In vitro STUDIES ON PALM PHYTONUTRIENTS: A NUTRIGENOMIC REVIEW

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ABSTRACT
This review discussed the nutrigenomics aspects of palm phytonutrients. Nutrigenomics is the study of the effect of nutrient intake in a specific diet on the regulation of gene expression. Nutrigenomics studies are done to develop personalised dietary, supplements and medicinal products. This can be achieved by either experimenting via in vitro, in vivo or clinical trials. However, researchers prefer to conduct in vitro studies as the data can be obtained faster than other modes of studies, primarily to assess dietary supplement safety. In vitro studies are performed outside of a living organism but in a controlled environment. Phytonutrients are natural chemicals that can be found in plant foods. Palm oil is one of the richest sources of phytonutrients. Palm oil phytonutrients include palm phenolic-enriched fraction (PEF), oil palm phenolics (OPP), and tocoferiol-rich fraction (TRF). These phytonutrients are used as health supplements and were proven to have nutritional benefits for consumers. In this review, we will discuss the nutrigenomics aspects of palm phytonutrients effect on cell proliferation, apoptosis and insulin resistance pathway, based on in vitro data published from 2010.

Keywords: apoptosis, cell lines, gene regulation, insulin resistance, tocotrienol.

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INTRODUCTION
Phytonutrients are bioactive food components that can act as supplements to prevent or/and treat diseases and other physiological disorders. Palm oil is one of the richest sources of phytonutrients, and this oil consists of 1% of phytonutrients, such as carotenes, phytoesters, squalene, ubiquinone, polyphenols, and phospholipids (Loganathan et al., 2010). All of these phytonutrients were reported by many researchers to give nutritional benefits to consumers, both healthy individuals and patients with certain diseases (Loganathan et al., 2010). Palm oil is also a source of vitamin E (Vit E), with its major constituents of 70% tocotrienols (T3) and 30% tocopherols (TCP), and contains 44% palmitic acid (C16:0) and 40% oleic acid (C18:1) (Montoya et al., 2014).

Nutrigenomics is the study of the impact of nutrition intake on genome expression (Elsamanoudy et al., 2016). This genome expression covers transcriptome, proteome and metabolome. According to Munshi and Duvvuri (2008), the research on nutritional genomics will explain the molecular basis on which specific genotypes react to dietary components, thus providing targeted treatment for disease prevention and cure. Nutrients and bioactive food compounds were proven tremendously in publications (Farhud et al., 2010; Fenech et al., 2011; Neeha and Kinth, 2013; Sales et al., 2014; Walker and Blackburn, 2004) that these two components can modify cellular pathways through gene expression. The identification of these gene-nutrient interactions will facilitate the recommendation of genotype-specific diets (Sales et al., 2014). For example, those people who are lactose intolerant could not break down the main milk sugar, known as lactose, effectively in dairy products. This group of people are advised to limit foods containing lactose or to consume lactase supplements or lactose-free dairy products (Swallow, 2003).
Many publications discussed the nutritional effects of consuming palm oil and its phytonutrients. Sundram et al. (2003) discussed several effects of the bioactive compounds in the phytochemistry of palm oil fruit on its nutritional and physiological benefits, such as its antioxidant activities, ability to lower cholesterol, anti-cancer effects and ability to prevent atherosclerosis. Balasundram et al. (2005) also discussed the antioxidant properties of palm fruit extracts. In 2014, May and Nesaretnam discussed the effects of palm oil on blood lipids and lipoproteins. Dong et al. (2017) published an article on the effect of red palm oil on vitamin A deficiency. There are also a few publications that focused on the phytonutrients themselves, such as the effects of tocotrienol-rich fraction (TRF) (Ali and Woodman, 2015; Mazlan et al., 2017; Meganathan and Fu, 2016) and also the effects on oil palm phenolics (OPP) consumption (Che Idris et al., 2014; Patten et al., 2015).

Even though there are many publications discussed on the nutritional benefits of consuming palm oil and its phytonutrients, most of these publications only focused either on its nutritional properties or one of its phytonutrients. None of them focused on how the experiments were conducted. Therefore, we believed that there is a need to discuss on how the experiments were conducted, to give an overview and guidance to other researchers for their future studies. There are two types of experiments conducted by researchers; in vitro and in vivo. Both are Latin words, in which in vitro means “within the glass”, and in vivo means “within the living”. In vitro studies are defined as experiments that are carried out in a controlled environment, outside of a living organism. In vivo studies are studies that are done on a living organism, such as plant and animal. In vitro studies are usually conducted using specific human or animal cells, bacteria and viruses. It is cheaper, easier and safer as compared to other studies. Even though the data obtained from in vitro studies give some insights and predictions on certain aspects, not all in vitro data reflects the living organism. Nevertheless, in vitro studies are preferable to assess dietary supplement safety as data could be obtained faster than in vivo studies.

Hence, this review aims to provide an integrated assessment of the available in vitro data on the nutritional genomic aspects of palm phytonutrients effect, based on studies published from 2010 onwards.

**NUTRIGENOMIC EFFECTS ON CELL PROLIFERATION**

A cell cycle is a process where cells replicate and make two new cells. The cell cycle has two stages: the interphase and the mitotic phase (M phase). The interphase consists of the Gap 1 (G1) phase, the synthesis (S) phase and the Gap 2 (G2) phase, while the M phase is where the division of the nucleus and cytoplasm occurs (Alberts et al., 2002) (Figure 1). However, cells may enter a specialised resting state, known as Gap 0 (G0) prior to G1, if the extracellular environments are unfavourable. The cell’s progression is regulated by a family of proteins known as the cyclin by activating the cyclin-dependent kinase (CDK) enzymes (Galderisi et al., 2003). Srivastava and Gupta (2006) demonstrated that chemotherapeutic and chemopreventive drugs can have antiproliferative effects by causing cell division to stop at specific cell cycle checkpoints.

In 2010, Kunnumakkara et al. used four types of pancreatic cell lines: BxPC-3, MIA PaCa-2, Panc-1 and MPanc-96; and discovered that gamma-tocotrienol (γ-T3) inhibited all of these four pancreatic cell lines from proliferating. They found that the inhibition was in a dose- and time-
dependent manner, in which they compared the proliferation rate using two concentrations of γ-T3; 10 mM and 50 mM; for 2, 4 and 6 days. The cell proliferation was almost entirely stopped at the concentration of 50 mM. The inhibition was because of the cell cycle arrest at the G_{1} phase. In addition, they discovered that the inhibition of cell proliferation by γ-T3 might be explained by the downregulation of cell proliferative gene products, such as cyclin D1, c-Myc and COX-2 (Kunnumakkara et al., 2010).

Wu and Ng (2010) treated human cervical carcinoma (HeLa) cells with three types of T3 (α-T3, γ-T3 and δ-T3) and α-TCPs discovered at the concentrations of 1, 2 and 3 mM, both α-T3 and γ-T3 significantly caused cell cycle arrest at G_{1}/S phase. However, they found that the effects of T3 on the distribution of the cell cycles depend on the cell cycle states. This is because α-T3 downregulated the expression of cyclin D3, p16 and CDK6 but does not have any effect on cyclin D1, P15, P21, P27 and CDK6. Contrarily, γ-T3 does not affect all these proteins.

Luna et al. (2011) used human intestinal fibroblast (HIF) isolated from Crohn’s disease (CD) patients in their experiment. They treated the cell with 1 mM of TRF and discovered that this concentration does not have any effects on the HIF proliferation. However, when they increased the concentration of TRF doses between 10 to 1000 mg/mL of a-TCP significantly increased the cell proliferation in the G_{2}/M phase after the treatment of TRF/a-TCP.

Concurrently, the same research group carried out the same treatment using human glioblastoma cells (DBTRG-05MG) to observe the protective effects of TRF and a-TCP against glutamate injury (Rati Selvaraju et al., 2014b). They injured the cells by using 180 mM glutamate. Their data showed that cell numbers increased with treatment of 100, 200 and 300 ng/mL of TRF and a-TCP, in the S phase.

In 2015, Ye et al. demonstrated the anti-cancer effect of δ-T3 on two different types of bladder cancer cell lines, which were the T24 and 5637 cell lines. The cell lines were treated with various concentrations of δ-T3, starting from 0, 50, 100 and 150 mM for 48 hr. Their analysis using flow cytometry revealed that treatment with δ-T3 of 100 mM and more, resulted in G_{1} phase arrest and a decrease in the number of cells in the S phase. They further investigated the amounts of protein expression for the cell cycle and discovered the elevated expression levels of cell cycle inhibitors, p21^{Waf1/Cip1} and p27^{kip1}, and decreased expression levels of Cyclin D1 after 24 hr of treatment with δ-T3 at all dosages in T24 and 5637 cells (Ye et al., 2015).

Ji et al. (2015) revealed that OPP was able to promote cell cycle arrest in the S phase for two types of pancreatic cancer cell lines: PANC-1 and BxPC-3; in a dose-dependent manner. For the PANC-1 cell line, the treatment group with 40 mL/mL of OPP, had roughly 28% of the cells in the S phase compared to 25% in the non-treated cells. The same pattern was shown in the BxPC-3 cell line treated with 50 mL/mL of OPP, where 52% of cells were found in the S phase, compared to the non-treated cells, with 21%.

**NUTRIGENOMIC EFFECTS ON APOPTOSIS**

Apoptosis is programmed cell death. It was first described back in 1972 (Kerr et al., 1972). Apoptosis is a homeostatic process that happens to maintain cell populations in tissues. It also occurs as a defence mechanism in the immune response or when cells are exposed to a disease or dangerous chemical reagents (Norbury and Hickson, 2001). During apoptosis, the cells shrink, with increased cytoplasm density, followed by organelles clumping with each other. These changes in cellular morphology are observable via light microscopy. Apoptosis involves the activation of caspases, a group of cysteine proteases and a cascade of events related to activate stimuli to the final demise of the cell (Elmore, 2007). Caspases’ function is to inactivate proteins that are important to cell survival (Kannan and Jain, 2000). There are two types of caspases activation pathways; extrinsic pathway and intrinsic
The extrinsic pathway is regulated by tumor necrosis factor (TNF) receptor, while the intrinsic pathway occurs in the presence of cellular stresses that involve cell organelles. Figure 2 shows the schematic representation of apoptotic events.

Wu and Ng (2010) showed that α-T3 and γ-T3 greatly induced apoptosis in the HeLa cell line, as the concentration of these two compounds increased. They started with 0 mM of α-T3 and γ-T3, followed by 1 mM, 2 mM and 3 mM. The HeLa cells were stained with annexin V-FITC and they found that the proportion of stained cells increased in both α-T3- and γ-T3-treated cells, through the fluorescence activated cell sorting (FACS) analysis. The presence of phosphatidylserine on the cell surface after 24 hr of treatment, proved that this both compounds triggered cell apoptosis.

Luna et al. (2011) also showed that TRF-induced apoptosis in a caspase-dependent process. They treated two HIF cell lines derived from CD patients and ulcerative colitis (UC) patients with 20 mM TRF. After 24 hr, they discovered an increase in the caspase-8, caspase-9 and caspase-3 activity. In addition, they also found that the pan-caspase inhibitor, zVAD-fmk, completely abolished HIF apoptosis, thus proving that the apoptosis of HIF caused by TRF is a caspase-dependent process.

In another study done by Lim et al. (2014), they treated 10 mM of β-T3 on the A549 cell line and 60 mM of β-T3 on the U87MG cell line to detect caspase-8 initiation. Cells were also preincubated for 30 min with 10 mM and 30 mM of the caspase-8 inhibitor z-IETD-fmk (Z-Ile-Glu-Thr-Asp-fmk) to determine whether caspase-8 was indeed involved. Both A549 and U87MG cell lines were shown to induce caspase-8 activity in response to β-T3. When compared to A549 cells, β-T3 consistently caused more caspase-8 activation in U87MG. An obvious reduction in β-T3 induced caspase-8 activity was also shown in the presence of z-IETD-fmk, which was somewhat linearly proportional to the concentration of z-IETD-fmk used (Lim et al., 2014).

In 2015, researchers discovered that phenolic-enriched fraction (PEF) enhanced cell survival by regulating the genes involved with oxidative stress, which leads to apoptosis (Oskoueian et al., 2015). They used aflatoxin B1 (AFB1)-treated chicken hepatocytes cells. In an untreated cell state, upregulation of pro-inflammatory mediators caused an imbalance between the anti-apoptosis gene, which leads to apoptosis. The genes were bcl2, bax, and caspase-3 effector. When the cells were treated with gradual concentrations of PEF from 0 mg/mL to 5, 10, 20 and 40 mg/mL for 24 hr, the bcl2 gene was increased. However, the expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB genes), the pro-inflammatory mediators; nitric oxide synthase (iNOS), tumour necrosis factor alpha (TNF-α), interleukin-1 beta (IL1β) and interleukin-6 (IL6); and bax gene, were found to be downregulated. This data showed that PEF regulated the imbalance between anti-apoptotic effectors, which results in cell survival enhancement. Figure 3 illustrates the cell survival events due to the PEF treatment.
In vitro STUDIES ON PALM PHYTONUTRIENTS: A NUTRIGENOMIC REVIEW

Phenolic-enriched fraction (PEF)

$iNOS$

$TNF\alpha$

$NF-k\beta$

$Cytochrome C$

$Caspase 9 activation$

$Caspase 3 activation$

$Bax$

$Bcl2$

Gene regulation

Nucleus

CELL SURVIVAL

Figure 3. The cell survival events due to the PEF treatment on the AFB1-treated chicken hepatocytes.

In addition, the PEF treatment was shown to upregulate the nuclear factor (erythroid-derived 2)-like 2 (nrf2) protein, while significantly downregulated the expression of heat shock protein 70 (Hsp70) and caspase-3 proteins. In 2011, nrf2 was reported to activate cellular antioxidant defence mechanisms and enhanced the production of antioxidant enzymes and heat shock proteins (El Golli-Bennour and Bacha, 2011). Hsp70 acts as a sensor of cellular redox exchanges, which act as antioxidant enzymes. Under oxidative stress, this protein can restore and maintain the redox homeostasis of cells.

Contrarily, Ye et al. (2015) revealed that the Bax’s expression level was increased in all four bladder cancer cell lines that were used: T24, 5637, J82 and UMUC-3, with the treatment of $\delta$-T3. The expression level started to increase as the $\delta$-T3 concentration increased from 0 mM to 50, 100 and 150 mM. However, the expression levels of Bcl-2, Bcl-xL and Mcl-1’s gradually decreased as the increase in the $\delta$-T3 concentration in all four bladder cancer cell lines that were used. The treatment of $\delta$-T3 for 24 hr on these cell lines resulted in the activation of Caspase-3 and cleavage of Poly (ADP-ribose) polymerase (PARP).

Ji et al. (2015) used human pancreatic cell lines, PANC-1 and BxPC-3, to study the potential effect of OPP as an anti-carcinogenic agent. Their findings correlate with the apoptotic pathway. Oil palm phenolics are the by-product of oil palm, recovered from aqueous waste in the milling process, containing various water-soluble phenolic compounds. Oil palm phenolics were shown to have valuable medicinal properties such as cardioprotective (Che Idris et al., 2014) and neuroprotective effects (Leow et al., 2013).

According to Ji et al. (2015), OPP reduced the production of anti-apoptotic genes, Bcl-XL, and survivin, in both cell lines gradually, as the OPP concentration increased from 0, 20, 30 to 40 mL/mL, post 72 hr treatment. On the other hand, OPP increased the expression of pro-apoptotic genes gradually, such as caspase-3, caspase-9, and PARP as the same concentration of OPP gradually increased after 72 hr treatment. This illustrates that OPP activates the apoptosis in the cancer cell lines by inducing the pro-apoptotic genes and inhibiting the cell survival genes; thus, OPP can slow down the migration and metastasis process in PANC-1 and BxPC-3 cell lines. An illustration of the apoptosis events is shown in Figure 4.

On another note, endoplasmic reticulum (ER) stress (EndoR stress) is an intrinsic pathway that leads to apoptosis. Endoplasmic reticulum was proposed to modulate apoptosis by sensitising mitochondria to various extrinsic and intrinsic death stimuli and commencing their own cell death signals. Endoplasmic reticulum stress is influenced by pharmacological agents that either hinder N-linked glycosylation, obstruct ER to Golgi transport, damage disulfide bond formation, or hamper ER Ca$^{2+}$ stores. There are three ER transmembrane receptors; pancreatic ER kinase (PKR)-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1) (Li et al., 2014). In normal conditions (non-stressed cells), all of these three sensors are in an inactive state through binding to the ER chaperone BiP. However, once ER is stressed, excessive unfolded proteins will gather in the ER lumen, causing BiP to dissociate from the ER stress transducer. This initiates the activation of unfolded protein response (UPR) and promotes cell survival (Bertolotti et al., 2000; Li et al., 2014; Zhang

Figure 4. An illustration showing how oil palm phenolics (OPP) initiates apoptosis to slow down the metastasis process on human pancreatic cell lines.
et al., 2010) (Figure 5). It was reported that apoptotic response occurs if the damage is too substantial and homeostasis could not be restored within a certain period (Breckenridge et al., 2003; Patil and Walter, 2001). Pancreatic ER kinase-like ER kinase has both pro-and anti-apoptotic functions, while ATF6 is anti-apoptotic and IRE1 is pro-apoptotic (Szegedi et al., 2006).

In a journal published by Comitato et al. (2016), they used HeLa cells to investigate the effect of T3 in gene expression that leads to the activation of EndoR stress and Ca-dependent. They demonstrated that γ- and δ-T3 activated a series of specific cellular responses leading to apoptosis. Among the differentially expressed genes during these treatments, seven genes were reported to function as markers of EndoR stress. Their data showed that at a concentration of 10 mg/mL, both γ-T3 and δ-T3 were able to increase the expression of heat-shock 70 kDa protein 5 (HSPA5) and asparagine synthetase (ASNS) after 24 hr. Heat-shock 70 kDa protein 5 was suggested to promote protein folding and inhibits protein aggregation in EndoR (Meunier et al., 2002), while ASNS is a transcriptional target of ATF4 in response to amino acid starvation through the GCN2/elf2α axis (Zhang et al., 2014).

Treatment with 10 mg/mL of γ-T3 specifically was shown to increase the gene expression of TNF receptor superfamily member 6 (Fas), x-box binding protein 1 (XBP-1), and C/EBP homologous protein (CHOP). Figure 6 shows the illustration of the apoptosis events due to the T3 treatment on the HeLa cells (Comitato et al., 2016).

Figure 5. The endoplasmic reticulum (ER) signalling pathways. 

Figure 6. The effects of γ- and δ-toco trienols treatments on HeLa cell line.
**Fas** is a death receptor family, also known by other names such as APO-1 and CD95 (Krammer, 2000). Fas expression will lead to the formation of the CD95 death-inducing signalling complex (DISC), which will eventually lead to either cell survival or cell death, depending on the downstream mechanism of DISC (Lavrik and Krammer, 2012). X-box binding protein 1 was reported to bind promoters of several genes involved in the UPR pathway and ERAD (ER-assisted degradation) to restore protein homeostasis and promote cytoprotection (Sano and Reed, 2013). Activation of CHOP cause changes in gene expression that favour apoptosis (such as the BCL2 gene).

In addition, treatment with only δ-T3 increased the expression of tribbles pseudokinase 3 (TRIB3) and stromal cell-derived factor 2-like 1 (SDF2L1). Tribbles pseudokinase 3 is one of the pro-apoptotic genes regulated when CHOP is overexpressed (Zhang and Kaufman, 2008). Stromal cell-derived factor 2-like 1 was reported to be involved in the regulation of caspases, which leads to apoptosis (Lorenzon-Ojea et al., 2016). Thus, Comitato et al. (2016) concluded that both treatments of γ-T3 and δ-T3 could affect UPR.

Studies that showed palm phytonutrients’ effect on apoptosis suggest that palm phytonutrients may act as an anti-tumour agent. In 2019, TRF was shown to positively affect the anti-tumour immune response (Abdul Hafid and Radhakrishnan, 2019). Bone marrow-derived dendritic cells were used. Tumour lysate from 4T1 murine mammary cancer cells was added to the dendritic cells. Researchers treated these cells with five types of treatment, with one treatment was given 8 mg/mL of TRF for 24 hr. The TRF treatment was able to promote the survival of T-cells in the tumours. It showed the slowest tumour growth and increased the rate of cell survival.

Four genes were differentially expressed, which were cysteine-rich protein 2 (CRIPT2), interferon-induced transmembrane protein 1 (IFITM1), mannose receptor C type 1 (MRC1), and special AT-rich binding protein 1 (SATB1). Interferon-induced transmembrane protein 1 (IFITM1), MRC1, and SATB1 were found to be down-regulated. However, at a confidence level of 99%, only SATB1 was reported to be significantly down-regulated. Studies have shown that the function of SATB1 is to fasten the specialised DNA sequences and bind to the matrix attachment region (MAR) (Kohwi-Shigematsu et al., 1997), which is involved in the loop domain organisation of chromatin remodelling. Special AT-rich binding protein 1 (SATB1) was also found to be engaged with prognostic outcomes of cancer patients (Alvarez et al., 2000). Thus, they concluded that TRF treatment could delay tumour growth, and SATB1 could be the next potential target gene in anti-tumour studies.

**NUTRIGENOMIC EFFECTS ON INSULIN RESISTANCE PATHWAY**

Insulin resistance is usually associated with obesity. Insulin promotes glucose uptake via the activation of the phosphatidylinositol 3-kinase pathway, which is responsible for most of the metabolic actions of insulin. Many genes are associated with insulin resistance. Among them is peroxisome proliferator-activated receptor gamma (PPARG), prospero homebox protein 1 (PROX1), insulin-like growth factor 1 (IGF-1), insulin receptor substrate 1 (IRS1), and many more.

In a journal published by Zaulkffali et al. (2019), the neuroblastoma cell line, SK-N-SH, was used to analyse the influence of vitamin D and E in enhancing insulin resistance in the neuronal-insulin resistance model. The Vit E was in the form of TRF. Treatment with 200 ng/mL Vit E alone and with the addition of 10 ng/mL and 20 ng/mL Vit D did not elevate the expression level of glucose transporter 3 (GLUT3) and glucose transporter 4 (GLUT4). These are insulin signalling markers. Glucose transporter 3 (GLUT3) is the main glucose transporter in the brain. Vitamin E alone was able to increase 2-D-glucose uptake significantly. In addition, treatment with the same concentration of Vit E alone and the combination of Vit E and D were shown to reduce the expression level of glycogen synthase kinase 3 beta (GSK3β) by almost 2-fold substantially. Vitamin E was found to also significantly reduce TAU expression level by 1-fold compared to the negative control. Vitamin E in the form of TRF was found to significantly downregulate reactive oxygen species (ROS) production after the induction of chronic insulin resistance, even though it did not substantially affect the insulin signalling molecules. Figure 7 illustrates the pathway of the insulin resistance model.

In another study, the Adfp (adipophilin) gene was expressed in human skeletal muscle (Phillips et al., 2005). Adipophilin was previously reported to stimulate the uptake of fatty acids (Gao and Serrero, 1999) and increased the storage of triacylglycerol (TAG) (Listenberger et al., 2007). Adipophilin was highly expressed when treated with palmitic acid and oleic acid, using a C2C12 cell line (De Wilde et al., 2010). The C2C12 cell line is a mouse muscle myoblast cell. This gene was expressed more in the oleic acid treatment as compared to palmitic acid. The expression of the Adfp gene is regulated by the peroxisome proliferator-activated receptors (PPAR) family. All three subtypes of PPAR, PPARα, PPARγ, and PPARβ/δ; increase the Adfp gene expression in a specific way. However, this gene was expressed the most through the activation of PPARα. Activation of PPARα reduces TAG level; thus, this correlates with the earlier findings in 1999 and 2007 (Gao and Serrero, 1999; Listenberger et al., 2007).
CONCLUSION

In vitro studies may lead to results that do not complement the situation of a living organism. However, this type of study allows a substance to study safely without compromising the health safety of animals and humans. Preliminary data obtained from here will be evaluated, and only those that appear to be beneficial will proceed to animal or human studies. The above review discussed the effects of palm phytonutrients on genetic modulation through in vitro experiments. Table 1 summarised the findings that were discussed from the year 2010 onwards.

The benefits of consuming palm oil and its phytonutrients have been widely discussed and published in journals, articles, or even through conferences and lectures. These benefits go beyond nutritional aspects, where many in vitro studies have reported findings indicating the potential for medicinal applications. We believed that the in vitro research that was published in all these previous publications could help and guide scientists to propose new projects that will be done in vivo and to investigate deeper in clinical research.

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REFERENCES


Che Idris, C A; Karuppaiah, T; Sundram, K; Tan, Y A; Balasundaram, N; Leow, S S; Nasruddin, N S and Sambanthamurthi, R (2014). Oil palm phenolics and vitamin E reduce atherosclerosis in rabbits. J. Funct. Foods., 7: 541-550. DOI: 10.1016/j.jff.2014.01.002.


Fenech, M; El-Soehemy, A; Cahill, L; Ferguson, L R; French, T A; Tai, E S; Milner, J; Koh, W P; Xie, L; Zucker, M; Buckley, M; Cosgrove, L; Lockett, T; Fung, K Y and Head, R (2011). Nutrigenetics and nutrigenomics: Viewpoints on the current status and applications in nutrition research and practice. J. Nutrigenet. Nutrigenomics., 4(2): 69-89. DOI: 10.1159/000327772.


Kunnumakkara, A B; Sung, B; Ravindran, J; Diagaradjane, P; Deorukhkar, A; Dey, S; Koca, C; Yadav, V R; Tong, Z; Gelovani, J G; Guha, S; Krishnan, S and Aggarwal, B B (2010). γ-Tocotrienol inhibits pancreatic tumors and sensitizes them to gemcitabine treatment by modulating the inflammatory microenvironment. Cancer Res., 70(21): 8695-8705. DOI: 10.1158/0008-5472.CAN-10-2318.


Luna, J; Masamunt, M C; Rickmann, M; Mora, R; España, C; Delgado, S; Llach, J; Vaquero, E and Sans, M (2011). Tocotrienols have potent antifibrogenic effects in human intestinal fibroblasts. Inflamm. Bowel Dis., 17(3): 732-741. DOI: 10.1002/ibd.21411


Oskoueian, E; Abdullah, N; Zulkifli, I; Ebrahimi, M; Karimi, E; Goh, Y M; Oskoueian, A and Shakeri, M (2015). Cytoprotective effect of palm kernel cake...


Ye, C; Zhao, W; Li, M; Zhuang, J; Yan, X; Lu, Q; Chang, C; Huang, X; Zhou, J; Xie, B; Zhang, Z; Yao, X; Yan, J and Guo, H (2015). δ-Tocotrienol induces human bladder cancer cell growth arrest, apoptosis and chemosensitization through inhibition of STAT3 pathway. **PLoS ONE,** 10(4): e0122712. DOI: 10.1371/journal.pone.0122712.


Zhang, J; Fan, J; Venneti, S; Cross, J R; Takagi, T; Bhinder, B; Djaballah, H; Kanai, M; Cheng, E H; Judkins, A R; Pawel, B; Baggs, J; Cherry, S; Rabinowitz, J D and Thompson, C B (2014). Asparagine plays a critical role in regulating cellular adaptation to glutamine depletion. **Mol. Cell.,** 56(2): 205-218. DOI: 10.1016/j.molcel.2014.08.018.
