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# Feasibility of Integrating Tripterygium wilfordii into Modern Cancer Therapy for Increased Efficacy with Minimal Toxicity

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# Feasibility of Integrating *Tripterygium wilfordii* into Modern Cancer Therapy for Increased Efficacy with Minimal Toxicity

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*Author's Note*

This research investigation was conducted under the guidance of Professor Mary Boyes from the Honors Writing Program and Dr. Yan Zhang from the VCU School of Pharmacy.

*Abstract*

Cancer is the second leading cause of death in the U.S., and millions of novel cancer cases are being diagnosed each year. While chemotherapy and ionizing radiation are effective treatments against these malignant tumors, the adverse effects that accompany such treatments are devastating. In order to find alternative treatment methods with less side effects, we turn to Eastern herbal medicine. Recent scientific research has found that *Tripterygium wilfordii*, an herbal medicine traditionally used to treat inflammation in China, contains compounds (triptolide and celastrol) that prevent the growth of solid tumors, induce apoptosis, and prevent metastasis of developed tumors. Investigations on these compounds on various cancer cells lines (in vitro and in vivo) have revealed insight into their mechanism, mode of action, and toxicity. In order to circumvent the potentially fatal side effects of triptolide and celastrol, it was proposed that roots of *T. wilfordii*, from which the compounds are extracted, be used as a treatment for cancer. Methods for testing the efficacy and toxicity of the roots on the different cell lines previously studied are outlined in this paper. If the results from the proposed experiment conflict with expectation, then future studies on combination drugs using triptolide and celastrol with other non-bioactive compounds within the roots should be done to develop new anti-cancer drugs with low toxicity.

Keywords: Eastern herbal medicine, alternative and complementary medicine, *T. wilfordii*, triptolide, celastrol, cancer, anti-angiogenesis, anti-proliferation, apoptosis

## *Feasibility of Integrating Tripterygium wilfordii into Modern Cancer Therapy for Increased Efficacy and Minimal Toxicity*

*Introduction*

Diseases are becoming more resistant to drugs being used on the market. The current solution to such problems is to develop stronger and more potent drugs to combat these diseases. However, accompanying these powerful drugs are adverse side effects that are proportional to the effectiveness. One disease that will arouse the most fear and concern among the general populace nowadays is cancer. According to the CDC, cancer is the second leading cause of death in the U.S, and the American Cancer Society reported that millions of new cancer cases are being diagnosed each year. Cancer is a disease due to a loss of cell division control, in which the cells divide continuously and do not respond to the appropriate signaling from its environment. The malignant cell will develop into a tumor and then begin to spread, or metastasize, to surrounding organs and organ systems along the blood stream. Current methods of cancer therapy include chemotherapy, which uses cytotoxic compounds to induce apoptosis within the cancer cells and/or reactivate the regulatory proteins in the cells, and ionizing radiation,

which uses potent electromagnetic waves and particles to bombard the tumors and cause DNA damage, causing it to stop replicating and potentially induce apoptosis. Although effective, chemotherapy and ionizing radiation also induce adverse side effects, such as hair loss, extraneous damage to healthy cells, and decreased immunity. In order to find alternative treatment methods with reduced side effects, here, we turn to Eastern medicine, more specifically Chinese medicine.

Chinese mythology tells of Emperor Yan, who lived more than four thousand years ago, as the first Chinese pharmacopeia and the founder of Chinese medicine. It was said that he personally compounded plants and herbs and tested their effects on himself. He eventually died by his hand when he unknowingly took an herbal poison (Grazoise et al. 2). The Chinese medicine that exists today has evolved much since the times of Yan, and its largest influence is Taoism, a philosophy and religion founded by Lao Tzu in the first century B.C.E. Chinese medicine incorporated the philosophy of Taoism into its practice, viewing illnesses and diseases as physical manifestations of an imbalance of qi, the life-force within the body. Analogous to the Five Elements of Chinese philosophy (wood, fire, earth, metal, and water), the ancient physicians also viewed the human body as consisting of five organ networks — the liver, the heart, the spleen, the lung, and the kidney. Different symptoms are associated with different organ networks, and treatments are prescribed by the differential symptoms and vitals. The most well-known methods of treatment in Chinese medicine are acupuncture and herbal medicine (Chen and Xu 226-227).

Acupuncture is used to alter the circulation of qi in order to restore the proper balance to the body, allowing it to heal itself. Acupuncturists embed needles into "specific locations (acupoints) along the channels that conduct the qi through the body" (Chen and Xu 227). Aside from acupuncture, Chinese medicine also has other ways of altering the flow of qi, including variants of acupuncture, such as moxibusion and tuina, and qi gong. Moxibustion is similar to acupuncture, with the exception that it uses a burning herb called moxa instead of needles. Tuina is another variant of acupuncture, where hand techniques are used to apply pressure on the acupoints or other specific locations to affect the circulation of qi. Qi gong is the practice of using breathing techniques and meditation to alter one's qi. This is can be done by the patient alone or alongside the physician who gives guided instruction as to how to direct the qi (Chen and Xu 227).

The other form of treatment, herbal medicine, is also quite renowned, even in Europe and in the United States. Grazoise et al. wrote that currently there are 11,146 plants species representing 2,309 genera and 383 families being used in Chinese medicine, along with 1,518 species of animals and 80 other substances for the preparation of herbal medicine (2). It is widely known that one main difference between Western and Eastern medicine (in this case, Chinese medicine, but this can be generalized to include ancient medical practices in most Far East countries) is that Western medicine uses single-compound drugs that affect the immediate symptoms whereas Eastern medicine uses herbs that "treat the underlying condition as defined by traditional diagnosis" (Chen and Xu 227). In addition, Chinese herbs are known for the lack of adverse side effects that typically accompany Western drugs (Chen and Xu 227). Many of the differences between Chinese and Western medicine are highlighted in Table 1 below.

| Table 1. Chinese Medicine vs. Western Medicine | | |
|---|---|---|
| Medical View | Chinese | Western |
| Diagnosis/Treatment | Philosophic | Scientific |
| Clinical Distinction | Wholesome | Local |
| Medicine | Natural | Chemical |
| Study Method | Human Experience | Clinical Lab Testing |
| Preventive View | Preventive | Sanitary |
| Treatment Method | Individualized | Standardized |
| Treatment Goal | "Cure" oriented | Reduction of Symptoms |
| Treatment Views | Natural | Invasive |

***Note:*** *From "Consumers' Perceptions of Chinese Vs. Western Medicine" by Piron, Ching, Peng, and Ching.*

Recent cancer research has gained interest in a plant used in Chinese medicine called *Tripterygium wilfordii* (Grazoise, Lila, and Raskin). *Tripterygium wilfordii* (雷公藤, lei gong teng; "thunder god vine") (see Figure 1) is a deciduous subshrub and climber native to east and southeast China, Japan, Korea, and northeast Myanmar ("*Tripterygium wilfordii* in Flora of China @ efloras.org"). The plant may be known to be toxic. However, its roots possess therapeutic effects and, when prepared properly can be used as an herbal medicine. It has been traditionally used for the treatment of fevers, chills, and inflammatory diseases. Research has shown that the roots of *T. wilfordii* contain two main bioactive compounds — triptolide (see Figure 2) and celastrol (see Figure 3). Because of their anti-inflammatory effects, research in developing these compounds into drugs for rheumatoid arthritis are taking place in China, but more recently, researchers have found that triptolide and celastrol possess other therapeutic effects. Triptolide and celastrol have also exhibited potent anti-tumor activities, making them strong candidates for novel anti-cancer drugs. However, despite their abilities to prevent tumor growth, induce apoptosis, and prevent metastasis of developed tumors, triptolide and celastrol also possess strong adverse effects, such as reproductive damage to both males and females, induce nausea, and cause diarrhea. Surprisingly the root as a whole is not toxic. Thus, to make use of the therapeutic effects of triptolide and celastrol, more research must be conducted using a crude extract of the roots of the plant rather than simply studying isolated compounds that are already known to produce damaging side-effects.

***Figure 1.*** *T. wilfordii (雷公藤, lei gong teng; "thunder god vine")*

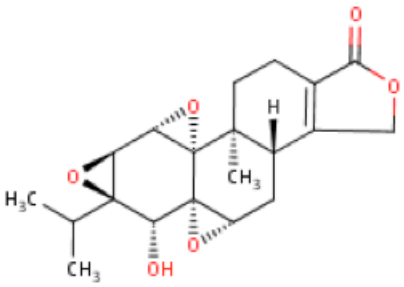

***Figure 2.*** *Triptolide ($C_{20}H_{24}O_6$, MW: 360.404)*

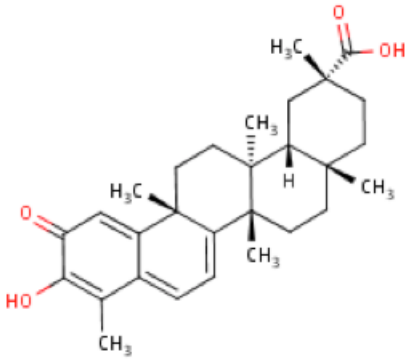

***Figure3.*** *Celastrol ($C_{29}H_{38}O_4$,MW: 450.6152)*

*riptolide for Cancer*

Triptolide (PG490) is a diterpenoid triepoxide natural product extracted from the roots of *T. wilfordii*. It exhibits potent anti-tumor activities through the inhibition of tumor formation and proliferation, the induction of apoptosis, and the inhibition of angiogenesis in a wide variety of cancer cells. The results of these combined effects localize the tumor by hindering metastasis and then eliminate it by killing the malignant cells. Therefore, triptolide is a strong drug candidate for future cancer therapy.

According to a literature review by Grazoise, Lila, and Raskin, triptolide has demonstrated anti-tumor activities in colorectal cancer, oral cancer, breast cancer, ovarian cancer, and many other solid tumors in both in vitro and in vivo studies (7). This property of triptolide to act on a wide variety of cancer cells makes it a very appealing anti-cancer agent. An experiment conducted by Yang et al. showed that triptolide inhibited the growth of four different solid tumor lines "with distinct origins and of different p53 status." These lines include B16 mouse melanoma, MDA-435 human breast cancer, TSU bladder cancer, and MGC80-3 gastric cancer (65). In the in vitro component of the experiment, Yang et al. found that "after 2 days of treatment, the proliferation of the tumor cells was significantly [($p<0.05$)] inhibited by [triptolide] in a dose-dependent manner" (67). Yang et al. also found that triptolide exhibited stronger potency than Taxol, a currently used chemotherapeutic in patients. At a concentration of 25 ng/mL (70 μM) triptolide inhibited growth more than Taxol at 100 ng/mL (117 μM). Additionally, Yang et al. noted that the maximum effects of triptolide only showed 3-4 days after the treatment (67). The superior potency of triptolide over currently used chemotherapeutic drugs is a great advantage. With stronger potency, lower dosages can be used, and thus, perhaps adverse effects can be mitigated to some extent. However, it must also be taken into consideration that perhaps the reason for the stronger potency of triptolide over Taxol may not be solely its pharmacodynamics. A confounding variable that was not examined is the cells' resistance to Taxol. It is not uncommon for continued usage of a drug to lead to drug resistance through metabolism.

For the in vivo study using mice, triptolide again inhibited the growth of all four cancer cell lines, but at different potency (Yang et al. 68). Yang et al. administered treatment of triptolide at 0.15 mg/kg/day i.p., which they found to be 60% of the maximum tolerated dosage. The B16 melanoma cancer cell line's growth was inhibited by 50% and the MGC80-3 gastric cancer cell line was inhibited by 90% when compared to the control (68). The results from this experiment suggested that triptolide is able to inhibit the proliferation of malignant tumors of many types, but its efficacy is cell-specific. In addition, due to the different status of p53, Yang et al. suggested that triptolide's mechanism of action regarding anti-proliferation does not in-

volve the tumor suppressor gene p53 (70).

A study of triptolide on human prostatic epithelial cells by Kiviharju et al. yielded similar results. When Kiviharju et al. treated cultures of low cell density with triptolide at a concentration of 1 ng/mL, "[c]omplete growth inhibition of all cell strains occurred, with half-maximal growth inhibition at ~0.1 ng/mL" (2668). The different cell strains were all prostatic epithelial cells, but they were taken from different locations. One strain was taken from an adenocarcinoma, and the other four were taken from the normal peripheral zone (2267). Given the results of this study when compared to the one by Yang et al., the proposition that triptolide's anti-tumor effect is cell-specific gains further support, and triptolide may be most potent on prostate cancer, since only a low concentration is needed to bring full inhibition in prostatic cells.

Another aspect of triptolide's effects on cancer is its ability to induce apoptosis. Kiviharju et al. conducted an analysis of triptolide's pro-apoptotic ability after witnessing the decline in cell viability. The researchers treated the E-CA-12 cell line (30% intraductal carcinoma/70% Gleason Grade 4) with triptolide for 3 days. Kiviharju et al. found that at 1 ng/mL, triptolide did not cause apoptosis during the 3-day period (2268). However, at higher concentrations (≥ 50 ng/mL), triptolide increased the apoptosis rate by 19% after 24 hours, and after 48 hours, "the number of apoptotic cells in the treated population increased substantially" (2669).

In contrast, after finding that triptolide inhibits the proliferation of tumor cells, Yang et al. performed a Western blot to examine triptolide's mechanism for inducing apoptosis. The researchers found that triptolide activated 2 key molecules in the apoptotic pathways: caspase-3 and Poly (ADP-ribose) polymerase (PARP) (69). After Yang et al. began to treat MDA-435 (human breast cancer) cells with triptolide, there was an increase in caspase-3 within 2 days and "a shift in PARP from its intact molecule into its subunit of Mr 89000, which peaked on the day 4 of the treatment" (69). Yang et al. reported that "Western blotting analysis also revealed that the treatment with [triptolide] for 3 days caused a significant reduction in c-myc and two pairs of cell cycle-promoting protein complexes, cyclinA/cdk2 and cyclinB/cdc2, and cyclin D1 as well the phosphorylated nonfunctional pRb" (69-70).

Triptolide can also inhibit angiogenesis, which is vital for growth and metastasis of tumors. This ability to inhibit angiogenesis also contributes to triptolide's overall anti-proliferative effects. In an in vivo study of the anti-angiogenesis effects of terpenoids in *T. wilfordii*, He et al. reported that triptolide, extracted by ethyl acetate from a 95% ethanol crude extraction, "inhibited 20% of vessel formation" with a concentration of 0.31 μM, and "inhibition reached a plateau of nearly 50% by 1.2 μM" in zebrafish models ("Antiangiogenic Activity of *Tripterygium wilfordii* and its Terpenoids" 64). Upon further analysis, He et al. found that triptolide inhibited angiogenesis by "selectively reduc[ing] both angpt2 and tie2 expression in a time- and dose- dependent manner" ("Antiangiogenic Activity of *Tripterygium wilfordii* and its Terpenoids" 64). He et al. noted that a different study of triptolide showed that it also inhibited inflammation, another result of angiogenesis, via inhibiting vegfa expression and production, and surmised that the discrepancy may be due to the usage of artificially induced inflammation and/or the difference in cell types used for the studies ("Antiangiogenic Activity of *Tripterygium wilfordii* and its Terpenoids" 68). Therefore, "triptolide might attenuate angiogenesis via distinct mechanisms" depending on the cell type ("Antiangiogenic Activity of *Tripterygium wilfordii* and its Terpenoids" 67).

He et al. then proceeded to test the hypothesis of whether the results on zebrafish could be applied to mammalian cancer cells as well. In their follow-up experiment, He et al. reported that "following 24-hour treatment, triptolide at 50 nM suppressed (vascular endothelial growth factor receptor-2) VEGFR-2 and Tie2 mRNA expression by more than 70% compared with the control" ("Triptolide Functions as a Potent Angiogenesis Inhibitor" 269). As He et al. has

hypothesized in their previous study, the mechanism of triptolide to inhibit angiogenesis does change according to cell type. In the zebrafish embryos, the genes inhibited were angpt2 and tie2, and in HUVECs, the genes were VEGFR-2 and Tie2. The inhibitory effects on these genes were both dose-dependent and time-dependent. Triptolide fully inhibited the expression of VEGFR-2 at 100 nM, and the maximum inhibition of both genes occurred 18-24 hours after treatment ("Triptolide Functions as a Potent Angiogenesis Inhibitor" 269-270).

While the studies done by He et al. showed promising results for the anti-angiogenic property of triptolide, there are some limitations to utilizing the information. First, the in vivo study done in 2009 was performed on Danio rerio, or zebrafish. While it is clear why zebrafish were chosen to be tested, it is difficult to generalize the findings to apply to humans, who are the intended recipients of the drug. The second study in 2010, an in vitro study, may have been an attempt to rectify this conundrum — and it does to some extent — but the lack of an in vivo study using human umbilical vein cells (HUVECs) or any other type of human cancer xenografts in mice to examine angiogenesis does not allow for the generalization of triptolide's anti-angiogenic activities in humans.

*Therapeutic Effects of Celastrol for Cancer*

Celastrol, or tripterine, is a quinine methide triterpenoid extracted from the roots of *T. wilfordii* and modulates many biochemical pathways and molecular targets, such as tumor necrosis factor-α (TNF-α), nuclear factor kappa B (NF-κB), cyclooxygenase-2 (COX-2), VEFG, Akt, C-X-C chemokine receptor type 4 (CXCR-4), pro-inflammatory cytokines, and chemokines. These molecules are involved in the initiation, proliferation, and progression of tumors; thus, celastrol, which interferes with the activities of these molecules, is another strong potential drug candidate for future cancer therapy. According to a literature review by Kannaiyan et al., "celastrol has been shown to inhibit the proliferation of various cancer cell lines including C6 glioma cell lines, human monocytic leukemia cell lines, melanoma cell lines, pancreatic cancer cell lines, 8266 myeloma cell lines, K-562, and human chronic myelogenous leukemia cell lines" (13). The wide range of effectiveness of celastrol is similar to triptolide and makes celastrol a strong drug candidate for cancer therapy.

Peng et al. conducted a study to investigate the anti-proliferative effects of celastrol in human monocytic leukemia cell U937 previously mentioned. Peng et al. treated the U937 cells with varying concentrations of celastrol, ranging from 0 to 2000 nM (3). In the untreated control group, the cells rapidly grew, reaching 2.4 times the initial quantity in one day. With a treatment of 400 nM, a difference in cell numbers began to appear between the experimental group and the control, and at 1600 nM, the numbers of cells was almost the same as the initial value. Further analysis of the mechanism behind the anti-proliferative property of celastrol revealed that celastrol's mechanism is dose-dependent. For dosages lower than 800 nM, "the total number of cells (living and dead) decreased, but dead cell numbers remained constant" and "with doses of 800 nM and higher, dead cells increased as dose increased" (Peng et al. 2). Based on these observations, Peng et al. concluded that at low concentrations, celastrol disrupted cell division, and at higher concentrations, celastrol also induced apoptosis, evident by the increasing number of dead cells. Peng et al. performed flow cytometry analysis and Western blotting analysis and found that the disruption of cell division was caused by the reduction in the levels of, cyclin dependent kinase 2 (CDK2), CDK4, and CDK6, thus arresting the U937 cells in G0/G1. Cyclin D1 was also down-regulated by celastrol, but it was shown to have no effect on the cell cycle arrest (2).

When comparing these anti-cancer effects of celastrol to those of triptolide, the results are the same: at low dosages, both triptolide and celastrol arrest cell cycle progression, and at high

dosages, they induce apoptosis. The difference in their chemical structure, however, changes the pathway that the two compounds accomplish these results. This variability proves advantageous and supports the implementation of an herbal treatment, containing both terpenoids with lower toxicity, as the variability will allow the herbal treatment to interfere in a diverse selection of biochemical pathways, making it more difficult for the tumors to develop a resistance to the treatment.

Like triptolide, celastrol is also capable of inducing apoptosis in tumor cells, although this aspect of celastrol's anti-cancer effect is less studied. Pang et al. noticed that in their in vivo study using a prostate cancer (PC-3) xenograft, "growth was strongly suppressed by [c]elastrol administration, suggesting [c]elastrol also has direct cytotoxic effects on cancer cells besides its anti-angiogenic effects seen on endothelial cells" (1956). Pang et al. proceeded to examine these other cytotoxic effects by performing a cell viability assay. The results showed that cells that were treated with celastrol exhibited a significant decrease in cell vitality. Pang et al. also found evidence of tumor cell apoptosis via the detection of full-length PARP1 and its large cleavage fragment (1956).

Celastrol also inhibits angiogenesis, thus preventing metastasis. The study by He et al., mentioned in the previous section, regarding anti-angiogenic activities of terpenoids also revealed that celastrol is capable of preventing angiogenesis. He et al. reported that celastrol "reduced vessel formation by more than 30% at 0.62 μM but killed 50% of the [zebrafish] embryos at higher concentrations" ("Antiangiogenic Activity of *Tripterygium wilfordii* and its Terpenoids" 66). He et al. concluded from this result that celastrol was less potent and less specific than triptolide ("Antiangiogenic Activity of *Tripterygium wilfordii* and its Terpenoids" 66).

In other studies of celastrol's anti-angiogenic effects, Sethi et al. and Pang et al. found that celastrol's ability to inhibit tumor angiogenesis is related to the down-regulation of VEGF. Sethi et al. found that "as indicated by electrophoretic mobility shift assay (EMSA), celastrol suppressed TNF-induced NF-κB activation in a dose-dependent manner" (2730). NF-κB is a transcription factor that regulates the activities of genes involved in cell proliferation, survival, angiogenesis, and invasion, and it regulates the expression of VEGF. Further experimentation by Sethi et al. revealed that the inhibition of NF-κB by celastrol is not cell-specific, as celastrol exhibited the same results in lung adenocarcinoma (H1299) cell lines and embryonic kidney (A293) cell lines. Sethi et al. also discovered that celastrol inhibits the constitutive activation of NF-κB in multiple myeloma (U266) and bladder cancer (253JBV) cell lines, which further demonstrates the lack of cell-specificity (2731) — an appealing characteristic of celastrol.

Similarly, Pang et al. found that celastrol inhibits VEGFs, which are also responsible for angiogenesis, in three different types of studies: in vitro, ex vivo, and in vivo. In the in vitro study, Pang et al. found that "treatments with 1 or 2 μmol/L [c]elastrol abolished the VEGF-induced tubule formation of HUVECs" (1955). In addition, Pang et al. witnessed that HUVECs treated with 1 μmol/L showed a significant decrease in VEGF-induced growth. Pang et al. concluded that "[c]elastrol could block VEGF-induced angiogenesis in vitro by inhibiting cell motility, cell proliferation, and endothelial tubular structure formation" (1955). For the ex vivo, Pang et al. examined the sprouting of vessels in aortic rings and found that "[c]elastrol antagonized the VEGF-induced sprouting in a dose-dependent manner, and 2 μmol/L [c] elastrol completely blocked microvessel sprouting" (1955) — further evidence that celastrol modulates VEGF and inhibit angiogenesis. For the in vivo study, Pang et al. found that Matrigel plugs, which appears dark with high concentration of RBC, became much lighter in color in the celastrol-treated group. Pang et al. used hematoxylin and eosin (H&E) staining and discovered that "[c]elastrol at a dose of 10 μg dramatically blocked VEGF-induced vasculature formation" (1956). The results from the study done by Pang et al. provided reasonable evidence

to conclude that celastrol does inhibit angiogenesis, and because the study contained an in vivo component using white mice, the results can be generalized to say that celastrol will have similar results in humans. Anti-angiogenic agents are now popular with the pharmaceutical field since preventing angiogenesis would mean stopping tumor growth and metastasis, making celastrol a fairly appealing candidate for future anti-cancer drug.

Pang et al. further investigated the exact mechanism of the anti-angiogenic property of celastrol using Western blotting assays. The results of the Western blot suggested that celastrol suppressed the phosphorylation of VEGFR2 in a dose-dependent manner and that perhaps the anti-angiogenic property is partially due to inhibition of VEGFR2. Pang et al. proceeded to examine the key molecules along this pathway and found that "[c]elastrol effectively suppressed VEGF-triggered activation of the mTOR signaling cascade, including AKT, mTOR, and S6K kinases in HUVECs in a concentration-dependent manner …, suggesting that celastrol inhibit[ed] tumor angiogenesis through the mechanistic target of rapamycin (mTOR) signaling pathway" (1956).

*Synergy between Terpenoids and Current Cancer Therapy*

In addition to the individual effects of the terpenoids (triptolide and celastrol), they are capable of acting in synergy with current methods of cancer therapy (chemotherapy and ionizing radiation). In these synergistic interactions, only low dosages of terpenoids are needed to be combined with low dosages of chemotherapeutic drugs or low intensities of ionizing radiation to achieve the desirable effects. The decreased concentrations of each element of the treatment will mitigate the induced adverse effects. Currently, exploiting these synergistic interactions is the most rational method of improving cancer therapy until further research is done on the terpenoids.

In an experiment by W. Wang et al. to explore the effect of triptolide treatment in conjunction with ionizing radiation (IR) on human pancreatic cancer cells, the researchers set up four levels of the independent variable: vehicle control, triptolide, IR, and triptolide+IR. W. Wang et al. found that triptolide at 25 nM lowered cell survival rate to only 52%, and IR at 4Gy allowed for 90% of the AsPC-1 cells to survive (4893). The experimenters then decided to combine the treatment at that concentration and intensity for the combined group. The results yield that "cell vitality was reduced to 21% when IR at 4 Gy was combined with triptolide at 25 nmol/L" (4893). The experimenters continued their in vitro study, combining triptolide at concentrations of 0, 3.125, 6.25, 12, 5, and 25 nmol/L with IR at doses of 0, 2, 4, 6, and 8 Gy on AsPC-1 cells for 3 weeks. The results from this revealed that cell growth inhibition correlated very strongly with the dose of triptolide ($r=0.988$, $p<0.001$) (W. Wang et al. 4894). The combined results of this in vitro study would suggest that triptolide dramatically potentiates the effects of the IR. The inhibition ratio for the combined treatment is much greater than that of triptolide or IR alone and the sum of inhibition. In addition, cell growth inhibition was shown to be strongly correlated with the dosage of triptolide, suggesting that perhaps triptolide is the key component in this synergistic interaction. From the data present, it stands to reason that triptolide potentiates the effects of IR by sensitizing the cells to radiotherapy.

For their in vivo study, W. Wang et al. injected pancreatic cancer cells into the hind legs of nude mice. After the tumor reached a minimum size of 100 mm3 (7 days after injection), W. Wang et al. divided the mice into four different groups and gave each group a different treatment: vehicle control (phosphate buffered saline (PBS)), triptolide (0.25 mg/kg, twice a week), ionizing radiation (10 Gy), or a combination of triptolide and IR, with triptolide following IR. As expected, triptolide and IR both significantly reduced the size of the tumor ($p<0.01$), but "the combined treatment was even more effective, with four out of eight tumors decreasing in

size from 100 mm3 to impalpable over about 3 weeks" (4895). After the 38 days of treatment, W. Wang et al. sacrificed the mice and massed the individual tumors. W. Wang et al. found that the tumors in the control group were large (approximately 1.7 g). The tumors of the single modality treatment group were about 0.2 to 0.3 g, and the tumors in the combined treatment group were 0.08 g ($p<0.01$ for combined vs. single) (4896). With such drastic difference in tumor size between the single modality treatment group and the combined treatment group and a small p-value to support the data, it is reasonable to conclude that triptolide can act in synergy with IR and produce even better results than each of them separately. In addition, because mice and humans are very similar biochemically, the data obtained by W. Wang et al. could be extrapolated and generalized to apply to humans.

Not only can triptolide act in synergy with IR and increase its efficacy, it can also potentiate the therapeutic effects of chemotherapeutic drugs. Chang et al. conducted a study to investigate the synergistic effects of triptolide and doxorubicin, a currently used chemotherapeutic drug. Chang et al. treated the HT1080 fibrosarcoma cell line with a control, triptolide (5 ng/mL or 20 ng/mL), doxorubicin (100nM), or a combination of triptolide at 5 ng/mL and doxorubicin at 100 nM. Chang et al. performed a cell viability assay using the trypan blue exclusion method and found that the combination of triptolide and doxorubicin "reduced cell viability by 65%" (2222). Chang et al. also reported that "cytotoxic synergy between triptolide and doxorubicin was … observed in A549 lung cancer cells, and triptolide also enhanced cell death by carboplatin, another topoisomerase II inhibitor, in A549 and HT1080 cells" (2222-2223). A Western blotting analysis revealed that triptolide induces post-transcriptional accumulation of p53 and reduced the doxorubicin-mediated accumulation of p21. This finding led Chang et al. to conclude that the inhibition of p21 is one of the causes of the decrease in cell vitality and increase in apoptosis. Since p21, a protein that normally activated to induce cell arrest, is inhibited and the tumor suppressor p53 is up-regulated, the malignant cells are proceeding along the cell cycle and eventually die because of p53-induced apoptosis. Had the cells been arrested by p21, however, induction of apoptosis would not have been possible.

Like triptolide, celastrol also acts in synergy with current methods of cancer treatment — chemotherapy and ionizing radiation. The studies by Zhu et al. ("Synergistic Anti-cancer Activity by the Combination of TRAIL/APO-2L and Celastrol"; "Up-regulation of Death Receptor 4 and 5 by Celastrol Enhances the Anti-cancer Activity of TRAIL/Apo-2L") and Sung et al. revealed that celastrol produces a synergistic effect when used in combination with tumor necrosis factor–related apoptosis-inducing ligand (TRAIL/APO-2L), a cytokine and chemotherapeutic agent currently in clinical trials. TRAIL/APO-2L is member of the TNF family that triggers apoptosis in a variety of cancer cell types. The experiment conducted by Zhu et al. demonstrated that "the combination [of celastrol and TRAIL/APO-2L] significantly improved the anticancer activities, as revealed by the synergistic inhibitory effects of cancer cell proliferation, the sensitized execution of apoptosis, and the enhanced in vivo antitumor efficiency" ("Synergistic Anti-cancer Activity by the Combination of TRAIL/APO-2L and Celastrol" 31). When testing for the anti-proliferative properties of the combined therapy, Zhu et al. found that the combination of TRAIL/APO-2L with celastrol resulted in a significantly lower half maximal inhibitory concentration (IC50 ) value for TRAIL/APO-2L than when TRAIL/APO-2L was used alone ("Synergistic Anti-cancer Activity by the Combination of TRAIL/APO-2L and Celastrol" 26).

To study the apoptotic effects of celastrol and TRAIL/APO-2L, Zhu et al. used flow cytometry and a mitochondrial membrane potential probe (JC-1). Zhu et al. found that the combination of TRAIL/APO-2L and celastrol in the OVCAR-8 cell line and the SW620 cell line caused a higher proportion of mitochondrial membrane depolarization, which activates

caspases to induce apoptosis, than either TRAIL/APO-2L or celastrol alone. Zhu et al. also used Western blotting analysis to investigate the effects of the combination treatment on the PARP and pro-caspases, and they found the combination treatment induced significantly higher cleavage of PARP and pro-caspase-3 ("Synergistic Anti-cancer Activity by the Combination of TRAIL/APO-2L and Celastrol" 26). The results collectively demonstrate that "celastrol sensitized TRIAL/APO-2L in activating caspase cascade and triggering apoptosis via death receptors and mitochondrial pathways" ("Synergistic Anti-cancer Activity by the Combination of TRAIL/APO-2L and Celastrol" 27).

The experiment performed by Sung et al. sought to validate the hypothesis that celastrol can modulate TRAIL-induced apoptosis and yielded similar results. The presence of celastrol enhanced TRAIL-induced apoptosis by sensitizing the breast cell lines MDA-MB-231, MCF7, and T57D to TRAIL. Sung et al. found that MDA-MB-231 was the most sensitive to TRAIL and that T47D cells were most resistant against TRAIL, and when the cells were treated with the combination treatment, the rate of apoptosis increased from 9% to 51% for MDA-MB-231 and from 8% to 38% for the cell line, T47D (11500-11501). Sung et al. also noted that "the combination of the two was highly effective in activation of all caspases and consequent PARP cleavage" after a Western blotting analysis (11501).

In addition, Sung et al. also found that celastrol down-regulates the expression of anti-apoptotic proteins related to TRAIL resistance and up-regulates the expression of Bax with the Western blotting analysis. After treating the MDA-MB-231 cells with varying concentrations of celastrol for 24 hours, Sung et al. found that celastrol down-regulated the expression of fas-associated protein with death domain-like IL-1β-converting enzyme-inhibitory protein (cFLIP), B-cell lymphoma 2 (Bcl-2), B-cell lymphoma-extra large (Bcl-xL), cellular inhibitor of apoptosis 1 (cIAP-1) , X-linked inhibitor of apoptosis protein (XIAP), and survivin in a time-dependent manner. The down-modulation of survivin and XIAP was less noticeable, but the down-modulation of cFLIP was very apparent in a dose-dependent manner. Sung et al. also found that celastrol up-regulated Bax in a time- and dose-dependent manner (11501). These findings elucidate one of the mechanisms by which celastrol induces apoptosis.

Zhu et al. also conducted an in vivo study for their experiment by injecting 95-D xenografts into nude mice. The researchers found that combination treatment decreased tumor growth by 67.0%, which is significantly higher than the inhibition of TRAIL/APO-2L at a dosage of 10 mg/kg every 2 days alone (41.0%) and celastrol at a dosage of 2 mg/kg every 2 days alone (39.4%) ("Synergistic Anti-cancer Activity by the Combination of TRAIL/APO-2L and Celastrol" 28). In another study later that year, Zhu et al. found that, on SW620 xenografts in nude mice, the combination treatment had an inhibition rate of 64.5%, while TRAIL/APO-2L had an inhibition rate of 26.3% and celastrol had an inhibition rate of 37.9% ("Up-regulation of Death Receptor 4 and 5 by Celastrol Enhances the Anti-cancer Activity of TRAIL/Apo-2L" 158). With the results of both in vitro and in vivo studies pointing to a significantly higher tumor growth inhibition and increased occurrence of apoptosis when using a combination of celastrol and TRAIL/APO-2L, it is reasonable to conclude that celastrol does indeed potentiate the effects of the chemotherapeutic agent TRAIL/APO-2L. With the increased efficacy demonstrated by the combined treatment, the dosages of the individual components would not have to be high, and thus adverse effects can be reduced.

As mentioned, celastrol is also capable of potentiating radiotherapy. In a study conducted by Dai et al., it was found that when PC-3 prostate cancer cells were treated with 0.4 μM of celastrol, the cells' sensitivity to ionizing radiation increased significantly ($p<.01$ at 2 Gy and $p<.001$ at 4 and 6 Gy) (1219). Dai et al. reported that "[c]elastrol achieved an enhancement ratio (ER) of 1.18 ± 0.02 and 1.38 ± 0.06 at a concentration of 0.2 and 0.4 μM, respectively"

(1219). Dai et al. also investigated the optimal schedule of administering celastrol and IR. They found that "pretreatment of celastrol for 1 h followed by irradiation … achieved a substantially greater ER (1.42 ± 0.03)" (Dai et al. 1219). Regrettably, Dai et al. did not perform any test of significance for this piece of data, and thus it is difficult to conclude whether there truly was a "substantial" difference between the various treatment schedules described.

However, based on this data, Dai et al. used this treatment schedule for the following in vivo study using PC-3 xenograft tumor model in mice. Dai et al. found that "the combination of celastrol with IR significantly suppressed tumor growth compared with IR alone ($p<.01$)" and "[b]y the last day, the median tumor volume after combination treatment had resulted in 40% tumor regression compared to IR alone ($p<.001$) and 80% tumor regression compared with untreated control" (1221). The combination of celastrol with IR also increased the tumor doubling time from 5.5 days in the control tumors to 6.0 days in the IR tumors and to 25 days in the tumors with combination treatment (Dai et al. 1221). Dai et al. observed that the mice suffered only a modest reversible weight loss as a result of the treatment (1221). The weight loss experienced by the mice in this study is minimal when compared to the full adverse effects of using radiotherapy, which include extraneous damage to surrounding tissues and organs (especially fast-growing cells), hair loss, and lowered immunity. By exploiting the synergy between celastrol and IR, cancer therapy can be improved very quickly to become more effective while minimizing the side-effects. Clinical trials on human subjects must be conducted before implementation, but if the results from this study by Dai et al. are accurate, then there is great promise for improving cancer therapy.

*Toxicity and Adverse Effects of T. wilfordii and Terpenoids*

One major obstacle in the medicinal usage of *T. wilfordii* and its terpenoids is their toxicity. Like with all other medications, in addition to the desired therapeutic effects, triptolide and celastrol also induce adverse effects, including reproductive damage, nausea, diarrhea, and other gastrointestinal abnormities. However, while triptolide and celastrol are known to possess strong toxicity capable of causing permanent damage, extracts of *T. wilfordii* have not been shown to possess significant toxicity.

Li et al.'s reported in a review on pharmacology and toxicology of triptolide that:

> According to data from the Chinese Food and Drug Administration (CFDA) (2004-2011.9), commercial preparations of Triptergygium wilfordii are responsible for 633 adverse reaction cases, including 53 severe cases, that involved reproductive toxicity, hepatotoxicity, and renal cytotoxicity, among other outcomes. Moreover, 266 clinical observations and follow up reports concluded that *Tripterygium wilfordii* caused a number of adverse effects, such as the damage to the digestive system (including liver injury and stomachache) and the endocrine and reproductive system. Additionally, 271 patients with rheumatoid arthritis reported side effects of *Tripterygium wilfordii*, namely digestive tract symptoms and irregular menstruation. (74)

At first glance, this report from the CFDA is a strong piece of evidence against the usage of extracts of *T. wilfordii*, but it should be noted that Li et al. did not mention what types of extracts were used. This lack of information creates a large fallacy in the argument that extracts of *T. wilfordii* are toxic. Depending on the chemical composition of the extracts, which is something that can be controlled through the usage of different solvents to produce the extract, the toxicity can easily be changed. Furthermore, the placebo effect must also be taken into account, which can lead to somatization of an imagined side-effect. In addition, Li et al. mentioned afterward that these adverse effects were mostly triptolide-induced toxicity.

Contrary to the reporting of the CFDA, a double-blind, placebo-controlled study by Tao

et al. using an ethanol/ethyl acetate extract of *T. wilfordii*, which is known to contain the strongest bioactive compounds (refer to He et al., "Antiangiogenic Activity of *Tripterygium wilfordii* and its Terpenoids"), to treat rheumatoid arthritis reported that the treatment "was well tolerated by most patients in this study" (1735). Tao et al. reported that "the frequencies of patients who developed ≥ 1 adverse event were 4 of 12, 6 of 12, and 5 of 11, respectively, for the placebo, low-dose extract, and high-dose extract groups" (1741). Although the data appear to show that patients with treated with extract from *T. wilfordii* develop side-effects, when subjected to a chi-square test of homogeneity, the calculated $\chi 2$ value was 0.725 with a p-value of 0.696, which is greater than any reasonable level of significance selected; thus, it is not reasonable to conclude that the adverse effects are caused by the ethanol/ethyl acetate extract any more than the placebo. Tao et al. also noted "[m]any of the side effects noted in patients treated with placebo as well as in those treated with extract, suggesting that the side effects may not be specifically associated with administration of the drug," which further supports that the ethanol/ethyl acetate extract of *T. wilfordii* does not necessarily induce adverse effects (1742).

However, there is one side effect of *T. wilfordii* that is known to exist: reproductive toxicity. According to a review by Qian et al., *T. wilfordii*'s anti-fertility effect on males was first documented in 1986. Qian et al. wrote that "in nine rheumatoid arthritis patients treated with the decoction of *Tripterygium wilfordii* and glycosides of T. wilfordi (GTW) for a total period of 2-56 months, necrospermia or azoospermia occurred" (121). The extract used to treat these patients is a "multi-glycosides" extract of *T. wilfordii*, hence GTW. The report of the reproductive adverse effects of GTW sparked great interest as a potential contraceptive, and researchers began to study the anti-fertility effects of GTW on male Wistar and Sprague Dawley (SD) rats, both done by Qian et al. in 1986 and 1987, respectively. Qian et al. found that:

When dosed with 10 mg/kg.d of GTW, 6 times a week, [the rats] became infertile after 8 weeks of dosing. There was a sharp decrease in the epididymal sperm motility and a moderate decrease in the sperm concentration. No apparent toxicity was seen and a full recovery of fertility was observed 4-5 weeks after cessation of treatment. (122)

Another study by Qian et al. in 1989 reported similar reversible results in the semen of male rheumatoid arthritis patients (Qian et al. 122).

Because triptolide is the main bioactive component of all the different extracts of *T. wilfordii*, including GTW (Chen F. et al. 2696), several studies on the adverse effects of triptolide have been conducted over the years. One of the more researched side effects is reproductive toxicity. Liu et al. conducted an experiment to examine the antifertility effects of triptolide on female SD rats, which mirrored the study done by Qian et al. in 1986. Liu et al. found that the toxic effects began to show starting at a concentration of 200 μg/kg. The 200- and 400- μg/kg group exhibited loss in body weight and in relative size of the ovary and the uterus. Liu et al. also performed a serum steroid and gonadotropin level analysis and found that "doses of 200- and 400 μg/kg of triptolide significantly increased both FSH and LH levels in serum, compared to their respective vehicle control" (3). One other effect that Liu et al. found in the rats was the increase in the duration of the metestrous and diestrous phases and the decrease in duration of the proestrous and estrous phases. Overall, Liu et al. found that these effects were absent in the 100 μg/kg group, suggesting that triptolide does not induce any noticeable effects at low concentrations. The collective results from the studies by Qian et al. and Liu et al. suggests that triptolide induces adverse effects on both the male and female reproductive system.

Strangely, although celastrol also demonstrates strong therapeutic potential for cancer and for a multitude of other diseases, such as rheumatoid arthritis and Alzheimer's, not many studies on the toxicity and adverse effects of celastrol have been done. The only explicit mentioning of the adverse effects of celastrol is its detrimental effects on zebrafish (Danio rerio). He et al. was

the first to note that celastrol at concentrations higher than 0.62 μM killed 50% of the embryos in the test group ("Antiangiogenic Activity of *Tripterygium wilfordii* and its Terpenoids" 66). These results were then iterated in a study by S. Wang et al. in 2010 investigating the toxicity of celastrol on the development of zebrafish. S. Wang et al. found that "embryos treated with 0.5-μM or higher concentrations of celastrol … displayed several developmental abnormalities, including no blood flow, edema in the pericardial sac, and tail malformation. Embryos exposed to 1.0 μM of celastrol had no blood flow in trunk vessels at 48 [hours past fertilization]" (62). The lack of blood flow is evident of the potent anti-angiogenic effect of celastrol, but the other side effects demonstrate that celastrol lacks the target specificity that is required of a drug. Its wide-acting nature, in this case, proves detrimental to the test subjects. However, when taken into account that test subjects were developing embryos, the weight placed on these side effects can be lessened. If celastrol is developed into a drug with the intended recipient being an adult human who has finished growing, then the abnormalities induced in developing embryos may be no longer applicable. Of course, that is not to say that celastrol does not possess any other adverse effects; they are, as of right now, undetermined.

*Proposed Herbal Treatment using the Roots of T. wilfordii*

Compared to the individual compounds found in *T. wilfordii*, the plant root itself, where the compounds are extracted, does not have such severe toxicity. Although the plant is originally used for anti-inflammatory purposes, *T. wilfordii* may also exhibit anti-cancer properties, since its components have anti-cancer properties. The effectiveness of using the whole plant may not be as great as the individual components; however, the toxicity can be mitigated. This hypothesis could be tested through an experiment, consisting of an in vitro study on different cancer cell lines and an in vivo study using rats as the animal model.

First, the herbal extract must be prepared following the procedure by He et al. ("Antiangiogenic Activity of *Tripterygium wilfordii* and its Terpenoids"). Four kilograms of the debarked root of *T. wilfordii* will be obtained. The root will be cut into smaller pieces and placed in a solution of 95% ethanol. The extraction will be performed three times with each lasting two hours. The ethanol liquid extract will then be evaporated in vacuo, and the mass of the remaining solute will be measured (62). The ethanol extract will be dissolved again in water at concentrations of 8 μg/mL, 16 μg/mL, 39 μg/mL, 78.5 μg/mL, 196 μg/mL, 392 μg/mL, 588 μg/mL, and 785 μg/mL. These concentrations were calculated based on the concentrations used in the studies by Yang et al., He et al. ("Triptolide Functions as a Potent Angiogenesis Inhibitor"), and Kiviharju et al. and the percent yield of triptolide and celastrol from an ethanol extraction indicated by He et al. ("Antiangiogenic Activity of *Tripterygium wilfordii* and its Terpenoids"). The listed concentrations above in addition to the control (0 μg/mL) will be used be on different cancer cell lines in vitro.

Five different types of cancers (see Table 2) were selected to be used as subjects for this experiment. All five of these cancers have been previously used in research on triptolide and celastrol, and they are listed as the common types of cancers to cause death in the U.S. (Center for Disease and Control and Prevention, " Cancer Statistics by Cancer Types").

The techniques to be used to analyze the results include a cell vitality assay, cell prolif-

| Table 2. Cancer Cell Lines for Herbal Treatment Experiment | |
|---|---|
| Breast cancer | MDA-345 (Yang et al.)<br>MDA-MB-231(Sung et al.)<br>MCF-7 (Sung et al.)<br>T47D (Sung et al.) |
| Colorectal cancer | SW620 (Zhu et al., "Up-regulation of Death Receptor 4 and 5 by Celastrol Enhances the Anti-cancer Activity of TRAIL/Apo-2L")<br>HT29 (Liu et al.; Wang, Z. et al.)<br>SW480 (Liu et al.; Wang, Z. et al.)<br>Caco 2 (Wang Z. et al.) |
| Lung cancer | A549 (Chang et al.) |
| Ovarian cancer | OVCAR-8 (Zhu et al., "Up-regulation of Death Receptor 4 and 5 by Celastrol Enhances the Anti-cancer Activity of TRAIL/Apo-2L") |
| Prostate cancer | AsP3-1 (Wang W. et al)<br>PC-3 (Dai et al.) |

eration assay, apoptosis assay, cell progression analysis, a Matrigel plug assay to assess the anti-angiogenic property, and Western blotting (immunoblotting) to analyze the activities of the different proteins involved in apoptosis, such as p53, p21, and PARP. The cell vitality assay will be performed using the trypan blue exclusion method. Cells will be incubated in 2% trypan blue solution. Afterward, the cells will be counted using a hemocytometer. The number of cells that retain the dye are recorded — these are the nonviable cells — and the total number of cells are also recorded (Kiviharju et al.; Chang et al.). The cell proliferation assay will be performed using an MTT assay like Zhu et al. described ("Synergistic Anti-cancer Activity by the Combination of TRAIL/APO-2L and Celastrol"; "Up-regulation of Death Receptor 4 and 5 by Celastrol Enhances the Anti-cancer Activity of TRAIL/Apo-2L"). The cells will be incubated in the dye, MTT and then dissolved in dimethyl sulfoxide (DMSO) for 4 hours. Afterward, they are analyzed using a plate spectrophotometer at 570 nm (Zhu et al., "Synergistic Anti-cancer Activity by the Combination of TRAIL/APO-2L and Celastrol"; Zhu et al., "Up-regulation of Death Receptor 4 and 5 by Celastrol Enhances the Anti-cancer Activity of TRAIL/Apo-2L"). The apoptosis assay will be performed using annexin V/ propidium iodide (PI) staining and FACS analysis (Chang et al.; He et al., "Triptolide Functions as a Potent Angiogenesis Inhibitor"; Zhu et al., "Synergistic Anti-cancer Activity by the Combination of TRAIL/APO-2L and Celastrol"; Zhu et al., "Up-regulation of Death Receptor 4 and 5 by Celastrol Enhances the Anti-cancer Activity of TRAIL/Apo-2L"). Cell cycle progression is analyzed by counting the number of cells in each phase of the cell cycle by flow cytometry (Peng et al.).

For the in vivo study, the model organism to be used is the BALB/C strain of laboratory mice, a common model system when testing human pharmaceutical products. The selected mice will be of the same age and vitality and will be split evenly between male and female. Then, the mice will then be stratified into males and females and will be randomly assigned to cancer groups. Within each group, they will be randomly designated a dosage of treatment (control, 78.5 mg/kg/day, 392.5 mg/kg/day, 785 mg/kg/day, 1177.5 mg/kg/day, 1570 mg/kg/day, 1962.5 mg/kg/day, 3925 mg/kg/day, 5887.5 mg/kg/day). These dosages were calculated

based on the dosages used by Yang et al. and He et al. ("Triptolide Functions as a Potent Angiogenesis Inhibitor") and the percent yield of triptolide and celastrol from a 95% ethanol extract by He et al. ("Antiangiogenic Activity of *Tripterygium wilfordii* and its Terpenoids").

The effectiveness of the drug will be determined by the mass and the volume of the tumor throughout the study. A toxicity study will be done simultaneously. Weight loss and other visible physical changes will be noted. Blood tests will be performed to analyze fluctuations in hormones, especially gonadotropins, and the presence of toxic byproducts from the metabolism of the *T. wilfordii* extract. Subjects will be taken out to humane end points and will be dissected and fully studied to determine whether the cause of advanced disease is related to drug administration. Surviving test subjects will be euthanized at the end of the study to record damage to the internal organs, especially the reproductive organs.

*Conclusion*

Cancer is a dangerous disease caused by a dysfunction of cell signaling regulation, leading to more deaths than violent crimes in the U.S. Current methods of treating cancer are chemotherapy and ionizing radiation, and although they are effective, the induced side-effects are severely damaging. In addition, with cancer cells growing more resistant to these forms of treatment, higher dosages and stronger drugs are being developed, leading to even greater adverse effects and toxicity. One approach taken to rectify this problem is to look for alternative treatment methods in Eastern medicine.

The herbal alternative proposed was the roots of *Tripterygium wilfordii*, known in China as lei gong teng (雷公藤) which translates to "thunder god vine." *T. wilfordii* has been used in Chinese medicine for fevers, chills, and auto-immune inflammatory diseases. Clinical trials are being conducted in China to develop extracts and bioactive compounds of the relatively non-toxic root of this plant into medications for rheumatoid arthritis. In recent years, these bioactive compounds triptolide and celastrol have also demonstrated strong anti-cancer effects, but they can induce detrimental side effects. One of the more severe side-effects is damage to the reproductive system of males and females and decreasing spermatogenesis and size of the ovaries and the uterus (Qian et al.; Liu et al.).

Therefore, to make use of the anti-cancer effects of the plant while minimizing adverse effects and toxicity, a solution was proposed to use a crude extract of the roots as a treatment for cancer. To test the effectiveness and safety of this treatment, an experiment was designed examine these two properties in vitro and in vivo. Previously used cancer cell lines will be used in this study, and analysis of efficacy and toxicity will be done using a variety of assays as in previous studies. The in vivo study will use tumor xenografts injected into white mice, a common model organism used for the development of new drugs. Effectiveness will be measured by the mass and volume of the tumor, and toxicity will be measured by physical changes such as weight loss, fluctuations in hormones, and presence of byproducts caused by metabolism of compounds within the extract of triptolide. If the treatment is proven to be ineffective and/or too toxic, then other types of extracts of *T. wilfordii* containing triptolide and celastrol can be tested. Research could also be taken in the direction of the development of a multi-component drug containing triptolide and/or celastrol and other non-bioactive compounds within *T. wilfordii* to mitigate the side-effects.

In addition, regardless of the results of this proposed experiment, more research on the plant must be conducted to understand the mechanisms of the interaction of triptolide and celastrol with the various types of cancers. Another area of research could focus on other related plants and herbs used in Chinese herbal medicine that are related to *T. wilfordii*. There may exist another plant that exhibits anti-cancer activities that have yet to be discovered. With the current conundrum faced by Western pharmaceutical practice with the increase in side-effects,

it is crucial to investigate other forms of medicine and to integrate them into modern practice.

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