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The mechanism of action of *Thymus* sp. for inducing apoptosis and fighting cancer

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Abstract

Cancer is one of the major reasons of death characterized by uncontrolled growth and spread of cancer cells due to internal and environmental agents. Different cases of cancer are increasing all over the world year by year. Recently, the use of the extracts and natural products from medicinal plants took a huge leap towards cancer curing. Chemoprevention of cancer through medicinal plants is gaining popularity for its relatively harmless action. This review is focused on the anti-tumor effect and mechanism of action of active substances present in the extracts from different *Thymus* sp. in inducing apoptosis and fighting cancer progress.

Keywords

Apoptosis, Chemoprevention, *Thymus* sp.

1. Introduction

During recent years, the medicinal plants have come more into the focus of phytomedicine. Their use has attracted the interest of scientists in exploring their potential anti-cancer activity. *Thymus* sp. (common thyme or garden thyme) is a species of flowering plant belonging to the mint family Lamiaceae. Thyme is native to southern Europe from the western Mediterranean to southern Italy. It is a bushy, woody-based evergreen subshrub with small, highly aromatic, grey-green leaves and clusters of purple or pink flowers [1].

Thyme has been used in foods mainly for the flavor and preservation. It used in folk medicine since the ancient Greeks, Egyptian and Romans. Flavonoids and phenolic compounds are common constituents of plants used in traditional medicine to treat a wide range of diseases. Thyme has been used widely as antispasmodic, expectorant in upper respiratory tract infections, *e.g.* bronchitis and pertussis, antithelmentic and antitussive agents [2,3]. In odontology, thymol a component of thyme is used as the main active antiseptic ingredient in chemotherapeutic mouthrinses against gingivitis [4]. A study by Szentandrassy et al. [5] suggested that thymus extracts are antimutagenic due to the potent antioxidant properties of thymol. Elhabazi et al. [6] evaluated the immunologic effects of *Thymus broussonetii* Boiss extract. The author showed that the extracts of this endemic species are of interest for two reasons: stimulation of the immunizing system and protection against the stress by a neurotropic activity. The immune boosting effect of thyme extracts appeared by the increased in vivo the number of leucocyte categories especially CD4+, CD8+ and NK cells. These results could be of practical importance in the field of phytotherapy in the treatment of some cases of human immunodeficiency such as cancer, leukaemia and AIDS. Different doses (40g and 20g crude drug/kg/d) from *Thymus quinquecostatus* C. extract and volatile oil were given to mice bearing Sarcoma 180 tumor caused significant tumor inhibition rates. The coefficient of spleen in group with extract was close to normal value, and its coefficient of thymus gland was near to the negative control group. The anti-tumor activity of the alcohol extracts was significantly higher than that of the control group and the tumor inhibition rate was depending on drug concentration. Depending on index of immunity, the extracts from *T. quinquecostatus* C. may have some influences on immunity as reported by Sun et al. [7].

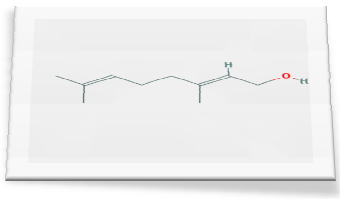
Carvacrol an antioxidant compound present in thyme

extracts exhibited potent antioxidant activity comparable to the known antioxidants, butylated hydroxytoluene (BHT) and α-tocopherol [8]. Furthermore, ingestion of these aromatic compounds may help to prevent oxidative damage such as lipid peroxidation which is associated with cancer, premature aging, atherosclerosis and diabetes [9]. Dietary sources of luteolin from thyme showed a variety of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial and anticancer activities. The ability of luteolin to inhibit angiogenesis, induce apoptosis and prevent carcinogenesis in animal models [10]. Ursolic acid extracted by dichloromethane and ethanol extracts of *Thymus mastichina* L. showed cytotoxicity with GI50 value of 6.8 microg/mL against the HCT colon cancer cell line. A fraction composed of equal mixture of oleanolic acid and ursolic acid displayed improved cytotoxicity with a GI50 of 2.8 microg/mL, suggesting a synergistic behavior. GI50 value is the concentration for 50% of maximal inhibition of cell proliferation [11]. Mouse lymphoma L5178Y cells were exposed to different concentrations of thyme extracts for 3h with and without metabolic activation (S9-mix). In the absence of S9, thyme extract was found to induce significant DNA damage without affecting the cell viability. The addition of S9 did not affect the DNA damaging effect of thyme [12]. Again Zu et al. [13] delineated that thyme essential oil exhibited the strongest cytotoxicity towards human prostate carcinoma cell (PC-3), human lung carcinoma (A549) and human breast cancer (MCF-7) tumor cell lines.

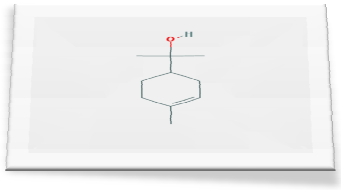
The present review focused on the mechanism of action by which thyme extract and its active compounds could induce apoptosis and control cancer invading.

2. Chemical Composition of *Thymus*

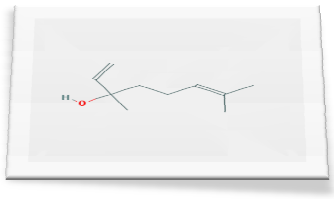
Thymus sp. show polymorphic variation in monoterpene production, the presence of intraspecific chemotype variation being common in the genus *Thymus*. Six chemotypes such as geraniol, α-terpineol, thuyanol-4, linalool, carvacrol, and thymol, which named after its dominant monoterpene have been extracted from thyme [14]. While, Shabnum & Wagay [15] identified many compounds from the *Thymus vulgaris* L. which are thymol, γ-terpinene, ρ-cymene, linalool, myrcene, α-pinene, eugenol, carvacrol, α-thujene and terpinene -4-ol. Gordo et al. [11] identified nine compounds from dichloromethane and ethanol extracts of *Thymus mastichina* L., which are sakuranetin, sterubin, oleanolic acid, ursolic acid, lutein, β-sitosterol, rosmarinic acid, 6-hydroxyluteolin-7-O-β-glucopyranoside, and 6-hydroxyapigenin-7-O-beta-glucopyranoside. The chemical formula of the different components from *Thymus* sp. revealed in the following figure.



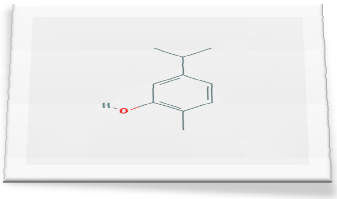
Geraniol, CID 637566



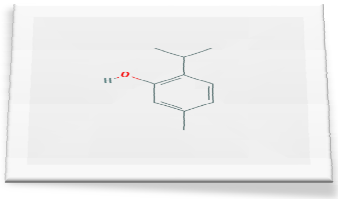
α-terpineol, CID 17100



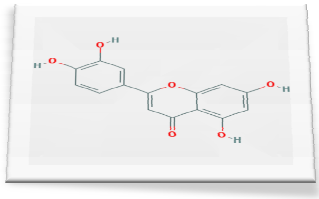
Linalool, CID 6549



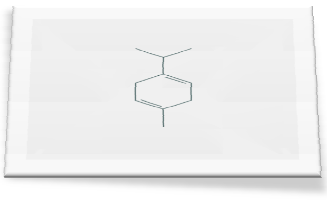
Carvacrol, CID 10364



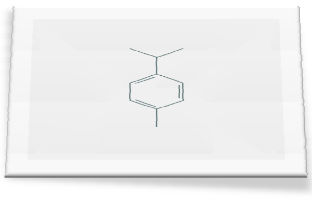
Thymol, CID 6989



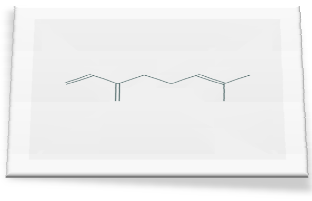
Luteolin, CID 5280445



γ-terpinene, CID 7461



P-cymene, CID 7463



Myrcene, CID 31253

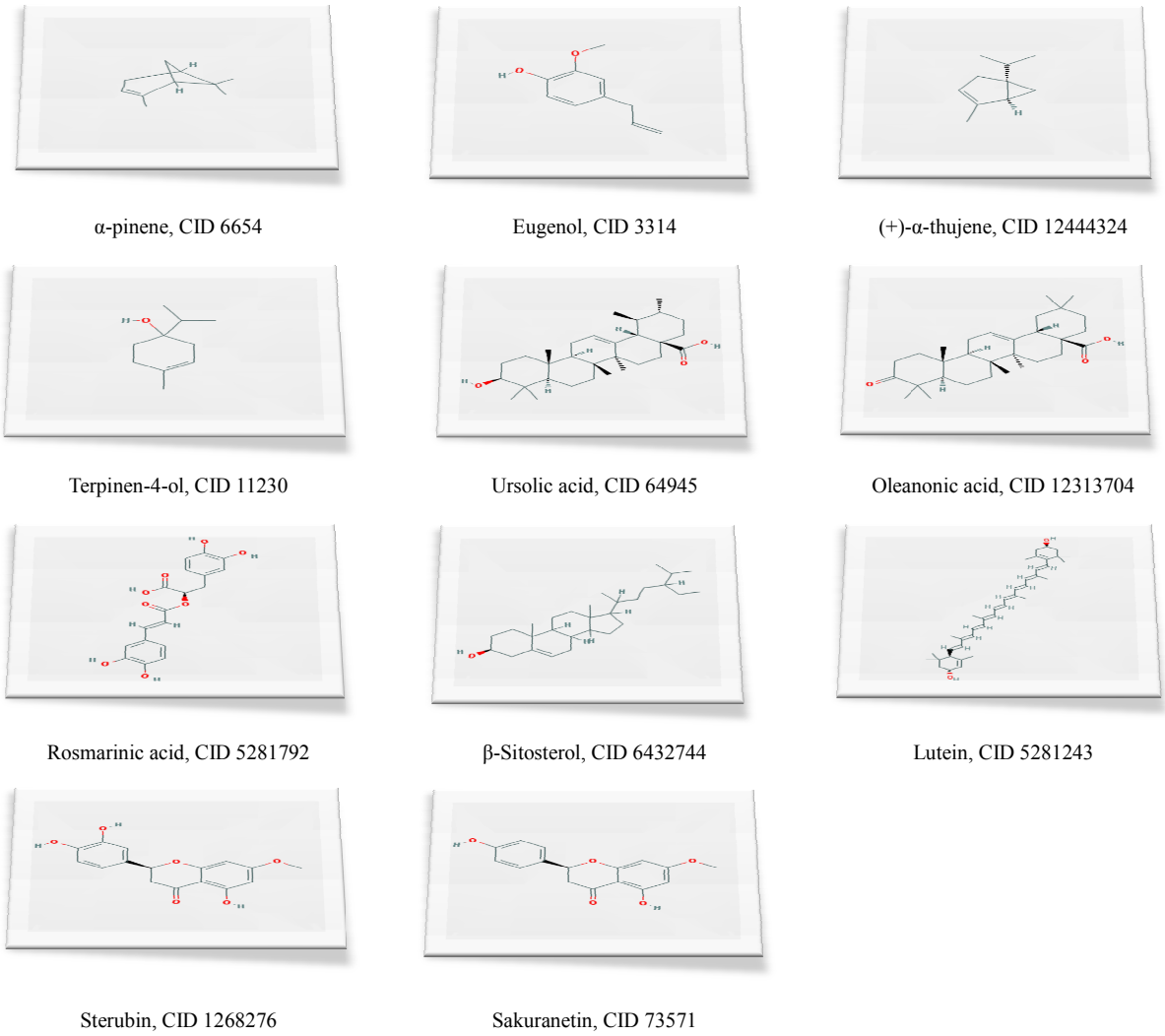


Fig 1. *Chemical structure of some component from Thymus sp. Chemical formula from Pubchem*

3. Apoptosis and Cancer Control Mechanism of Action

The molecular mechanisms responsible for the curative power of *Thymus vulgaris* against hydrogen peroxide- and 2,3-dimethoxy-1,4-naphthoquinone-induced DNA damage, appered by the elevation levels of enzymatic and non-enzymatic antioxidants superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) in human HepG2 cells and hepatocellular carcinoma (HCC) in rats [16]. These enzymes constitute the enzymatic antioxidant defense system in the cells as a result of *T. vulgaris* treatment [17]. The effects of thyme (*Thymus vulgaris* L.) leaves and its phenolic compounds, thymol and carvacrol, on the activities of 7-ethoxycoumarin O-deethylase (ECOD) and glutathione S-transferase (GST) and quinone reductase (QR), were investigated. Dietary administration thyme caused slightly but significantly higher ECOD, GST, and QR activities by 1.1-1.4-fold. Thymol (200 mg/kg) treatment resulted in significantly higher ECOD, GST, and QR activities by 1.3-1.9-fold, and carvacrol (200 mg/kg) treatment caused significantly higher ECOD, GST, and QR activities by 1.3-1.7-fold. Thymol-treated animals had significantly higher protein levels of GST alpha and GST micro subclasses, and carvacrol-treated animals had significantly higher levels of GST micro. These results imply that thyme contains bifunctional inducers (i.e., substances capable of inducing both phase I enzymes such as ECOD and phase II enzymes such as GST and QR, and that thymol and carvacrol may account for the effects of thyme [18].

Bozkurt et al. [19] evaluated the effects of *Thymus serpyllum* on apoptosis and epigenetic events in breast cancer cells. Breast cancer is one of the most common malignancies and increasing evidence supports that it is not only a genetic but also an epigenetic disease. Epigenetics investigates changes in gene expression caused by mechanisms that do not involve alterations in DNA sequence. DNA methylation and histone acetylation are the most widely studied epigenetic changes in cancer cells. *T. serpyllum* extract induced significant cytotoxicity in breast cancer cells (MCF-

7 and MDA-MB-231) but not in normal cells. It also induced apoptosis and inhibited the DNA methyl transferase (DNMT) and histone deacetylase (HDAC) activities in MDA-MB-231 cells. The data suggested that *T. serpyllum* may be a promising candidate in the development of novel therapeutic drugs for breast cancer treatment [19].

Cytotoxicity of *T. vulgaris* L. essential oil (EOT) was investigated on the head and neck squamous cell carcinoma (HNSCC) cell line, UMSCC1. The IC_{50} of ETO recorded 369 µg/ml [20].The aromatic compounds and oily components of thyme have shown cytotoxic activity against breast cancer [21] and ovarian adenocarcinoma IGR-OV1 cells and their counterparts resistant to chemotherapy [22]. EOT cytotoxicity might be due to its lipophilic compounds that accumulate in cancer cell membranes and increase their permeability, resulting in leakage of enzymes and metabolites [23]. Aside from its anticancer property, thyme extract has also shown immune-stimulatory effects involving leukocyto- and thrombocytopoesis [24]. This pharmacological property of thyme might be clinically beneficial as an adjuvant therapy during chemotherapy in cancer or immunocompromised patients to overcome leukopenia. The anti-tumor effect of the Moroccan endemic thyme (*Thymus broussonettii*) essential oil (EOT) was investigated in vitro using the human ovarian adenocarcinoma IGR-OV1 parental cell line OV1/P and its chemoresistant counterparts OV1/adriamycin (OV1/ADR), OV1/vincristine (OV1/VCR), and OV1/cisplatin (OV1/CDDP). All of these cell lines elicited various degrees of sensitivity to the cytotoxic effect of EOT. The IC50 values were 0.40, 0.39, 0.94, and 0.65% for OV1/P, OV1/ADR, OV1/VCR, and OV1/CDDP, respectively. Intra-tumoral injection of EOT significantly reduced solid tumor development in DBA-2/P815 (H2d) mouse model. By the 30th day of continuous EOT treatment, the tumor volumes of the animals were 2.0, 1.3 and 0.85 cm(3) after injection with 10, 30, or 50 microL per 72 h (six times), respectively, as compared to 3.88 cm(3) for the control animals. This effect associated with a marked decrease of mouse mortality. The author revealed these results as a result of carvacrol component in EOT [22].

The genes associated with interferon signaling, *N*-glycan biosynthesis or ERK5 signaling differentially regulated upon EOT treatment [20]. Among the down-regulated genes, UBE2C encodes a member of the E2 ubiquitin-conjugating enzyme family, which is required for the destruction of mitotic cyclins and for cell cycle progression [25]. This down-regulation might explain one aspect of the cytotoxic activity of EOT. Another down-regulated gene was CDC20, which acts as a regulatory protein in the cell cycle and is required for nuclear movement prior to anaphase and chromosome separation [25]. Here again, EOT showed influence on cell cycle progression by down-regulating a critical mitosis regulatory protein.

While, OAS2 gene upregulation contributes to this process of cell growth control. It encodes a member of the 2-5A synthetase family, responsible for controlling cell growth, differentiation and apoptosis [25]. Interferons are a family of cytokines with potent antiproliferative, antiviral and immunomodulatory properties [26]. *N*-Glycans (oligosaccharides) play crucial roles in glycoproteins functions, *e.g.* epidermal growth factor (EGF) and transforming growth factor-β (TGF-β) receptors [27,28]. EOT might be able to induce the growth arrest of cancer cells through targeting *N*-glycan biosynthesis. ERK5, belonging to the mitogen-activated protein kinase (MAPK) family, is expressed in a variety of tissues and is activated by a range of growth factors, cytokines and cellular stresses. ERK5 signaling is important in endothelial cells for preventing apoptosis, regulating tumor angiogenesis and cell migration [29]. The influence of *T. vulgaris* in ERK5 signaling provides a novel target for anticancer therapy as an anti-angiogenic agent [30].

Another pathway in the mechanism of action of thyme to fight cancer is the reduction of NF-kβ and activating protein-1 (AP-1) activity [10,31]. Thyme infusion exhibited the potential to inhibit cell growth and to reduce IL-8 levels in HT29 colon and PC3 prostate cancer cells. The regulation reported in PC3 treated with thyme might point to the NF-κβ as the molecular target underlying the effect of this infusion [32] which related to its phenolic content.

Many studies have identified NF-κβ as a direct link between inflammation and cancer [10, 33] and thus render this family of transcription factors as a key molecular target for both prevention and treatments of cancers. The NF-κβ family of transcription factors is crucial mediators of cellular stress, immune, and inflammatory responses [34]. Although NF-κβ is essential in normal physiology, several human disorders involve inappropriate regulation of NF-κβ. Several chronic degenerative diseases [35] and various human cancers [36] have been associated with an aberrant upregulation of NF-κβ activity [31]. Both the NF-κβ activation and cytokine profile of tumor-associated macrophages are closely linked to tumor growth [37]. In vertebrates, the NF-κβ family is comprised of five structurally related proteins [p65 (REL A), p50, p52, c-REL, REL B] that form heterodimers and homodimers. Normally, the NF-κβ dimers are sequestered in the cytoplasm by binding to inhibitory factors. Two pathways lead to the delocalization of NF-κ dimers to the nucleus and consequent transcriptional regulation of target genes [38]. The classic NF-κβ signaling pathway is activated by pro-inflammatory stimuli, bacterial or viral infection, and various forms of stress and environmental toxins [39] and is essential for the innate immune system and anti-apoptotic signaling. The alternative NF-κβ pathway plays a critical role for both the development and maintenance of the adaptive immune system and is activated by members of the tumor necrosis factor (TNF) family other than TNF-α [38]. NF-κβ target genes code for proteins that are central players in inflammation, activation of the immune system, and anti-apoptotic signaling, which can be involved in both the promotion and progression of cancers [40]. With the central role of NF-κβ activation in inflammation, cancer, and anti-apoptosis, substances with the ability to inhibit NF-κβ

activation may be valuable in a generally healthy diet, in chemoprevention [41], and in combination with chemotherapy [42].

A combined extract of clove, oregano, thyme, walnuts, and coffee synergistically inhibited lipopolysaccaride (LPS)-induced NF-kβ activation in a monocytic cell line, compared with the sum of effects from the single extracts. Transgenic NF-kβ luciferase reporter mice were given a single dose of the combined extract and subsequently challenged with LPS. NF-kβ activation was monitored by in vivo imaging for 6 hours and NF-kβ activity in organs and the expression of immune-related genes in many organs were increased. The extract decreased whole body LPS-induced NF-kβ activity the first 6 hours by 35% compared with control mice. Organ-specific NF-kβ activation was inhibited in intestine, liver, testis, and epididymis of the mice receiving the combination extract. In addition, dietary plants reduced the expression of genes related to inflammation, cell migration, and proliferation in liver. Therefore the authors showed that dietary plants may be potent modulators of NF-kβ signaling both in vitro and in vivo [31].

Thyme and oregano essential oils in combination decreased the levels of IL-1β and IL-6, as well as inflammation-related tissue damage in a model of colitis [43], both of which may be a result of NF-κβ inhibition in the colon. In addition, thyme may induce the level of endogenous cytoprotective proteins in the liver [18]. The reproduction of alpha-fetoprotein (AFP) in adults is associated with hepatocellular carcinoma. The possible explanation for the re-initiation of AFP synthesis by neoplastic hepatocytes includes either increased transcription of AFP gene or post-translational modification affecting AFP production [44].

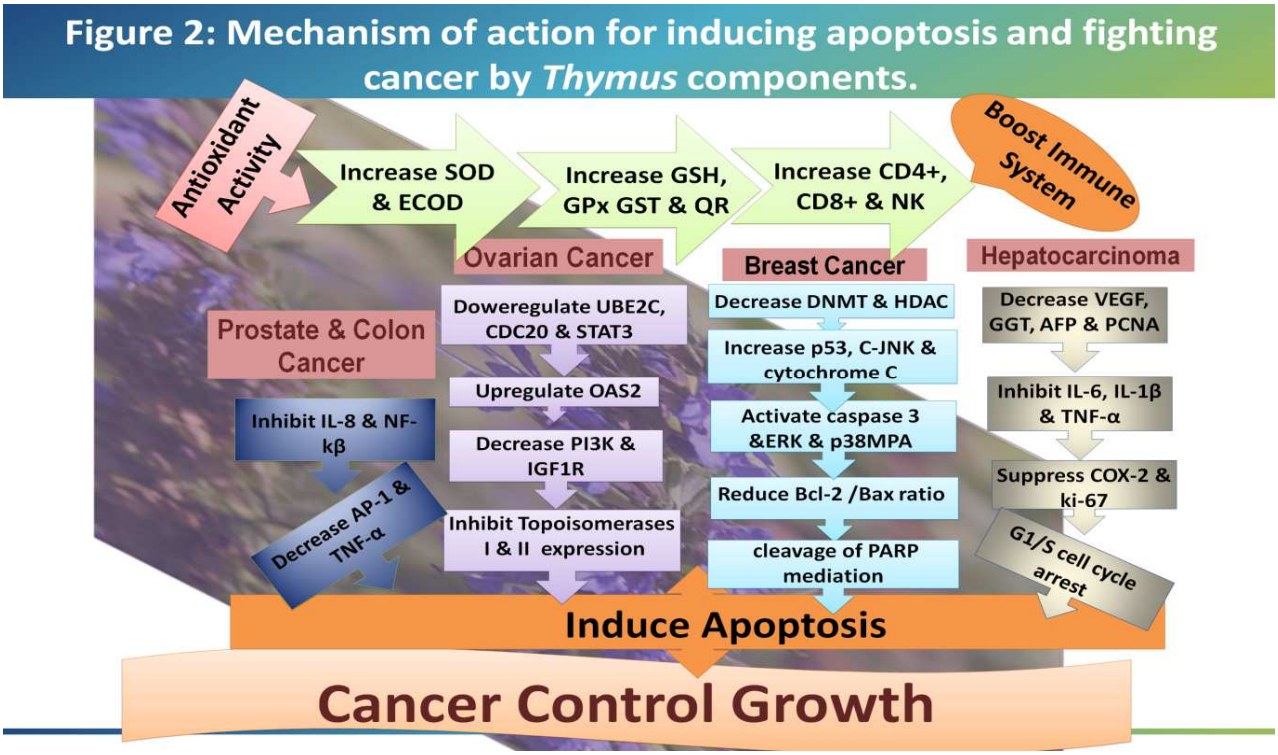


Fig 2. *Summarized the pathways by which thyme fight different types of cancer*

Hamzawy et al. [45] and Ahmed et al. [46] indicated that treatment of hepatocellular carcinoma (HCC) with carvacrol showed significant decrease in AFP serum level in rats. The possible explanation for this improvement could be attributed to the anti-inflammatory effect exhibited by carvacrol which is responsible for decreasing COX-2 overexpression that regulates AFP production [47]. Another possible explanation for such decrement could be due to the antioxidant properties of carvacrol that attenuates the oxidative stress [48,49]. It has been reported that carvacrol helps parenchymal cell regeneration in liver, thus protecting membrane integrity and thereby minimizing enzyme leakage [50]. Carvacrol treatment in HCC group significantly decreases serum level of VEGF [51,52] and markedly decreases the expression of gamma glutammyl transferase (GGT) gene [48]. The maintenance of GSH by carvacrol was mainly because of inactivation of ROS *via* its radical scavenging effects and retention of enzymic antioxidants [49]. Such modulation of GSH activity leads to reduction in the GGT activity. Thus, carvacrol could prevent the oxidative damage of liver tissue and hence decrease the inflammation and necrosis that leads to oval cell proliferation which is the main cause for GGT gene expression [48].

The expression of Ki-67 protein is well known in neoplastic human tissues [53]. This increase may be caused by the fact that post transcriptional regulatory mechanisms suppress protein synthesis until cells are at the G1/S boundary. Thus, the large increase in the proportion of

hepatocytes expressing the Ki-67 protein at this time suggests that this cell-cycle associated protein may play a crucial role near the G1/S border and during S phase in cells traversing the cell cycle for the first time since leaving G0 [54]. On the other hand, Cui et al. [55] found that cyclooxygenase-2 deletion (C2D) significantly inhibits Ki-67 expression in HCC xenografts, indicating that cyclooxygenase-2 (COX-2) overexpression promotes G1/S transition of the cell cycle in HCC xenografts *via* a series of complicated signaling, and this means that COX-2 gene enhance the expression of Ki-67. Another clarification reported by Tuncer et al. [56] who delineated that the decrease in Ki-67 expression could be possibly due to the inhibitory effect of carvacrol on the proinflammatory cytokine TNF-α, IL-6 and IL-1β which in turn decreases COX-2 expression [17, 45].

Carvacrol treatment revealed formation of DNA fragments which provides substantial evidence of induction of cell death by apoptosis [57], the increase in the number of apoptotic cells after carvacrol treatment indicated by cell shrinkage, chromatin condensation, and nuclear fragmentation. Again a decrease in the number of positive cells for proliferating cell nuclear antigen (PCNA) expression in liver tissue due to carvacrol administration were reported [46]. Yin et al. [57] revealed that the antiproliferative effect of carvacrol in HepG2 cells could be attributed to the inhibition of extracellular signal-regulated kinase (ERK) phosphorylation which is known to enhance the cell proliferation.

Latest research showed that the antiproliferative effect of carvacrol in metastatic breast cancer cells (MDA-MB231) is based on the activation of the classical apoptosis responses, including a decrease in mitochondrial membrane potential, an increase in cytochrome C release from mitochondria, a decrease in Bcl-2/Bax ratio, an increase in caspase 3 activity, cleavage of poly-(AD-Pribose)-polymerase (PARP), increase in p53 and fragmentation of DNA, which belong to the mitochondrial pathway of apoptosis [17,58]. Moreover, Yin et al. [57] suggested that mitogen-activated protein kinase (MAPK) pathway is the other possible mechanism for apoptosis induction by carvacrol as it could activate phosphorylation of p38 MAPK. The MAPK pathway, especially c-jun N-terminal kinase (JNK), extracellular-signal-regulated kinases (ERK), and p38, has various functions in the apoptosis of various cancer cells [59]. The activation of JNK and/or p38 MAPK induces cell death and apoptosis [60].

Treatment with luteolin induce apoptosis in Chinese hamster ovary AA8 cells and prevent tumor growth by inhibiting of topoisomerases I and II which are the targets of anti-cancer drugs that act to inhibit these enzymes by blocking the reaction that reseals the breaks in the DNA, stabilization of p53, and inhibition of Phosphatidylinositol 3-kinase; PI3K (cell proliferation stimulant), signal transducer and activator of transcription; STAT3 (cell proliferation stimulant), insulin-like growth factor; IGF1R (strong mitogens, cancerous cell chemoresistant) and human epidermal growth factor receptor 2; HER2 (oncogene) are possible mechanisms involved in the biological activities of luteolin [10,61].

References

[1] Grigore, A., Paraschiv, I., Colceru-Mihul, S., Bubueanu, C., Draghici, E., and Ichim, M. 2010. Chemical composition and antioxidant activity of Thymus vulgaris L. volatile oil obtained by two different methods. Romanian Biotechno Lett 15(4): 5436-5443.

[2] Broucke, C.V. 1983. The therapeutic of Thymus species. Fitoterapia 4:171-174.

[3] Blumenthal, M., Busse, W.R., and Goldberg, A. 1998. Therapeutic Guide to Herbal Medicines. In: The Complete German Commission E Monographs: Austin, Tex, American Botanical Council, pp. 219-220, 1998.

[4] Clavero, J., Baca, P., Gonzalez, P.M, and Valderrama, M.J. 2006. Efficacy of chlorhexidine-thymol varnish (Cervitec) against plaque accumulation and gingival inflammation in a geriatric population. Gerodonto 23: 43-47.

[5] Szentandrassy, N., Szentesl, P., Nanasi, P.P., and Cszernoch, L. 2003. Effect of thymol on kinetic properties of Ca and K currents in rat skeletal muscle. BMC pharmacol 3: 9-17

[6] Elhabazi, K., Dicko, A., Desor, F., Dalal, A., Younos, C., and Soulimani, R. 2006. Preliminary study on immunological and behavioural effects of Thymusbroussonetii Boiss., an endemic species in Morocco. J Ethnopharmacol 103(3):413-419.

[7] Sun ,Z.X., Sun, J.H., Cheng, S., Ma, Q.W., Guo, S.L., and Zhang, J.B. 2003. Original studies on anti-tumor and immunological effect of extracts from Thymus quinquecostatus Celak in mice. Zhong Xi Yi Jie He Xue Bao 1(3):209-210.

[8] Lee, K.G., and Shibamoto, T. 2002. Determination of antioxidant potential of volatile extracts isolated from various herbs spices. J Agric Food Chem 50:4947–4952

[9] Verma, R.J. and Nair, A. 2001. Ameliorative effect of vitamin E on aflatoxin-induced lipid peroxidation in the testis of mice. Asian J Androl 3:217–221

[10] López-Lázaro, M. 2009. Distribution and biological activities of the flavonoid luteolin. Mini Rev Med Chem 9(1):31-59

[11] Gordo, J., Máximo, P., Cabrita, E., Lourenço, A., Oliva, A., Almeida, J., Filipe, M., Cruz, P., Barcia, R., Santos, M., and Cruz, H. 2012. Thymus mastichina: chemical constituents and their anti-cancer activity. Nat Prod Commun 7(11):1491-1494.

[12] Demma, J., Engidawork, E., and Hellman, B. 2009. Potential genotoxicity of plant extracts used in Ethiopian traditional medicine. J Ethnopharmacol 122(1):136-142.

[13] Zu, Y., Yu, H., Liang, L., Fu, Y., Efferth, T., Liu, X., and Wu, N. 2010. Activities of ten essential oils towards Propionibacterium acnes and PC-3, A-549 and MCF-7 cancer cells. Molecules 15(5):3200-3210.

[14] Thompson, J., Chalcha, J., Michet, A., Linhart, Y., and Ehlers, B. 2003. Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of Thymus vulgaris chemotypes. J Chem Ecol 29(4):859-880

[15] Shabnum, S. and Wagay, M.G. 2011. Essential Oil Composition of Thymus Vulgaris L. and their Uses. J Res

Develep 11: 83 -94.

[16] Kozics, K., Klusová, V., Srančíková, A., Mučaji, P., Slameňová, D., Hunáková, L., Kusznierewicz, B., and Horváthová, E. 2013. Effects of Salvia officinalis and Thymus vulgaris on oxidant-induced DNA damage and antioxidant status in HepG2 cells. Food Chem 141(3):2198-206.

[17] Abdel-Aziem, S.H., Hassan, A.M., El-Denshary, E.S., Hamzawy, M.A., Mannaa F.A., and Abdel-Wahhab, M.A. 2013. Ameliorative effects of thyme and calendula extracts alone or in combination against aflatoxins-induced oxidative stress and genotoxicity in rat liver. Cytotech 65(4):1-14

[18] Sasaki, K., Wada, K., Tanaka, Y., Yoshimura, T., Matuoka, K., and Anno, T. 2005. Thyme (*Thymus vulgaris* L.) leaves and its constituents increase the activities of xenobiotic-metabolizing enzymes in mouse liver. J Med Food 8:184–189.

[19] Bozkurt, E., Atmaca, H., Kisim, A., Uzunoglu, S., Uslu, R., and Karaca, B. 2012. Effects of Thymus serpyllum extract on cell proliferation, apoptosis and epigenetic events in human breast cancer cells. Nutr Cancer 64(8):1245-50.

[20] Sertel, S., Eichhorn, T., Plinkert, P.K., and Efferth, T. 2011. Cytotoxicity of Thymus vulgaris essential oil towards human oral cavity squamous cell carcinoma. Anticancer Res 31(1):81-87.

[21] Zava, D.T., Dollbaum, C.M., and Blen, M. 1998. Estrogen and progestin bioactivity of foods, herbs, and spices. Proc Soc Exp Biol Med 217: 369-378.

[22] Ait M'barek, L., Ait Mouse, H., Jaâfari, A., Aboufatima, R., Benharref, A., Kamal, M., Bénard, J., El Abbadi, N., Bensalah, M., Gamouh, A., Chait, A., Dalal, A., and Zyad, A. 2007. Cytotoxic effect of essential oil thyme (Thymus broussonettii) on the IGR-OV1 tumor cells resistant to chemotherapy. Braz J Med Biol Res 40(11):1537-44.

[23] Sikkema, J., de Bont, J.A., and Poolman, B. 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiol Rev 59: 201-222.

[24] von Ardenne, M. and Reitnauer, P.G. 1981. The elevation of the leucocyte and thrombocyte counts produced by a thyme extract in the peripheral blood as compared to that caused by 2-cyanoethylurea (author's transl). Pharmazie 36: 703-705.

[25] Genecards.org (2010): http://www.genecards.org/index.shtml: Accessed: 01.01.2010.

[26] Katsoulidis, E., Kaur, S., and Platanias, L.C. 2010. Deregulation of interferon signaling in malignant cells. Pharmaceuticals 3: 406-418.

[27] Takahashi, M., Tsuda, T., Ikeda, Y., Honke, K., and Taniguchi, N. 2004. Role of N-glycans in growth factor signaling. Glycoconj J 20: 207-212.

[28] Lau, K.S. and Dennis, J.W. 2008. N-Glycans in cancer progression. Glycobiol 18:750-760.

[29] Roberts O.L., Holmes, K., Muller, J., Cross, D.A., and Cross, M.J. 2009. ERK5 and the regulation of endothelial cell function. Biochem Soc Trans 37: 1254-1259.

[30] Krill, D., Madden, J., Huncik, K., and Moeller, P.D. 2010. Induced thyme product prevents VEGF-induced migration in human umbilical vein endothelial cells. Biochem Biophys Res Commun 403(3-4):275-281.

[31] Paur, I., Balstad, T.R., Kolberg, M., Pedersen, M.K., Austenaa, L.M., Jacobs, D.R. Jr., and Blomhoff, R. 2010. Extract of oregano, coffee, thyme, clove, and walnuts inhibits NF-kappaB in monocytes and in transgenic reporter mice. Cancer Prev Res (Phila). 3(5):653-663.

[32] Kaliora, A.C., Kogiannou, D.A., Kefalas, P., Papassideri, I.S., and Kalogeropoulos, N. 2014. Phenolic profiles and antioxidant and anticarcinogenic activities of Greek herbal infusions; balancing delight and chemoprevention? Food Chem 142:233-241.

[33] Pikarsky, E., Porat, R.M., Stein, I., Abramovitch, R., Amit, S., Kasem, S., Gutkovich-Pyest, E., Urieli-Shoval, S., Galun, E., and Ben-Neriah, Y. 2004. NF-κB functions as a tumour promoter in inflammation-associated cancer. Nature 431:461–466.

[34] Karin, M., Yamamoto, Y., and Wang, Q.M. 2004. The IKK NF-κB system: a treasure trove for drug development. Nat Rev Drug Discov 3:17–26.

[35] Mattson, M.P. and Camandola, S. 2001. NF-κB in neuronal plasticity and neurodegenerative disorders. J Clin Invest 107:247–254.

[36] Karin, M., Cao, Y., Greten, F.R., and Li, Z.W. 2002. NF-κB in cancer: from innocent bystander to major culprit. Nat Rev Cancer 2:301–310.

[37] Maeda, S., Kamata, H., Luo, J.L., Leffert, H., and Karin, M. 2005. IKKβ couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell 121:977–990.

[38] Bonizzi, G., and Karin, M. 2004. The two NF-κB activation pathways and their role in innate and adaptive immunity. Trends Immunol 25:280–288.

[39] Pahl, H.L. 1999. Activators and target genes of Rel/NF-κB transcription factors. Oncogene 18:6853–6866.

[40] Karin, M. and Greten, F.R. 2005. NF-κB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol 5:749–759.

[41] Surh, Y.J. 2003. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 3:768–780.

[42] Nakanishi, C. and Toi, M. 2005. Nuclear factor-κB inhibitors as sensitizers to anticancer drugs. Nat Rev Cancer 5:297–309.

[43] Bukovska, A., Cikos, S., Juhas, S., Il'kova, G., Rehak, P., and Koppel, J. 2007. Effects of a combination of thyme and oregano essential oils on TNBSinduced colitis in mice. Mediators Inflamm 2007:23296.

[44] Motalleb, G., Hanachi, P., Fauziah, O., and Asmah, R. 2008. Effect of Berberis vulgaris fruit extract on alpha-fetoprote gene expression and chemical carcinogen metabolizing enzymes activities in hepatocarcinogenesis rats. Iran J Cancer Prev 1:33-44.

[45] Hamzawy, M.A., El-Denshary, E.S., Hassan, N.S., Manaa, F., and Abdel-Wahhab, M.A. 2012. Antioxidant and hepatorenoprotective effects of Thyme vulgaris extract in rats during aflatoxicosis. Global J Pharmacol 6:106-117.

[46] Ahmed, H.A., Shousha, W.Gh., El-Mezayen, H.A., Ismaiel, N.N., and Mahmoud, N.S. 2013. In vitro antitumor potential of carvacrol against hepatocellular carcinoma in rat model. World J Pharm Pharmaceut Sci 2(5): 2367-2396.

[47] Hotta, M., Nakata, R., Katukawa, M., Hori, K., Takahashi, S., and Inoue, H. 2010. Carvacrol,a component of thyme oil, activates PPAR alpha and gamma, and suppresses COX-2 expression. J Lipid Res 51:132–139.

[48] Jayakumar, S., Madankumar, A., Asokkumar, S., Raghunandhakumar, S., Gokula-dhas, K., Kamaraj, S., Divya, M.G., and Devaki, T. 2012. Potential preventive effect of carvacrol against diethylnitrosamine-induced hepatocellular carcinoma in rats. Mol Cell Biochem 360:51–60.

[49] Aeschbach, R., Loliger, J., Scott, B.C., Murcia, A., Butler, J., Halliwell, B., and Aruoma, O.I. 1994. Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxyltyrosol. Food Chem Toxicol 32: 31–36.

[50] Canbek, M. 2008. Effects of carvacrol on defects of ischemia-reperfusion in the rat liver. Phytomed 15:447–452.

[51] Łoboda, A., Cisowski, J., Zarêbski, A., Wa, J.A., Nunez, D.R., Kypriotakis, Z., Heinrich, M., and Dulak, J. 2005. Effect of plant extracts on angiogenic activities of endothelial cells and keratinocytes. J Physiol Pharmacol 56: 125-137.

[52] Nangia-Makker, P., Tait, L., Hogan, V., Miller, F., and Raz, A. 2006. Inhibition of breast cancer progression by a medicinal herb Ocimum sanctum, P Am Assoc Canc Res 2170: 47.

[53] Sawhney, N. and Hall, P.A. 1992. Ki-67 structure, function and new antibodies. J Pathol 168:161-162.

[54] Gerlach, C., Sakkab, D.Y., Scholzen, T., Daßler, R., Alison, M.R., and Gerdes, J. 1997. Ki-67 Expression During Rat Liver Regeneration After Partial Hepatectomy. Hepatology 26:573-578.

[55] Cui, W., Hu, S.X., Tang, Z., and Hu, K. 2007. In Vivo Effects of Cyclooxygenase-2 Deletion on Cellular Signaling in Hepatocellular Carcinoma Xenografts in Nude Mice. J Cancer Mol 3: 49-54.

[56] Tuncer and, E., Unver-Saraydin, S., Tepe, B., Karadayi, S., Ozer, H., Sen, M., Karadayi, K., Inan, D., Elagoz, S., Polat, Z., Duman, M., Turan, M. 2013. Antitumor effects of Origanum acutidens extracts on human breast cancer. J Buon 18(1): 77-85.

[57] Yin, Q.,Yan, F., Zu, X.,Wu, Y., Wu, X., Liao, M., Deng, S., Yin, L., and Zhuang, Y. 2012. Antiproliferative and pro-apoptotic effect of carvacrol on human hepatocellular carcinoma cell line HepG-2. Cytotech 64(1):43-51.

[58] Arunasree, K. 2010. Antiproliferative effects of carvacrol on a human metastatic breast cancer cell line, MDA-MB231.Phytomed 17:581–588.

[59] Yoon, S. and Seger, R. 2006. The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. Growth Factors 24:21–44.

[60] Chang, H.L., Wu, Y.C., Su, J.H., Yeh, Y.T., and Yuan, S.S. 2008. Protoapigenone, a novel flavonoid, induces apoptosis in human prostate cancer cellsthroughactivationofp38mitogen-activated protein kinase and c-Jun NH2-terminal kinase 1/2. J Pharmacol Exp Ther 325:841–849.

[61] Cantero, G., Campanella, C., Mateos, S., and Cortés, F. 2006. Topoisomerase II inhibition and high yield of endoreduplication induced by the flavonoids luteolin and quercetin. Mutagenesis 21(5):321-325.