies with more specific targets are needed to improve treatment effectiveness. (Ghassan and Abou-alfa, 2004, Park et al., 2006) There are no prospective controlled studies showing that systemic chemotherapy prolongs the survival of liver cancer patients compared with supportive care. (Park et al., 2006, Ghassan and Abou-alfa, 2004, Wirth et al., 2005)

Antihormonal therapy with tamoxifen or otreotide does not provide better patient survival. Therapeutic strategies based on molecular targets through pathway intervention signal transmission and apoptotic regulation, offering new hope for more effective treatment options. Potential targets for systemic chemotherapy strategies in liver cancer include mechanisms of oxidative and inflammatory stress, growth factors, cell cycle checkpoints, oncogene viruses, telomere shortening, carcinogenesis, stem cells, angiogenesis and antiapoptosis. (Wirth et al., 2005, Park et al., 2006, Ghassan and Abou-alfa, 2004) Dysregulation of apoptosis influence the process of carcinogenesis, progression of the tumor and tumor resistance to radio-chemotherapy, therefore the development of anticancer agents by inducing apoptosis is very potential to be developed. Understanding in genetics and treatment has improve a lot, but liver cancer remains a deadly disease causing high mortality. Further studies are needed to identify agents that have more effective activity against liver cancer.

Cancer occurs because of fundamental changes in cell physiology, one of the common characteristics of which is avoiding apoptosis, therefore the target of developing cancer therapy agents is directed to induction of apoptosis. (Cha and DeMatteo, 2005, Ho et al., 2009) Neoplastic cell accumulation can occur not only because of activation of oncogenes that promote tumor growth or inactivation of tumor suppressor genes that suppress growth, but also because of dysregulation in genes that control apoptosis. As cell growth is controlled by growth and suppressor genes, cell survival is also controlled by genes that promote and inhibit apoptosis (Kumar et al., 2003, Ho et al., 2009)

Apoptosis is a form of programmed cell death that depends on the results of intracellular gene expression. The apoptosis process is divided into two phases, initiation phase and execution phase. Apoptosis initiation occurs because of signals transmission from different pathways, namely the extrinsic pathway (death receptor pathway) and intrinsic pathway (mitochondrial pathway). These two pathways eventually activate the caspase

enzyme and relate to each other in several stages. (Kumar et al., 2003, Xu et al., 2010, Liu et al., 2011)

Apoptosis is induced by a cascade of sequences of molecular events initiated through many pathways that eventually activate caspase enzymes (Kumar et al., 2003, Wirth et al., 2005, Chiu et al., 2003) Apoptosis processes can be divided for two phases, namely (Kumar et al., 2003, Martin) initiation phase, when the caspase enzyme is catalyzed to become active and the execution phase, when the caspase enzyme causes cell death. Apoptosis initiation occurs because of signals from different pathways, namely the extrinsic pathway (death receptor pathway) and intrinsic pathway (mitochondrial pathway). Both of these pathways eventually activate caspase enzymes and relate to each other in several stages. (Kumar et al., 2003) Intrinsic pathway involves one of the pro and antiapoptotic molecules including Bcl-2.

Intrinsic signaling pathways are initiated by increased mitochondrial permeability and release of proapoptotic molecules into the cytoplasm, without the involvement of death receptors. Hormones, growth factors and other survival signals stimulate antiapoptotic production from the Bcl-2 protein group. Two proteins which are the main antiapoptosis molecules are Bcl-2 and Bcl-x. Both of these antiapoptotic proteins are normal in the mitochondrial membrane and cytoplasm of the cell. (Kumar et al., 2003, Ho et al., 2009, Xu et al., 2010, Robotin et al., 2009) There is an increase in expression of Bcl2 and IAP in liver cancer, which is an antiapoptotic protein resulting in apoptosis inhibition via intrinsic pathways. (Chang Y., 2011, Bassiouny A et al., 2008, Yildiz L et al., 2008)

The principal of intrinsic pathway is the existence of a balance between pro-apoptotic molecules such as Bax, Bak, and Bim with antiapoptotic molecules such as Bcl2 and Bcl-xl, which control mitochondrial permeability and release of factors that induce cell death in normal mitochondria. Mitochondria play an important role in this pathway by releasing cytochrome c, which eventually forms a complex with apoptosis-inducing factor 1 (APAF-1), procaspase-9 and ATP. Inside this complex, procaspase-9 is activated to caspase-9, which then triggers caspase-3 as executor caspase. (Kumar et al., 2003, Ho et al., 2009, Xu et al., 2010) In our study, induction of cell apoptosis was investigated through one of the intrinsic pathways which would be proven biochemically with changes in the Bcl2 expression.

Bcl-2 is a protein family that is a CED-9 homologue found in mammals. Bcl-2 was first

discovered in B-cell lymphoma as a proto oncogen. Bcl-2 gene overexpression is a protective mechanism to deal with various stimuli that cause cell death. Bcl-2 gene is responsible for synthesizing protein bcl-2. The Bcl-2 protein family consists of antiapoptosis groups, namely bcl-2, bcl-xl, bcl-w, Mcl-1, Nr13, and A1 / Bfl1, as well as proapoptosis groups namely Bax, Bak, Bok, Diva, Bcl-xs, Bik, Bim, Hrk, Nip3, Nix, Bad, and Bid. These proteins are characterized by the Bcl-2 homology (BH) domain in their structures, namely BH1, BH2, BH3, and BH4 (Lu et al., 2011, Cagle and Allen, 2009, Marschitz et al., 2000)

The proapoptosis group has two subfamily, multidomain groups (Bax, Bak, Bok, Diva, and Bcl-xs) and groups that only have BH3 domains (Bik, Bim, Hrk, Nip3, Nix). This relative ratio between pro protein and antiapoptosis determines cell sensitivity to various apoptotic stimuli. Proapoptosis proteins that have been studied well are Bax and Bid. Exposure to various apoptotic stimuli causes translocation of Bax from the cytosol to the mitochondrial membrane. Bax oligomerization in the mitochondrial membrane together with other proapoptotic proteins, Bak eventually releases cytochrom-c from within the mitochondria to the cytosol. Other proapoptotic proteins, especially those that only have BH3 domains, play a role in the Bax-Bak oligomerization process in the mitochondrial membrane. Bcl-2 antiapoptotic protein plays a role in inhibiting Bax-Bak oligomerization in the mitochondrial membrane and eventually inhibits the release of cytochromic cytosol (Marschitz et al., 2000, Lu et al., 2011, Cagle and Allen, 2009)

Hepatocarcinogenesis is a slow process, genomic changes that progressively alter hepatocellular phenotypes and produce cellular intermediates that develop into hepatocellular carcinoma (Thorgeirsson and Grisham, 2002, Cha and DeMatteo, 2005) In liver cancer pathogenesis, apoptotic dysregulation occurs in the form of low Fas expression which inhibits caspase-8 and caspase-3 activation which ultimately inhibits the apoptosis process through the extrinsic pathway, and an increase in Bcl2 and IAP expression which inhibits caspase 9 activation and caspase 3 which ultimately inhibits the apoptosis process through intrinsic pathways. (Kumar et al., 2003, Ho et al., 2009, Xu et al., 2010, Bassiouny A et al., 2008, Cagle and Allen, 2009, Yildiz L et al., 2008) Based on this thought, a study was conducted to observe Bcl2 gene profile as one of the genes that play a role in the liver cancer pathogenesis.

Natural polyphenols are a large group of compounds derived from plants, which are chemically characterized by two or more phenol units (Dai and Mumper, 2010, Fraga and Oteiza, 2011) Some chemists know the term polyphenols as White-Bate-Smith Swain-Haslam which is described in several characteristics. (Quideau et al., 2011) This definition does not include low molecular weight structures, which have been shown to have potential benefits for human health. There are thousand compounds derived from plants with higher biological potential, which only have one or more aromatic rings and at least two hydroxyl groups, categorized as polyphenols. (Sies, 2010)

Natural polyphenols are secondary metabolites derived from plants that are produced as defense agents against various types of stress, such as ultraviolet radiation, pathogenic aggression, low soil fertility, changes in ambient temperature and drought. (Dai and Mumper, 2010, Manach et al., 2004) Based on its chemical structure, there are four main classes of polyphenols: phenolic acids, flavonoids, stilbenes, and lignans (Manach et al., 2004, Bravo, 1998) Knowledge and implications of these compounds on human health including their effects on cancer (Kampa et al., 2007, Guo et al., 2009, Korkina et al., 2009) nervous system protection, (Zhao, 2009, Gutierrez-Merino et al., 2011) cardiovascular system dysfunction and damage, (Sies, 2010, Grassi et al., 2009) metabolic syndrome, (Agouni et al., 2009, Cherniack, 2011) diabetes, (Milne et al., 2007) aging, (Queen and Tollefsbol, 2010, Accomando et al., 2010) and various pathologies (Tejasari M et al., 2014) and condotion related to inflammation. (Accomando et al., 2010) Polyphenols have been used for thousands of years as traditional medicine in eastern countries. However, the inclusion of these compounds in western medicine is still a pending issue, perhaps because of lack information and still not widely known scientifically. (Rodriguez et al., 2013, Dashwood, 2007)

Annona muricata Linn is a plant generally known as soursop or graviola which contain a large group of phytochemicals that naturally have anticancer activities with high selectivity between cancer cells and normal cells. (Fraga and Oteiza, 2011, Song et al., 2014, Martin, 2006, Young et al., 2005, Sayers, 2011) There are much studies show that the active ingredients of soursop leaves have strong anticancer activity on various types of cancer cell lines. (He et al., 2010, Xiao et al., 2011, Coloma et al., 2002a). Other studies also report that active from the leaves of scales proved to be able to induce

apoptosis, but research on the ability of soursop leaves to induce apoptosis in liver cancer cells and its mechanism of action has not been widely carried out. (Coloma et al., 2002b)

This study aims to test the effect of administration of pure compounds (SF-1603) isolated from soursop leave, to analyze its ability to induce apoptosis in liver cancer cells, and explore its mechanism of action in inducing apoptosis by analyze Bcl2 mRNA expression in liver cancer cell line to determine potential pathways as a new molecular target of liver cancer therapy.

2 MATERIAL AND METHODS

Materials used in this study was the pure compound isolated from the soursop leaves code SF-1603, HepG2 cell line (HB-8065TM) from the American Type Culture Collection (ATCC), Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), penicillin, streptomycin and trypsin for cell culture.

2.1 HepG2 Cell Line Culture

HepG2 cell line used in this study were less than 15 passages. Cell were seeded into well in Medium (DMEM / F12) containing 10% Fetal Bovine Serum, previously release cells using trypsin 0.05% - EDTA 0,53mM, then added to the growth medium into a cell suspension. The cells were counted using a hemocytometer and planted with a cell density of 25,000 cells/mL and observed at 0, 24, 48 and 72 hour at 37oC with 5% CO2 atmosphere. There were control group and intervention group given SF-1603 with concentration of 0,5xIC₅₀, IC₅₀ and 2xIC₅₀. Determination of IC₅₀ concentration was done using 3-4-5-dimethylthiazol-2yl-2,5-difenil tetrazolium bromide (MTT) method.

2.2 Measurement of mRNA Expression using Quantitative Real-time Polymerase Chain Reaction

This study using real-time PCR for quantitative analysis. The working principle of real time PCR is similar to conventional RT-PCR with the fundamental difference that is: (i) Analysis of amplicons using fluorescent reporter and not using conventional gel electrophoresis, (ii) amplicon can be analyzed from each cycle, and not only when the end point. This study uses SsoFast™EvaGreen

SUPERMIX containing a mixture of ready-made for the qPCR reaction except the primer and template, ie 2x reaction buffer with dNTPs, sso7d-fusion polymerase, MgCl₂, EvaGreen dye and stabilizers.

2.3 Statistical Analysis

All quantitative data are representative of at least three independent experiments. Values are expressed as mean±SD. Statistical analyses were conducted using independent test, ANOVA test and simple linear regression. The statistical package IBM SPSS Statistics 21 for Windows was used in the analysis.

2.4 Implications of Ethical Aspects

This study has obtained ethical approval from Medical Research Ethics Committee Medical Faculty Padjadjaran University No.988 / UN6.C2.1.2 / KEPK / PN.

3 RESULTS AND DISCUSSION

The group with 0.5xIC₅₀ administration dose showed the of Bcl2 mRNA expression in the treatment group was much different from the control group. In general, in the control group there were not many changes, there was only a slight increase in the expression from the 48th to 72nd hours, whereas in the treatment group there were quite dynamic changes. During the first 24 hours there was only a slight decrease in Bcl2 mRNA expression in the treatment group, whereas the control group did not experience changes in expression level. From the 24th to the 42nd hours there was a sharp increase in the expression level of treatment groups until it reached the highest level, while the control group only showed very little increase. From the 48th hour to the 72nd hour there was a sharp decrease in the level of expression in the treatment group to the lowest point while the control group showed a slight increase in expression.

In the group with the administration of IC₅₀ dose, it was seen that the expression of Bcl2 mRNA in the treatment group was significantly different from the control group. There were not many changes in the control group, there was only a slight increase in expression from 48 hours to 72 hours, whereas in the treatment group there was a quite dynamic change. In the first 24 hours there was only a slight decrease in Bcl2 mRNA expression in the treatment group while the control group did not experience changes in expression level. From the 24th to the 42nd hours

there was a sharp increase in the expression level until it reached the highest score, while the control group only showed very little increase. From the 48th hour to the 72nd hour there was a sharp decrease in the level of expression in the treatment group to the lowest point while the control group showed a slight increase in expression.

The group with the administration of 2xIC₅₀ doses showed that overall the expression of Bcl2 mRNA in the treatment group was lower than the expression of the control group. In the first 24 hours there was a decrease in Bcl2 mRNA expression in the treatment group, whereas in the control group there was no change in expression level. From the 24th to the 72nd hours there was an increase in the expression of Bcl2 mRNA in the treatment and control groups, with the expression level of the treatment group being around 3 times lower than the control group. The lowest Bcl2 mRNA expression occurred at the 24th hour and the highest was at 72 hours, both in the treatment and control groups.

The expression profile of Bcl2 mRNA in HepG2 cell line culture after administration of pure compounds SF-1603 can be seen in Figure 1.

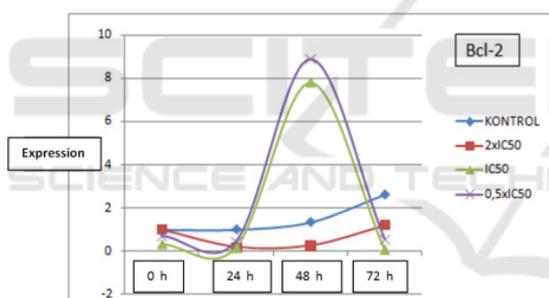


Figure 1: Bcl-2 expression profile based on observation time in all groups, on HepG2 cell line culture after administration of pure compound SF-1603.

The normality test was performed using the Kolmogorov-Smirnov Test and the test results with $\alpha = 5\%$ showed that at 95% confidence level, all data were normally distributed. ($p > 0.05$) To find out the differences in of Bcl-2 mRNA expression after administration of pure SF-1603 compound at each dose and time of observation, we used dependent test and analysis of variance (ANOVA) method by making decisions using the F distribution table. Based on statistical calculations using ANOVA method with $\alpha = 5\%$ obtained F count value compared with Ftable value as shown in Table 1 which showed that the $F_{\text{count}} > F_{\text{table}} (0.05)$. It can be concluded that at 95% confidence level there were significant differences in Bcl-2 expression between

each group in all doses and time of observation ($p < 0.05$).

Table 1: Test of different expressions of Bcl-2 in HepG2 cell Line culture between groups

Group	mRNA Bcl-2 expression	
	F count	F table:
		(0.05)
Control group		
0.5xIC ₅₀ group	21.3872	2.81647
IC ₅₀ group	8741	
2xIC ₅₀ group		

ANOVA (Analisis of varians)

To determine the dose and time that produces the optimum Bcl-2 mRNA expression for apoptosis initiation, we used an average comparison test. Figure 2&3 showed the administration of SF-1603 soursop leaf pure compound on HepG2 cell line culture resulted in optimum Bcl-2 gene expression for apoptosis initiation at a dose of 2xIC₅₀ at 24 hours.

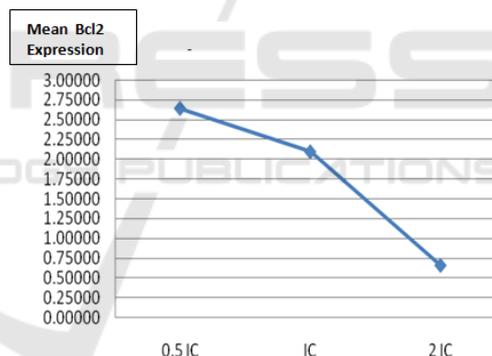


Figure 2: Effect of Doses on Bcl-2 Gene Expression on All Observations time, After Giving Soursop Leaf Pure Compounds to HepG2 Cell Line Culture.

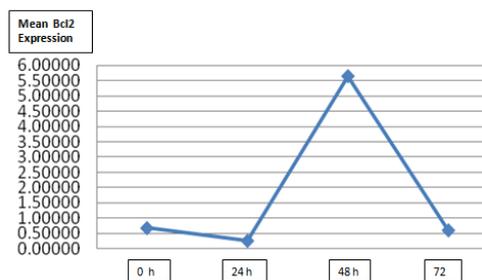


Figure 3: Effect of Time Factors on Bcl-2 Genes in All Doses, After Giving Soursop Leaves Compound to HepG2 Cell Line Culture.

Various chemical compositions have been isolated from various parts of soursop plants such as leaves, roots, bark, flesh and seeds. Some phytochemicals have been reported to be isolated and characterized from various parts of soursop plants and one of them isolated from soursop leaves has strong anticancer activity and is able to induce apoptosis in liver cancer cells. (Tejasari et al., 2018)

Suppression of Bcl-2 gene expression will initiate the process of apoptosis through intrinsic pathways. This apoptotic pathway occurs due to increased mitochondrial permeability and release of proapoptotic molecules into the cytoplasm, without the involvement of death receptors. Several growth factors and other survival signals stimulate antiapoptosis production from the Bcl-2 protein group (Martin, Wirth et al., 2005, Kumar et al., 2003, Albert and Johnson, 2002)

Lots of proteins that belong to this group which all play a role in regulating the apoptosis process. One of the proteins which is the main antiapoptosis is Bcl-2 protein. This antiapoptotic protein is present in the mitochondrial membrane and cytoplasm of cells. When the cell receives a survival signal or experiences stress, Bcl-2 will disappear from the mitochondrial membrane and be replaced by proapoptotic proteins such as Bak, Bax, and Bim (Kumar et al., 2003, Albert and Johnson, 2002). When Bcl-2 and / or Bcl-x level decreases, permeability of the mitochondrial membrane will increase so that some proteins are released which can activate the caspase cascade. One of these proteins is cytochrome c, which is known to play a role in mitochondrial respiration. In cytosol, cytochrome c binds to a protein called Apaf-1 (apoptosis activating factor-1, homologous with Ced-4), and this complex activates caspase-9. Bcl-2 and Bcl-x may also directly inhibit Apaf-1 activation so that in the absence of Bcl-2 and Bcl-x, Apaf-1 activation can occur (Albert and Johnson, 2002, Kumar et al., 2003). is the caspase cascade initiation process. It can be concluded that the essence of this intrinsic pathway is the balance between proapoptotic molecules and protective molecules that control mitochondrial permeability and the release of factors that induce cell death that are normally in mitochondria. (Kumar et al., 2003).

The study conducted in 2005 was reported that in liver cancer pathogenesis, there was an increase in the Bcl2 expression which is an antiapoptotic protein and strengthened by a study in 2008 which stated that Bcl2 activation played a role in the progression towards liver cancer. (Bassiouny et al., 2008,

Coloma et al., 2002, Chang and Xu, 2000, Yildiz et al., 2008, Wu et al., 1995, Liu and 2009, 2009)

Inhibition of apoptosis through the intrinsic pathway involving the Bcl-2 gene is one of the pathways involved in the pathogenesis of liver cancer. The ability of SF1603 soursop leaf pure compound to suppress the expression of Bcl-2 can be seen in figure 1 which showed the expression level of Bcl-2 was lower than the control group in all treatment groups. This is reinforced by the graph in figure 2&3 which showed the optimum point of expression of Bcl-2 by calculating the dose and time factors. Statistical test with ANOVA in Table 1 strengthens the evidence with conclusions on 95% confidence levels there are significant differences in Bcl-2 expression between each group in all doses and time of observation ($p < 0.05$), in HepG2 cell line culture after the administration of soursop leave pure compounds SF-1603.

To measure the correlation strength of Bcl2 expression with apoptosis level, a simple correlation test was calculated using the Pearson formula, and presence coefficient correlation between Bcl2 mRNA expression and the level of apoptosis is $r=0,558$, which means the strength of the correlation is strong. With the ability of SF-1603 soursop leave pure compound in suppressing the expression of Bcl-2, this compound can be used as a candidate for HCC therapy agent by making the Bcl-2 gene as a molecular target of therapy to initiate the HCC apoptosis induction process.

4 CONCLUSIONS

The study conclude that the novel soursop leaves active compound (SF-1603) is a powerful anticancer that affect Bcl2 gene expression in apoptosis induction on liver cancer cell, so it can be used as a candidate for new therapeutic agent for liver cancer treatment with Bcl2 as a new molecular target

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REFERENCES

- Accomando, S., Pellitteri, V. & Corsello, G. 2010. Natural Polyphenols As Anti Inflammatory Agents. *Fornt Biosci*, 2, 318-31.
- Agouni, A., Lagrus-Lak-Hal, A., Tesse, A., Mulder, P. & Al, E. 2009. Red Wine Polyphenols Prevent Metabolic And Cardiovascular Alterations Associated With Obesity In Zucker Fatty Rats (Fa/Fa). *Plos One*, 4, E5557.
- Albert & Johnson 2002. *Molecular Biology Of The Cell*, New York., Garland Science Taylor And Francis Group Company.
- Bassiouny, A., Bassiouni, N., Nosseir, M., Zoheiry, M., El-Ahwany, G., F. F. S. & Al., E. 2008. Circulating And Hepatic Fas Expression In Hcv-Induced Chronic Live Disease And Hepatocellular Carcinoma. *Medscape J Med.*, 10, 130.
- Bravo, L. 1998. Polyphenols: Chemistry, Dietary Source, Metabolism And Nutritional Significance. *Nutr Rev*, 56, 317-33.
- Bruix, J. & Sherman, M. 2005. . Management Of Hepatocellular Carcinoma. *Hepatology*, 42, 1208-36.
- Cagle, P. & Allen, T. C. 2009. Apoptosis. *Basic Concept Of Molecular Pathology*. Springer Dordrecht Heidelberg.
- Cha, C. & Dematteo, R. 2005. *Molecular Mechanisms In Hepatocellular Carcinoma Development*. Practise&Research Clinical Gastroenterology, 19, 25-37.
- Chang, Y. & Xu, Y. 2000. Expression Of Bcl-2 Inhibited Fas-Mediated Apoptosis In Human Hepatocellular Carcinoma Bel-7404 Cells. 10.
- Cherniack, E. 2011. Polyphenols: Planning The Seeds Of Treatment For The Metabolic Syndrome Nutrition, 27, 617-23.
- Chiu, H., Chih, T., Hsian, Y., Tseng, C., Wu, M. & Wu, Y. 2003. Bullatacin, A Potent Antitumor Annonaceous Acetogenins, Induces Apoptosis Through A Reduction Of Intracellular Camp And Cgmp Levels In Human Hepatoma 2.2.15 Cells. . *Biochemical Pharmacology.*, 65, 319-327.
- Coloma, A., Guadan-O, A., Ine'sa, C., Martinez-Diazb, R. & Cortesc, D. 2002a. Selective Action Of Acetogenin Mitochondrial Complex I Inhibitors. *Z Naturforsch*, 57c, 1028-1034
- Dai, J. & Mumper, R. 2010. Plant Phenolics: Extraction, Analysis And Their Antioxidant And Anticancer Properties. *Molecules*, 15, 7313-52.
- Dashwood, R. 2007. *Frontiers In Polyphenols And Cancer Prevention*. *J Nutr*, 37, 267s-269s.
- Fraga, C. G. & Oteiza, P. I. 2011. Dietary Flavonoids : Role Of (-)-Epicatechin And Related Procyanidins In Cell Signaling. *Free Radical Biology & Medicine*, 51, 813-23.
- Ghassan, K. & Abou-Alfa, M. 2004a. *Current And Novel Therapeutics For Hepatocellular Carcinoma*. American Society Of Clinical Oncology.
- Ghassan, K. & Abou-Alfa, M. 2004b. . *Current And Novel Therapeutics For Hepatocellular Carcinoma*
- Grassi, D., Desideri, G., Groce, G., Tiberti, S., Anggio, A. & Al, E. 2009. Flavonoids, Vascular Function And Cardiovascular Protection. *Curr Pharm Des*, 15, 1072-84.
- Guo, W., Kong, E. & Meydani, M. 2009. Dietary Polyphenols, Inflammation And Cancer. *Nutr Cancer*, 61, 807-10.
- Gutierrez-Merino, C., Lopez-Sanchez, C., Lagoa, R., Samhan-Arias, A., Bueno, C. & Al, E. 2011. Neuroprotective Actions Of Flavonoids. *Curr Med Chem*, 18, 1195-1212.
- He, H., Wu, X., Yu, B., Liu, K., Zhou, G., Qian, G. & Al, E. 2010. The Effect Of Desacetylurvaricin On The Expression Of Tlr4 And P53 Protein In Hepg 2.2.15. *Journal Of Hepatitis*, 11, 364-367.
- Ho, H., Pok, S., Streit, S., Ruhe, J. E., Hart, S., Lim, K. & Al., E. 2009. Fibroblast Growth Factor Receptor 4 Regulates Proliferation, Anti-Apoptosis And Alpha-Fetoprotein Secretion During Hepatocellular Carcinoma Progression And Represents A Potential Arget For Therapeutic Intervention. . *J.Jhep*, 50, 118-127.
- Huynh, H., Ngo, V., Koong, H., Poon, D., Choo, S., Toh, H. & Al., E. 2010a. Azd6244 Enhances The Anti-Tumor Activity Of Sorafenib In Ectopic And Orthotopis Models Of Hcc. *J.Jhep*, 52, 79-87.
- Huynh, H., Ngo, V., Koong, H., Poon, D., S, S. C., Toh, H. & Al, E. 2010b. Azd6244 Enhances The Anti-Tumor Activity Of Sorafenib In Ectopic And Orthotopis Models Of Hcc. *Jjhep*, 52, 79-87.
- Kampa, M., Nifli, A., Notas, G. & Castanas, E. 2007. Polyphenols And Cancer Cell Growth. *Rev Physiol Biochem Pharmacol*, 159, 79-113.
- Korkina, L., Luca, C. D., Kostyuk, V. & Pastore, S. 2009. Plant Polyphenols And Tumors: From Mechanism To Therapies, Prevention And Protection Againts Toxicity Of Anti-Cancer Treatments. *Curr Med Chem*, 16, 3943-65.
- Kumar, V., Cotran, R. & Robbin, S. 2003. *Pathologic Basic Of Disease*, Philadelphia, Saunders-Elsevier.
- Lencioni, R., Crocetti, L., Petruzzi, P., Vignali, C., Bozzi, W., Pina, C. & Al, E. 2008. Doxorubicin-Eluting Bead-Enhanced Radiofrequency Ablation Of Hepatocellular Carcinoma:A Pilot Clinical Study. *J.Jhep*, 49, 217-222.
- Liu, C. & 2009, Y. Z. 2009. Effect Of Annonaceous Acetogenin On Proliferation And Apoptosis Of Raji Cell. *Chinese Journal Of Information On Traditional Medicine*. .
- Liu, Q., Chen, J., Liu, L., Zhang, J., Wang, D., Ma, L. & Al, E. 2011. The X Protein Of Hepatitis B Virus Inhibits Apoptosis In Hepatoma Cell Through Enhancing The Methionine Adenosyltransferase 2a Gene Expression And Reducing S-Adenosylmethionine Production. *The Journal Of Biological Chemistry*, 286, 17168-17180.
- Lu, Y., Zhang, Y., Jia, Z., Wu, W. & Lu, Z. 2011. Hepatocellular Carcinoma Hepg2 Cell Apoptosis And Caspase-8 And Bcl-2 Expression Induced By Injectable Seed Extract Of Coix Lacryma-Jobi. *Hepatobiliary Panreat Dis Int.*, 10.

- Manach, C., Scallbert, A., Morand, C., Remesy, S. & Jimenez, L. 2004. Polyphenols: Food Sources And Bioavailability. *Am J Clin Nutr*, 79, 727-47.
- Marschitz, I., Tinhofer, I., Hittmair, A., Egle, A., Kos, M. & Greil, R. 2000. Analysis Of Bcl-2 Protein Expression In Chronic Lymphocytic Leukemi. *Am J Clin Pathol* 113, 219-229.
- Martin, K. Targeting Apoptosis With Dietary Bioactive Agents. *Experimental Biology And Medicine*, 231, 117-129
- Martin, K. R. 2006. Targeting Apoptosis With Dietary Bioactive Agents. *Experimental Biology And Medicine*, 231.
- Milne, J., Lambert, P., Schenk, S., Carney, D., Smith, J. & Al, E. 2007. Small Molecule Activators Of Sirt1 As Therapeutics For The Treatment Of Type 2 Diabetes. *Nature* 450, 712-16.
- Park, S., Lee, Y., Han, S., Kwon, S., Kwon, O., Kim, S. & Al, E. 2006a. . Systemic Chemotherapy With Doxorubicin, Cisplatin And Capecitabine For Metastatic Hepatocellular Carcinoma. *Bmc Cancer*, 6, 10.1186/471-2407-6-3.
- Park, S., Lee, Y., Han, S., Kwon, S., Kwon, O., Kim, S. & Al, E. 2006b. Systemic Chemotherapy With Doxorubicin, Cisplatin And Capecitabine For Metastatic Hepatocellular Carcinoma. *Bmc Cancer*, 6, 10.1186/1471-2407-6-3.
- Queen, B. & Tollefsbol, T. 2010. Polyphenols And Aging. *Curr Aging Sci*, 3, 34-42.
- Quideau, S., Deffieux, D., Douat-Casassus, C. & Pouyssegu, L. 2011. Plant Polyphenols: Chemical Properties, Biological Activities And Synthesis. *Angew Chem Int Ed Engl*.
- Robotin, M., Kansil, M., Howard, K., George, J., Tipper, S., Dores, G. & Al, E. 2009a. Antiviral Therapy For Hepatitis B-Related Cancer Prevention Is More Cost-Effective Than Cancer Screening. *Jhep*, 50, 990-998.
- Robotin, M., Kansil, M., Howard, K., George, J., Tipper, S., Dores, G. & Al, E. 2009b. Antiviral Therapy For Hepatitis B-Related Cancer Prevention Is More Cost-Effective Than Cancer Screening. *J.Jhep*, 50, 990-998.
- Rodriguez, M. L., Estrela, J. M. & Ortega, A. L. 2013. Natural Polyphenols And Apoptosis Induction In Cancer Therapy. *J Carcinogene Mutagene*, S6.
- Sayers, T. 2011. Targeting The Extrinsic Apoptosis Signaling Patways For Cancer Therapy. *Cancer Immunol Immunother*, 60, 1173-80.
- Sherman, M., Burak, K., Maraun, J., Metrakos, P., Myers, R., Guindi, M. & Al, E. Multidisciplinary Canadian Concensus Recommendations For The Management And Treatment Of Hepatocellular Carcinoma. *Current Oncology*, 18.
- Sies, H. 2010. Polyphenols And Health: Update And Perspectives. *Arch Biochem Biophysics*, 501, 2-5.
- Song, Y., Zhang, A., Yan, G., Han, Y. & Wang, X. 2014. Plant-Derived Natural Products As Leads To Anti-Cancer Drugs. *J.Med.Plant Herb.Ther.Res*, 6.
- Tejasari, M., Sastramihardja, H., Abdurachman, S. & Prasetyo, D. 2018. Anticancer Activity Of Novel Soursop Leaves Active Compound (Sf1603) Through Apoptotic Induction In Liver Cancer *Malaysian Journal Of Fundamental And Applied Sciences*, 14, 226-234.
- Tejasari M, S. H., Abdurrachman Sa, Prasetyo D 2018. Anticancer Activity Of Novel Soursop Leaves Active Compound (Sf-1603) Through Apoptotic Induction In Liver Cancer. *Malaysian Journal Of Fundamental And Applied Sciences* 14, 226-234.
- Tejasari M, S. N., Sastramihardja H, Djanuarsih I 2014. The Role Of Soy In Preventing Apoptosis In Liver Injury. *International Journal Of Research In Pharmaceutical And Nano Science*, Issn: 2319 – 9563, 3, 373-379.
- Thorgeirsson, S. & Grisham, J. 2002. Molecular Pathogenesis Of Human Hepatocellular Carcinoma. *Nature Genetics*, 31.
- Wirth, T., Kuhnel, F., Fleischmann-Mundt, B., Woller, N., Djojsubroto, M., Rudolph, K. & Al, E. 2005a. Wirth T, Kuhnel F, Fleischmann-Mundt B, Woller N, Djojsubroto M, Rudolph K, Et Al. Telomerase-Dependent Virotherapy Overcomes Resistance Of Hepatocellular Carcinomas Against Chemotherapy And Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand By Elimination Of Mcl-1 *Cancer Res*. *Aacrjournal* 65, 7393.
- Wirth, T., Kuhnel, F., Fleischmann-Mundt, B., Woller, N., Djojsubroto, M., Rudolph, K., Manns, M., Zender, L. & Al, E. 2005b. Telomerase-Dependent Virotherapy Overcomes Resistance Of Hepatocellular Carcinomas Against Chemotherapy And Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand By Elimination Of Mcl-1. *Cancer Res*. *Aacrjournal* . 65, 7393.
- Wu, F., Zeng, L., Gu, Z., Zhao, G., Zhang, Y., Schwedler, J., McLaughlin, J. & Sastridihardjo, S. 1995. New Bioactive Monotetrahydrofuran Annonaceous Acetogenins, Annonamuricin C And Muricatocin C, From Leaves Of *Annona muricata*. *J Nat Prod* 58, 909-15.
- Xiao, Q., Qiu, Y., Zhou, G., Mao, C., Li, Z., Yao, Z. & Al, E. 2011. Potent Antitumor Mimetics Of Annonaceous Acetogenins Embedded With An Aromatic Moiety In The Left Hydrocarbon Chain Part. *J Med Chem*, 54, 525-533.
- Xu, L., Qian, G., Tang, L., Su, J. & Wang, J. 2010. Genetic Variations Of Hepatitis B Virus And Serum Aflatoxin-Lysine Adduct In High Risk Hepatocellular Carcinoma In Southern Guangxi, China. *J.Jhep.*, 53, 671-676.
- Yildiz, L., Baris, S., Aydin, O., Kefeli, M. & Kandemir, B. 2008. Bcl-2 Positivity In B And C Hepatitis And Hepatocellular Carcinoma. *Hepatogastroenterology* 55, 2207-10.
- Young, L., Hantz, H. & Martin, K. 2005. Resveratrol Modulates Gene Expression Associated With Apoptosis, Proliferation, And Cell Cycle In Cells With Mutated Human C-Ha-Ras, But Does Not Alter C-Ha-Ras Mrna Or Protein Expression. *J.Nutr Biochem*, 16, 663-74.
- Zhao, B. 2009. Natural Antioxidant Protect Neuron In Alzheimers Disese And Parkinsons Disease. *Neurochem Res*, 34, 630-38.