

# Verbascoside—A Review of Its Antitumor Activities

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## Abstract

Cancer is a set of diseases including abnormal growth of cells that can spread to another tissue. Verbascoside (or acteoside) is a naturally occurring, water-soluble secondary metabolite with significant biological properties, which is distributed widely in plant kingdom. Verbascoside is pharmacologically active compounds with many recent evidences that support its biological activities and safety. This review focuses on the recent studies that concerned with the antitumor activities of verbascoside alone and as a synergistic agent as well as nanoparticle. It also shows the latest advances in its antitumor effects, cytotoxic selectivity and its efficiencies in treating cancer, *in vitro* and/or *in vivo*.

## Keywords

Verbascoside, Phenylethanoids, Phenylpropanoids, Glycosides, Cytotoxic, Antitumor

## 1. Introduction

In spite of the significant technological advances in diagnosis and treatment of tumors in the past few decades, cancer (CA) transpires to be one of the key causes of death around the world [1], and considered as a second leading cause of mortality, globally, 9.6 million deaths was recorded by WHO in 2018 [2]. Cancer may harm various organs and occurs in different tissue levels. Prostate, lung colorectal, liver and stomach CAs are the most common kinds of CA in men, while breast, cervical, thyroid, lung, and colorectal CAs are the most common types among women [1]. Moreover, blood CA is the most common CA in children [3]. The common causes of CA are lifestyle parameters (90% - 95%)

like food habits of having carbonated beverages, poor nutrition, smoking, junk food and excess alcohol consumption. In addition to the genetic factors (5% - 10%) [4]. Now days, when the Corona virus became pandemic, individuals with cancer may be more likely to have Corona virus disease (COVID-19) [5].

Many options are available for cancer treatment starting with surgical removal and radiation treatment of the large accumulated or sized biomass of cancer. The next option to surgery and radiation is treatment with systemic chemotherapy. Chemotherapeutic agents include alkylating agents ((e.g doxorubicin), antimetabolites (e.g., cytrabine), anti-tubulin agents (taxanes), hormones, molecular targeting agents and phytochemicals [5].

Phytochemicals are naturally occurring compounds from plants which serve as vital resources for cancer therapy and production of novel drugs. Many examples of phytochemicals used in cancer treatment include podophyllotoxin analogs, taxol analogs, vinca alkaloids such as vinblastine and vincristine and polyphenols. The most common mechanism of phytochemicals is regulating molecular pathways in cancer cells [6]. Polyphenols have been extensively examined for their possible benefits such as antioxidant, anti-angiogenic, antiproliferative antitumor, hypoglycemic and immunomodulatory properties that have increase in their applications in the functional foods, pharmaceutical and cosmetic industries [7]. Phenolic compounds can rapidly scavenge free radicals like hydroxyl radicals, superoxide radicals, peroxy radicals, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and reactive oxygen species (ROS) by transferring single electron or hydrogen atoms to free radicals. These mechanisms are the main physicochemical factors to evaluate the antioxidant efficiency of plant polyphenols [8].

Although many enhancements have been made, several semi-synthetic or synthetic derivatives of anti-tumor drugs are depend on natural molecules with anti-neoplastic properties. In this field, the most contribution of anti-tumor molecules belongs to plant secondary metabolites, specially phenolic compounds [9]. Plant polyphenols can inhibit CA development by modulation of some of signal transduction pathways that takes place in cancer cells. The key antitumor mechanisms involve modulation of some apoptotic proteins like cyclooxygenase-2, NF-κB, endothelin-1 and STAT3 and modulation in the pro inflammatory cytokines [10]. Various polyphenols are from plant origins representing around 8000 diverse structures. They have at least one or more aromatic rings containing, at least, one or many hydroxyl groups, that connected to glycosides or esters, enhancing their stability. Phenolic compounds include various molecules, like flavonoids, lignans, stilbenes, tannins, and, phenolic acids (hydroxycinnamic acids and hydroxybenzoic acids) [11].

Phenylethanoid-phenylpropanoid glycosides (PPGs) are widely distributed compounds in the kingdom of plants, most of these compounds isolated from medicinal plants, which are soluble in water and organic solvents [12]. This kind of glycosides are not specific to any organ in the plant, they have been isolated from plant leaves, aerial parts, barks, roots, callus and cell cultures [13] [14]. Most

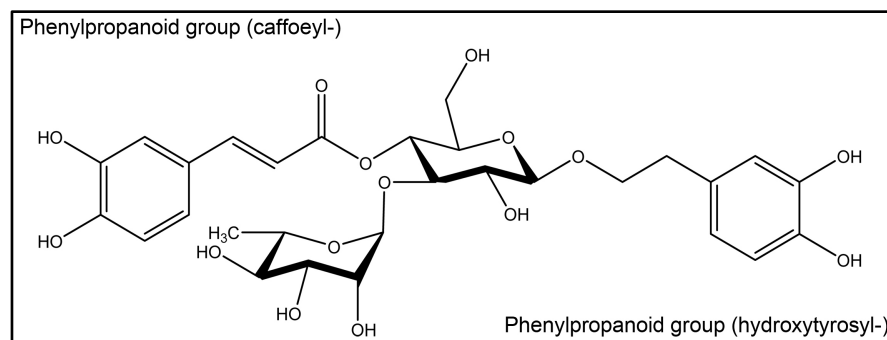
of these glycosides reported yet, were isolated from Plantaginaceae, Orobanchaceae Scrophulariaceae, Lamiaceae, Oleaceae and Lamiaceae families [15].

The basic structure characterized by 3 parts which are hydroxyphenylethyl (C6–C2) moiety, cinnamic acid moiety (C6–C3) and sugar part (which is usually di or trisaccharide) to which the first two moieties attached by the glycosidic linkage [16] as shown in (Figure 1) [17]. In few past years, attention has been growing about this type of glycosides, verbascoside (VERB) in particular, due to the high number of literatures that define its evident part in the prophylaxis and treatment of different disorders and diseases. [18]

This review was conducted to focus on verbascoside and its *in vivo* and/or *in vitro* anticancer activities against different types of tumors or cancer cell lines. It also extends to show the possible anticancer mechanism alone or in combination with other anticancer drugs. In addition, it discusses shortly some new applications of VERB with nanotechnology. Furthermore, this study attracts attention to the high efficiency and safety of VERB in order to enter the clinical field.

## 2. Chemistry and Biosynthetic Pathway of Verbascoside (VERB)

VERB is an important secondary metabolite that distributed in different plant species, consist of four moieties, phenylethyl alcohol, caffeic acid, rhamnose and glucose. The biosynthetic pathway of VERB remains to be completely elucidated. The first steps are well-known, but numerous intermediates and key enzymes with their corresponding genes remain to be unknown. Existing knowledge of this biosynthetic pathway, which is depend on experiments that including the feeding of plant with precursors labelled with specific isotope [19]. The biosynthesis starts with generation of tyrosine and phenylalanine precursors through the shikimic acid pathway [20]. The hydroxytyrosol part of VERB is synthesized from tyrosine through tyramine and also from dopamine, however, its caffeoyl part is biosynthesized from phenylalanine via cinnamic acid pathway [21]. Dopamine molecule is combined into phenylethanoid-phenylpropanoid glycoside by oxidation to aldehyde, then reduction to the alcohol, followed by  $\beta$ -glycosylation [22]. The sugar part is disaccharide (rhamnose and glucose) the phenylethanoid and phenylpropanoid parts attached to the rhamnose part by ether and



**Figure 1.** VERB structure.

ester bond respectively [19].

Many bioactivities of VERB are discovered till now, it has a wide range of pharmacological activities. The plants that are rich in these glycosides known for their powerful antioxidant activities [23]. Many researchers used variety of tumor models to identify the antitumor activities of PPGs. VERB, well-known PPG, which has different in vitro and in vivo clinical activities on various diseases [24] [25], with evidence-supported bioactivities including neuroprotection [26], antidiabetic [27], antioxidant [28], anticancer [29] antiinflammatory, anti-androgen, antimicrobial [30] [31] [32]. Toxicity studies on VERB showed its high level of safety as oral LD50 less than 2000 mg/kg, which give it high level of safety [33].

### 3. Anticancer Activities

VERB showed anticancer activities against many cancer cell lines as the following below.

#### 3.1. Brain Cancer

Glioblastoma is one of the most prevalent malignant tumors in gliomas, it constitutes about 45.5% of primary malignant tumors of brain [34]. SHP-1 is one of tyrosine phosphate protein, which is considered as a unique tumor suppressor gene that involved in different hallmarks of glioblastoma and other types of cancer. The key mechanism by which SHP-1 limits cancer progression and development is the capability to weaken signaling pathways, which control cell proliferation, migration, survival and invasion [35]. SHP-1 activities can be inhibited by small interfering RNA (siRNA) [36].

In 2018, Chinese researchers studied the anticancer activities of VERB by targeting protein tyrosine phosphatase (SHP-1) and STAT3. The result showed lower activity VERB when administered together with si-SHP-1 (siRNA + SHP-1) and higher cell migration and invasion in comparison to that shown in the VERB-treated mice [37]. Moreover, the survival rate in the mice group treated with combination of temozolomide (TMZ) and VERB was less than that in the groups treated with VERB or TMZ alone with higher apoptosis. The results indicated that si-SHP-1 could partially inverse the effect of VERB, by decreasing the expression of SHP-1 and increasing the expression of p-STAT3, (p-STAT3 which is often associated with cell survival, proliferation, and transformation) [38]. The results demonstrated that VERB enhanced the TMZ-induced inhibition on U87 cell (brain tumor cell line) invasion and migration [39]. Moreover combination of TMZ with VERB offered a therapeutic capability for treatment of glioblastoma. This synergistic effect of VERB on the therapeutic mechanisms of TMZ was explained through mitogen-activated protein kinases (MAPK) pathway. This synergistic effect also was demonstrated on C6 cell line from rat glioblastoma treated by TMZ, with VERB. Following co-treatment, the results showed higher expression levels of LC3 and LAMP1 (autophagy markers) [40],

and higher conversion rate of LC3-I to LC3-II (autophagosome generation sign), leading to enhancement of autophagy. Additionally, the combination therapy showed expression levels of p-ERK, P-38 and p-JNK (MAPKs that activated in response to stress stimuli) higher than treatment with TMZ only [37].

### 3.2. Colorectal Cancer

Colorectal cancer (CRC), the second most common cancer in the United States, is associated with a high prevalence of mutations [36]. VERB has high synergistic effect with 5-Fluorouracil (5-Fu) against colorectal cancer cells. Resistance of colorectal cancer cells was previously related to upregulation of AKT/PI3K pathway in these tumor cells [41], and therefore, suppression of this pathway has been suggested for sensitizing cancer cells to conventional treatment. The gene expression of Bcl-xL, p53, Bcl-2, Bax, Akt, PI3K and caspase-3,8,9 were appraised in Caco-2 cells treated with 5-Fu + VERB as a combined treatment. The combined treatment resulted in greater Bax expression level than 5-Fu (10  $\mu$ M) and VERB (0.1  $\mu$ M) alone. Moreover, VERB + 5-Fu-treated cells showed more than 1 fold lower P-AKT/total AKT ratio than that of the control cells, which suggest an improvement in colon cancer cells to 5-Fu [42]. The combined treatment indicated a significant decrease in PI3K that reached to 89.14% compared to control cells. In case of the caspases-3, 8 and 9, the combined treatment increased these caspases by 7.1 fold, while the increase was 6.7 fold in case of using 5-Fu only compared to control [43].

In another study, human colorectal cancer LoVo, HCT-116, SW620 and HT-29 cell lines were used for in vitro study, and HCT-116 cells were used for in vivo study using nude mice. VERB showed that with increasing doses (20, 40, and 80 mg/kg/day), the inhibition rates of tumor weight were 42.79%, 53.90%, and 60.99%, respectively. Particularly, at higher dose, the anti-tumor efficiency of VERB was similar to 5-Fu. VERB also improved the expression of Bax, HIPK2 and p53 (pro-apoptotic proteins) in tumors [44], and decreased the expression of Bcl-2 (anti-apoptotic protein) in a dose-dependent manner. When VERB administered together with p53-specific inhibitor (PFT-a) the rate of apoptosis is highly reduced, this strongly proposed that the apoptosis induced by VERB is the result of p53-HIPK2 signaling pathway activation [45].

### 3.3. Lung Cancer

Pulmonary cancer is the primary cause of mortality from the cancer worldwide (18.41% of cancer deaths) and results in more deaths than colorectal, cervical and breast cancers combined [46] about 15% of lung cancer patients are remain alive five years after diagnosis, because about 70% of patients have progressive disease at the diagnosis time [47].

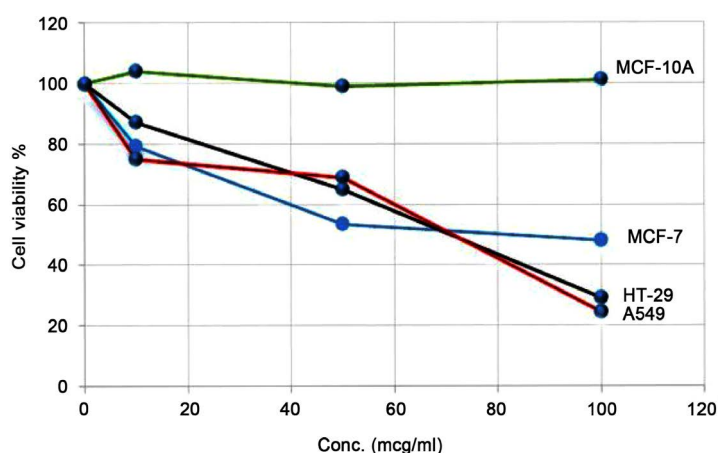
The antitumor mechanisms for VERB on the viability of A549 (adenocarcinomic cell from human alveoli), HT-29 (cancer cell from human colon), and MCF-7 (human breast cancer cell line) cells, and mechanisms included cell cycle

arrest, modification in apoptosis and intracellular ROS level (that have antitumor effect in high level) were observed [48]. The reduction in the cell viability was concentration-dependent, at 100  $\mu\text{g}/\text{mL}$ , the cell viability reduced by VERB as 60.9%, 65.6%, 68.6% in A549, HT-29 and MCF-7 cells respectively. The  $\text{IC}_{50}$  of MCF-7 cell was lower than other cells (*i.e.* A549 and HT-29), which indicated higher sensitivity of these cells to the antitumor activity of VERB, as shown in (Figure 2). The rate of apoptosis increased by 2.3, 2.5 and 7.5-times in A549, MCF-7 and HT-29 cells respectively in comparison with control cells. VERB also showed an increase in the ROS production in all cancer cells with maximum effects from 1 to 2 hours after treatment with VERB (100  $\mu\text{g}/\text{mL}$ ), A549 pulmonary cells showed the highest sensitivity, with ROS levels that increased by 9.4%, 21.3%, and 37.7%, after 2, 3, and 24 hrs incubation periods, respectively [49].

### 3.4. Liver Cancer

The largest organ in the human body, the liver, that can be exposed to many conditions and routines (e.g. viral infection, toxins and alcohol abuse) that lead to its inflammation and damage, which can be advanced to liver carcinoma; the excessive production of pro-inflammatory cytokines is the main cause of inflammation and damage of liver [50].

The inhibitory action of VERB against the production of tumor necrosis factor (TNF- $\alpha$ ) and interleukin-6 (IL-6) compared to silymarin was estimated. At first HepG2 cells (cancer cell line from human liver) treated with VERB at 40, 60 and 80  $\mu\text{M}$ , then the cells treated with alcohol for inducing the cytokines production (*i.e.* TNF- $\alpha$  and IL-6); the maximum inhibition (in comparison with control) shown at 80  $\mu\text{M}$  VERB concentration that is time-independent. The inhibition of IL-6 and TNF- $\alpha$  by VERB was assessed to be 37.9% and 28.3% respectively, while silymarin at 100 $\mu\text{M}$  showed 29.9% and 38.7% inhibition of



**Figure 2.** Conc-response curve, which show the  $\text{IC}_{50}$  for each type of cells. MCF-7 shows to have higher sensitivity to the treatment with lower  $\text{IC}_{50}$ , while MCF-10A shows no remarkable changes after treatment, which indicate the high selectivity of VER.

TNF- $\alpha$  and IL-6 respectively [33].

VERB showed a high efficacy against hepatocellular carcinoma (HCC) after analyzing the proliferation and migration of cells in HLF, JHH-7, and BEL7404 xenografts (liver tumor cell line from human) in mice. Additionally, angiogenesis inhibition by VERB was also analyzed using human umbilical vein endothelial cells (HUVECs). The kallikren-related peptidase (KLK) has been proposed as a tumor biomarker in the diagnosis as well as in the prognosis of different cancers, due to its disturbed expression [51]. VERB also has the ability to exert an antitumor activity in HCC cell lines and in mice transfecting with HUVECs by increasing p53 levels and inhibiting KLK and angiogenesis and also prohibit the cell proliferation in all the three cell lines. However, the prohibition potency together with sorafenib (anticancer) as a co-treatment was stronger than that of sorafenib or VERB alone. Moreover, VERB + sorafenib showed higher wound healing inhibition than single treatment which indicates the high efficiency of VERB in prevention of cell migration. In the case of angiogenesis, which is linked to tumor growth, progression and metastasis, the inhibition of HUVECs migration, alignment and elongation, and formation of vessel-like structures were also highly efficient with co-treatment [52].

### 3.5. Breast Cancer

The VERB effect is shown to be similar to that of the known phytoestrogens such as resveratrol, which indicate that VERB has plant estrogenic action [53]. VERB has anti-breast cancer activity, which could antagonize the upregulation of estrogen response element (ERE) luciferase by estradiol, this because VERB has competitive effect with estradiol by blocking the receptors of estrogen in HeLa cells. VERB interacted with Sterile Alpha Motif Domain 3 (SAMD3), which is protein-coding gene, resulting in its phosphorylation, which in turn could hinder the cell proliferation. Additionally, VERB shown to promote the expression of activator protein 1 (AP-1), which can regulates a number of cell processes, including proliferation, differentiation and apoptosis; activated AP-1 can reduce the gene expression MYC (an oncogene) and CDk6, which is an important key in the progression of the cell cycle [54].

In turkey, some researchers isolated VERB from *Phlomis nissolii* and studied the ability of VERB for apoptotic induction in the breast cancer cell lines. VERB IC<sub>50</sub> for MCF-7 and MDA-MB 231 cell lines after exposure to different concentrations of VERB, as shown in (Table 1).

**Table 1.** VERB IC<sub>50</sub> for MCF-7 and MDA-MB 231 cell lines.

The time of exposure to VERB	IC <sub>50</sub> ( $\mu$ M) for MCF-7	IC <sub>50</sub> ( $\mu$ M) for MDA-MB 231
24 hr	0.127	0.159
48 hr	0.217	0.258
72 hr	0.282	0.256

After using different concentrations of VERB (100, 48, 25, 10, 1, 0.5 and 0.1  $\mu\text{M}$ ), the result showed that only 100  $\mu\text{M}$  was effective with highest cytotoxic activity on MCF-7 after 72 hr and highest cytotoxic activity on MDA-MB 231 after 24 hr, 48 hr and 72 hr [55].

### 3.6. Hematologic Cancer

The dynamics of hematopoietic stem cell may precede many blood cancers, involving myelodysplastic syndrome, myeloproliferative neoplasms, chronic lymphocytic leukemia and acute myeloid leukemia [56]. Kyung-Won Lee and his group revealed that VERB prevented the growth of HL-60 cells (human leukemia cell line) in a time and concentration-dependent style with an IC<sub>50</sub> of 30 mM. Additionally, flow cytometric analysis revealed that VERB blocked progression of cell cycle at the G1 phase in HL-60 cell [57]

Nanotechnology is the use of material on an atomic, molecular or supramolecular scale for different purposes. Medicinally, nanotechnology plays an advance role in enhancing the absorption, bioavailability and efficacy of drugs [58]. It has been shown that nanocarriers loaded with antioxidant can be used in various formulations with a high and controlled-release antioxidant effect that would meet the modern requests of consumers [11]. Recently, Chinese scientists developed a new system for better delivery of VERB for enhancing its chemotherapeutic effect against drug-resistant type of leukemia cells (K562/A02, KA). A novel combination product of poly n-isopropyl acrylamide with gold nanoshells, showed a better role of drug delivery [59]. VERB nanoparticle (200 nm) shown obvious tumor inhibitory effects by increasing the expression of initiator caspases (e.g. caspase 3,8,9) inside the KA cells with smaller size of tumor in comparison with control group and those treated with VERB alone, and the result proved that this delivery system enhance the anticancer activity of VERB [60].

### 3.7. Skin Cancer

Polyphenols of plant origin, known for their antioxidant, anti-inflammatory, antimutagenic, and antiproliferative characteristics *in vitro* and *in vivo* [61]. Protective effects of polyphenols in green tea against UVB-induced skin cancer [62], pro-cyanidins in grape seeds [63], curcumin, silymarin and genistein were confirmed to be potent protective agents against initiation and progression steps of UVA and UVB carcinogenesis on the mouse model [64] [65] [66] [67]. However, because of their poor gastrointestinal absorption, little bioavailability, and enhanced metabolism; the clinical chemoprotective worth of VERB and other polyphenols through oral route is still questioned [68].

Moreover, VERB has shown to be more toxic in A5 cells (multistage cancer of mouse skin) than C5N cells (non-carcinogenic). It was found that VERB differentially reduced the phosphorylation of ribosomal protein S6 and enhanced the phosphorylation of p53, Creb1, Stat6, Fak1 and pro-apoptotic Stat1 in A5 com-



pared to C5N cells, as well as, VERB resulted in suppression of the MMP-2 and MMP-9 activities in the A5 cells. This indicate that VERB has evidenced selectivity toward tumor cells [69].

### 3.8. Prostate Cancer

Prostate cancer is one of the most common carcinoma in men [70]. There are many chemotherapeutic agents for treating prostate cancer, which are extremely toxic to the normal tissues [71]. To resolve this problem, co-therapy of a chemotherapeutic agent with a remedy possessed anti-proliferative effect. Several studies have revealed that natural secondary metabolites that have a cinnamic acid moiety possess anti-proliferative effects on tumor cell lines [57].

VERB has ability to promote rat prostate apoptosis, and inhibit benign prostatic hyperplasia (BPH). VERB with high-dose treatment can results in apoptosis in the rat prostate cells, which is significantly higher in comparison with model group [72]. Treatment with VERB significantly prevented cell proliferation, and migration capabilities of men prostate tumor cell line (e.g. PC-3 cells and Du-145) by suppression of HMGB1/RAGE pathway, which resulted in downregulation of TGF- $\beta$ -associated epithelial-mesenchymal transition (EMT) progression [73].

## 4. Miscellaneous

DNA is under continuous stress, which results from cellular metabolism or environmental factors [74]. Reactive oxygen species (ROS) cause DNA damage through oxidative damage, hence, playing an important role in the initiation of tumor [75]. The oxidative DNA stress has been implicated in the induction of several diseases including inflammation, heart disease and cancer [76].

*Abeliophyllum distichum* (AAD) is a Korean plant that rich in VERB [77]. A recent Korean study clarified the essential role of AAD in preventing the oxidative damage of DNA. The results showed that AAD eliminated 1,1-Diphenyl-2-picrylhydrazyl (DPPT) and 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) free radicals in dose-dependent manner, IC 50 for AAD and control (L-ascorbic acid) were 8.8 and 5.0  $\mu\text{g/ml}$  respectively in case of DPPT, and 6.47 and 10.49  $\mu\text{g/ml}$ , respectively, in case of ABTS. ADD also enhanced the cell viability compared with control cells after exposure to  $\text{H}_2\text{O}_2$ -induced damage [78].

VERB considered as strongest antioxidant recognized in Australian olive mill waste [79]. VERB Hydroxytyrosol and oleuropein at 10  $\mu\text{M}$  significantly decreased the proliferation of gastric adenocarcinoma (AGS cells) by 19, 27 and 16%, respectively. Though, the ethyl acetate extract of olive mill waste (biophenols extract) was more potent as anti-proliferative than VERB alone [80].

VERB also showed antitumor activity against oral squamous cell carcinoma (OSCC) by decreasing the vitality and metastasis of HN6 and HN4 cancer cells, while stimulating apoptosis. VERB efficiently inhibited activation and downstream

of nuclear factor (NF)- $\kappa$ B and expression of Bcl-2/Bcl-XL, resulting in high rate of OSCC cell apoptosis, consequently, mRNA and matrix metalloproteinase-9 expression have been suppressed, thus, VERB inhibiting metastasis of cancer cell [81].

#### 4.1. VERB and Nanotechnology

Based on the advantage of nanotechnology in cancer therapeutics in improving the pharmacokinetics and reducing the systemic toxicities of chemotherapies assuming the selective targeting and delivery of these anticancer drugs to tumor tissues [82]. So the beneficial effect of medicinal agent-loaded nanoparticles have become highly concluded [83]. Many researches studies the application of VERB loaded nanoparticles in treatment of cancer and the results were better than without nanoparticles. VERB carried on nickel nanoparticles showed a synergistic effect on the of apoptosis induction in doxorubicin-resistant human erythro-leukemic cell line (K562). Observations demonstrate that nickel have ability to facilitate the VERB uptake into K562 cells. Additionally, *in vivo* study indicated that the tumor growth in the mice could be efficiently inhibited by these nanoparticles. Thus, VERB-Nickel can serve as a novel approach to sensitively led the tumor cells to effective chemotherapy [77]. VERB carried on gold nanoparticles (Au) also were studied and demonstrated that VERB-Au nanoparticles provided an effective strategy to control tumor cell growth [29].

#### 4.2. Safety and Side Effects of VERB

VERB has a wide range of biological and pharmacological activities so studying its side effects and toxicity is important. [18]. VERB has oral LD50 less than 2000 mg/kg, which give it high level of safety [33]. A single intraperitoneal VERB injection at 1, 2 and 5 g per kg did not induce deaths and side effects in mice. Therefore, LD50 value of VERB was found to be greater than 5 g per kg, and a substance with a LD50 in range of 1 - 5 g per kg is considered low-toxic [84]. Additionally, *in vitro*, VERB showed no cytotoxic effects on HepG2 and NIH cell at concentrations up to 400  $\mu$ M, as well as, VERB did not cause significant changes in hematological and biochemical and histopathological parameters [85]. Further wide studies may be required to set up on the possible adverse effects of VERB.

### 5. Conclusions

This review summarized the latest studies that proved the antitumor activities of VERB. As a polyphenolic compound, VERB showed a powerful antioxidant and antiproliferative efficiencies, alone and as a synergistic agent. VERB has ability to be more effective when given together with chemotherapeutic agent (e.g. TMZ, 5-Fu, cisplatin, sorafenib, etc.). Moreover, lower effective dose of chemotherapeutic agent is needed with co-treatment, and hence, less toxic effects of chemotherapy. For instance, IC50 of 5-Fu was highly reduced from 1.1990  $\mu$ M

when used as a single treatment to 0.1875  $\mu\text{M}$  when combined with 0.1  $\mu\text{M}$  VERB. VERB + 5-Fu-treated cells, showed the lowest p-AKT levels, in comparison with VERB, control and 5-Fu-treated cells.

Resistant cells characterized by modified membrane transport, improved DNA repair, defects in apoptotic pathway, modification of proteins, target molecules and pathway mechanisms, like enzymatic deactivation [86]. One way to overcome the multidrug-resistant tumor cell is the nanoparticle, which enhances the bioavailability of anticancer agent inside the tumor cell. VERB nanoparticle significantly stimulated apoptosis-related caspases expression in tumor cells, which might offer a new chemotherapeutic approach in cancer treatment such as leukemia. ROS is like a sword with two edges, at low levels, ROS enhances the survival of tumor cell [87], while at high level, ROS can overwhelm tumor growth by activation of cell cycle inhibitors [88]. Additionally, VERB has high ability to increase ROS inside the tumor cell like A549, HT-29 and MCF-7 that showed time-dependent generation of ROS in tumor cells (1 - 24 hours) [49].

Collective data with the evidences showed that VERB is active compound with high selectivity, no toxicity in animals and no mutagenic effects and it seems to be possible for the future use of the co-therapy of VERB and chemotherapy in clinic [69] [89]. Also loading of VERB on nanoparticles can provide a good tool for delivery of VERB to cancer sites [9].

In spite of the fortune of laboratory data, which is accessible that describe the anti-tumor activities of VERB after *in vitro* results additional to the animal models, many questions remain unsettled with respect to actual clinical applications. Evidence-based human researches in large scale with precise therapeutic settings are important. More intensive studies are necessary to settle the clinical potential of VERB, thus permitting its acceptance as a therapeutic compound. VERB is also has a special structure that suggested an interesting scaffold with many reactive sites for combinatorial chemistry.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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