

# **Dammarane Sapogenins**

Novel Nontoxic Anti-Cancer Agents

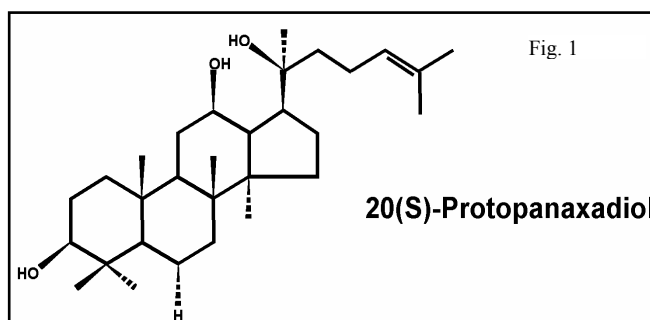
## I. Background: Dammarane Saponin Research and Development

### 1. Anticancer Activities of Dammarane Saponin

Medical research has determined that the principle pharmacological activities of Araliaceous plants are derived from their saponin content. Further research has confirmed that saponin, the metabolites of saponin, are a class of compounds that are directly responsible for these pharmacological activities. These compounds are referred to as Dammarane Saponin.

Araliaceous saponin are metabolized into Dammarane Saponin by  $\beta$ -glucosidase bacteria in the gastrointestinal tract. All Dammarane Saponin share a pharmacologically active tetracyclic triterpene backbone. Figure 1 shows the structure of protopanaxadiol, a Dammarane Saponin with recognized cytotoxic anticancer properties.

Dammarane Saponin have strong anticancer properties, and are considered non-toxic. These qualities make Dammarane Saponin ideal candidates for anticancer therapy. At low dosages, Dammarane Saponin arrest the cell division of cancer cells, and induce malignant cells to differentiate, resembling benign cell morphology. At high dosages, Dammarane Saponin can induce apoptosis in cancer cells through multiple mechanisms.



Protopanaxatriol an isolate of Dammarane Saponin, has demonstrated strong immunomodulating action. Studies show that dendritic cells treated with protopanaxatriol enhance the activation and differentiation of T cells. Many other lines of evidence support the anticancer action of Dammarane Saponin:

1. Dammarane Saponin arrest cancer cell growth and induces differentiation at low dosages.
2. Dammarane Saponin induce cancer cell apoptosis through multiple pathways at high dosages;
3. Dammarane Saponin inhibit P-gp transporters.
4. Dammarane Saponin inhibit the growth of estrogen-sensitive breast cancer by blocking estrogen receptors.

### 2. Progresses in Dammarane Saponin Research

Since the mid 1990's, a great deal of research has been focused on Dammarane Saponin. With recent advances in technology, the isolation and standardization of these active ingredients for therapeutic use is now possible. Extensive pre-clinical, and clinical studies on these isolates have already been conducted.

In 2002, pilot clinical studies on Dammarane Saponin were conducted at UCLA by Dr. Jerome Block. Considering the long history of safe traditional use of Araliaceous plants, the FDA waived its Investigation of New Drug (IND) mandate for Dammarane Saponin for these studies. The study results indicated that Dammarane Saponin were well tolerated with no adverse effects reported. In these studies, the quality of life in patients with pancreatic cancer was significant improved, with stabilization of their disease during the 8-month course of Dammarane Saponin treatment. Dr. Block's findings, along with a large body of existing evidence convinced the FDA, and the standard toxicity studies for these proposed drugs were exempted.

In February 2003, following an extensive literature and operation review at Panagin Pharmaceuticals, the National Research Council of Canada (NRC) granted financial support to Dammarane Saponin research programs at Panagin. This support has brought Dammarane Saponin research to a new level of development.

In August 2003, the federal government of Canada organized three rounds of project review meetings on Dammarane Sapogenins. The panel reviewed the progresses in Dammarane Sapogenins research and development conducted by Pegasus. Based on their promising findings, funding for further clinical research in Dammarane Sapogenins was granted through Industry Canada. This was the first federally funded clinical project of its kind awarded to R&D in Dammarane Sapogenins.

In 2006, Pegasus Pharmaceutical Group Inc. received a site license from Health Canada's Natural Health Product Directorate (NHPD), which certifies Good Manufacturing Practice. In 2007, Dammarane Sapogenin preparations were granted with Product Licenses from Health Canada NHPD. The licenses ensure that these products comply with the highest degree of quality and standardization.

Intravenous Dammarane Sapogenin formulas have been approved as provisional drugs in a major hospital, in Fujian Province, China. The preclinical studies required by Chinese SFDA have been completed, showing that intravenous Dammarane Sapogenin therapy has great potential in cancer treatment.

Currently, Panagin is actively promoting the global development of Dammarane Sapogenins through collaborations with many cancer research institutes in Canada, USA, Europe, Japan, China, HongKong, and Taiwan. Multi-centre clinical studies for the efficacy of Dammarane Sapogenins on cancer therapy are underway.

## II. The Mechanisms of Action of Dammarane Sapogenins *In Vitro*

### 1. Dammarane Sapogenins Arrest Cancer Cell Growth and Induce Differentiation at Low Dosages

All cells, whether healthy or malignant, undergo three phases before entering mitotic division (M) – the Gap-1 phase (G1), the Synthesis phase (S), and the Gap-2 phase (G2) (Figure 2). Dammarane Sapogenins can arrest the cell division at the G1-S transition phase in wild type p53 cancer cells, and at G2 phase in p53 mutated cancer cells. As a consequence, cancer cells stop dividing and tumors stop growing.

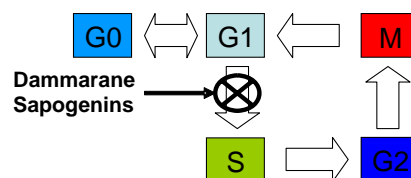
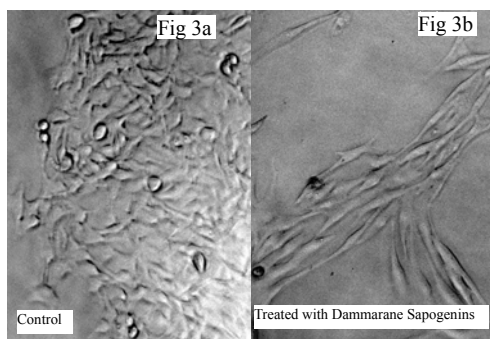


Fig. 2 Dammarane Sapogenins Cause arrest of cell division cycles

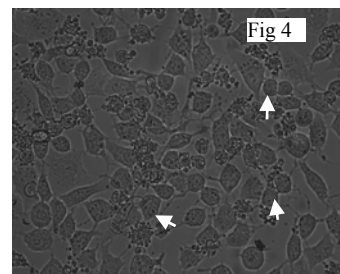


Furthermore, Dammarane Sapogenins can induce malignant cells to differentiate into cells resembling benign cell morphology. The tightly arranged, round, or irregular-shaped cells are malignant melanoma (Fig. 3a). Following a 48-hour administration of Dammarane Sapogenins, the differentiated, spindle-shaped phenotype (Fig. 3b), resemble benign melanocyte morphology.

In summary, above studies demonstrated that low dose Dammarane Sapogenins can induce cancer cell differentiation, and inhibit cancer cell division. Therefore, Dammarane Sapogenins are prospective for the use in the prevention and treatment of cancers.

### 2. Dammarane Sapogenins Induce Cancer Cell Apoptosis through Multiple Pathways at High Dosages

Apoptosis, also referred to as programmed cell death, is a distinct cellular process where cell membrane reshapes to form small spheric bodies called apoptotic membrane blebbing (Arrows in Figure 4). With the administration of Dammarane Sapogenins, the number and frequency of membrane blebs are greatly increased, indicating an accelerated apoptotic process. This pro-apoptotic capability of Dammarane Sapogenins involves a cascade of intracellular signalling pathways.



Dammarane Sapogenins activate apoptosis in cancer cells through the following 3 mechanisms (also shown Figure 5):

1. Activation of multiple Caspase pathways, leading to apoptosis in cancer cells;
2. Inhibition of Akt phosphorylation, which in turn inhibits the survival pathways in cancer cells;
3. Amplification of free radical production in cancer cells, which also trigger apoptosis in cancer cells.

The activation of any one of the above-mentioned pathways will lead to apoptosis in cancer cells. The activation of all three pathways simultaneously will initiate cancer cell apoptosis at an increased rate.

### 2.1. Dammarane Sapogenins Activate Multiple Caspases to Induce Apoptosis in Cancer Cells

Apoptosis is a physiological process that brings about natural cell death. Apoptosis can be achieved through various physiological means in cancer cells, as shown in Figure 5. These processes can prevent aberrant cells from proliferating and potentially developing into malignant tumors.

Among these different physiological processes, the Caspase Pathway is one of the most important and well documented. Caspases are a class of zymogen, pre-enzymes responsible for activating apoptosis.

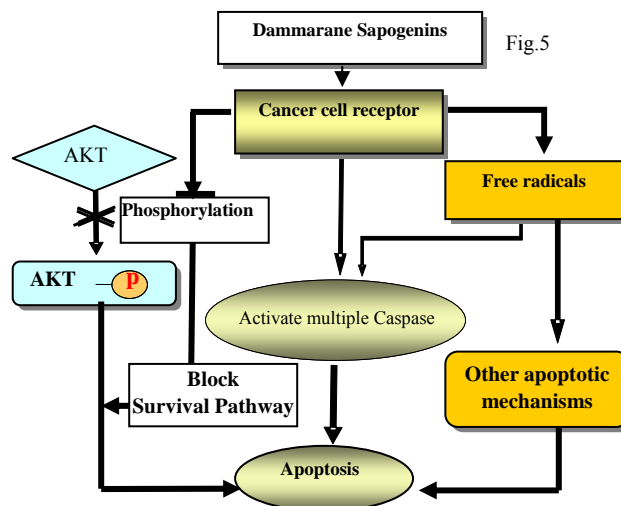
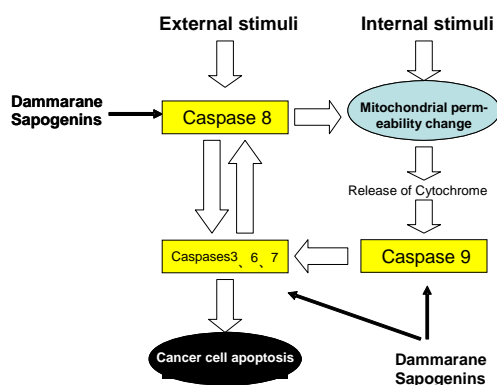


Fig. 6. Dammarane Sapogenins activate Caspases to cause apoptosis in cancer cells.



Zymogens become active enzymes through proteolytic cleavage, which results in a cascade of zymogen to enzyme activation. Caspases eliminate abnormal cells, including cancerous cells, by activating the apoptosis process through a series of biochemical processes called signal transduction.

Caspases are divided into 2 groups according to their functions: the initiator enzymes, and the executor enzymes. As shown in Figure 5, caspase 8 is an upstream initiator, transducing the death signals that activate caspases 3, 6 or 7. Caspases 3, 6, and 7 are the executors, whose actions result in the death of cancer cells through apoptosis. Cytochrome C and caspase 9 are also involved in zymogen activation. Likewise, they transduce death signals down stream to caspases 3, 6, and 7. However, genetic mutations in cancer cells interrupt these pathways, disrupting apoptosis. In result, many cancer cells survive harsh environments, such as

exposure to chemotherapeutic drugs.

Dammarane Sapogenins directly activate many caspase pathways, including caspases 8 and 9 (the initiators) and caspase 3, 6 and 7 (the executors). Directly initiating the “executor” caspases activates apoptosis without the mediation of their up stream initiators. In this way, Dammarane Sapogenins short cut apoptotic mechanisms, leading to the rapid programmed cell death of cancer cells.

### 2.2 Dammarane Sapogenins Cause Apoptosis in Cancer Cells through Other Pathways

Dammarane Sapogenins can also bring about apoptosis in certain cancer cells that cannot be activated through caspases. This indicates that Dammarane Sapogenins have apoptotic mechanisms independent of caspase pathways.

● Dammarane Sapogenins Increase Free Radicals within Cancer Cells, Leading to Apoptosis

Recent studies have discovered another mechanism for Dammarane Sapogenins mediated apoptosis. Free radicals within cancer cells are significantly increased following treatment with Dammarane Sapogenins. Most cancer cells have elevated concentrations of free radicals following 1 hour of Dammarane Sapogenins administration. Free radicals can activate various apoptotic pathways through caspases dependent and caspase independent mechanisms.

● Dammarane Sapogenins Inhibits Akt Activity, which Inhibits the Cells' Survival Pathway

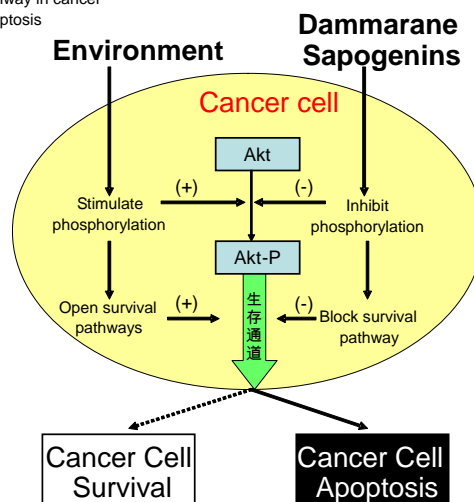
Cancer cells are constantly under attack by the body's immune system, anti-cancer drugs, etc.. Studies have found that cancer cells survive this grueling environment by keeping their survival pathway open. In normal cells this survival pathway is usually closed, and is only activated provisionally while the cell is subjected to temporary stressors. However, the survival pathway in cancer cells is kept open indefinitely, allowing them to resist immunological and pharmacological assaults. Genetic degradation in cancer cells, such as damage to the PTEN gene, can activate this cancer cell survival pathway.

Akt is a kinase that inhibits cancer cell apoptosis by opening survival multiple pathways (Figure 7). Akt is activated through the process of phosphorylation. Once activated, not only does Akt inhibits apoptosis, it also stimulates cell growth. Therefore, one way to inhibit the survival pathway is to down regulate Akt activity.

PTEN gene expression normally inhibits Akt activation, which then leads to apoptosis. However, a PTEN mutation that results in the loss of functional expression would no longer inhibit Akt activation. As a result, cellular apoptosis is disrupted.

Dr. William Jia and his team at the University of British Columbia discovered that Dammarane Sapogenins effectively inhibit the survival pathway in cancer cells lacking PTEN expression. There is evidence that the level of Akt phosphorylation is significantly reduced in cancer cells treated with Dammarane Sapogenins. Furthermore, Dammarane Sapogenins arrest Akt biosynthesis in certain cancer cells.

Fig. 7. Dammarane Sapogenins block survival pathway in cancer cells to cause apoptosis



2.3 Dammarane Sapogenins Are Effectively Inhibit Cancer Cells with Various Mutations.

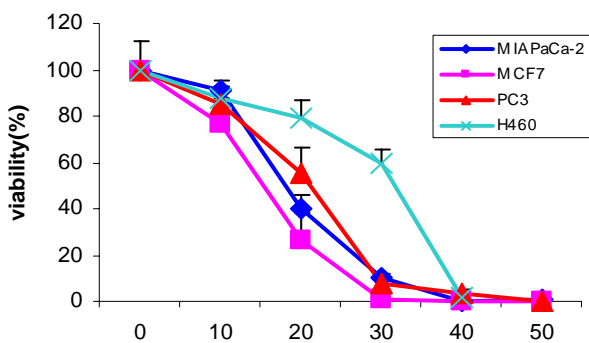


Fig. 8 concentration(ug/ml)

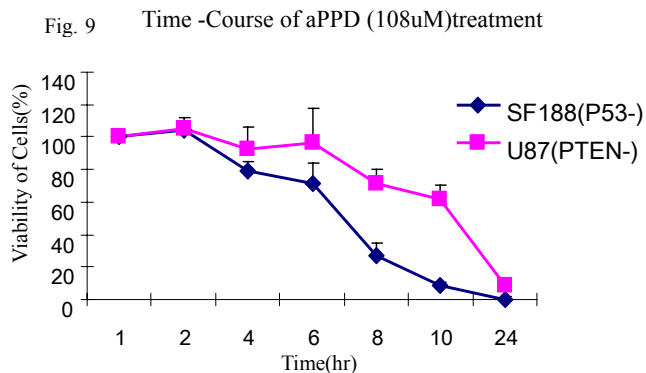


Fig. 9 Time -Course of aPPD (108uM)treatment

Dammarane Sapogenins and its analogs affect a wide spectrum of cancer cells. Dr. Jia's laboratory found that Dammarane Sapogenins exhibited anticancer activity in all cancer cell lines tested. As shown in Figure 8, Dammarane Sapogenins and its analogs exhibited cytotoxicity in a range of cancer cells, including human pancreatic cancer (MIAPaCa-2), breast cancer (MCF-7), prostate cancer (PC3), and lung cancer (H460).

In addition, Dammarane Sapogenins induce apoptosis in cancer cells with different genetic backgrounds. Figure 9 shows a typical example of the cytotoxicity of Dammarane Sapogenins in two different brain cancer cell lines, exhibiting different genetic mutations. SF188 lacks the p53 gene, while U87 has lost the PTEN gene. p53 is a tumor suppressor gene that codes for a protein involved in regulating gene expression. The normal expression of p53 arrests cellular division, which eventually results in apoptosis. However, p53 is prone to mutation. This mutation can cause normal cells to become cancerous. Furthermore, some chemotherapeutic drugs require the normal function of p53 in order to be effective. Therefore, a mutation of p53 may lead to multidrug resistance.

PTEN mutations are also common in cancer cells. As previously mentioned, PTEN regulates the survival pathway through the activation of Akt. Once the PTEN gene is lost, cancer cells can become drug resistant through the activation of Akt. Dammarane Sapogenins exhibit anticancer effects regardless of whether the normal functions of the p53 or PTEN genes have been compromised.

### 3. Dammarane Sapogenins Inhibit P-gp

Polyglycoprotein (P-gp) is a transporter protein located on cell membranes. It functions by pumping toxic substances, including anticancer drugs, out of the cell. In this way, P-gp protects cells by maintaining low intracellular concentration of cytotoxic drugs. The over expression of P-gp on the cancer cell membrane is one of the principal mechanisms for multi-drug resistance (MDR).

Dr. William Jia's laboratory at UBC studied the effects of Dammarane Sapogenins on P-gp. These studies indicate Dammarane Sapogenins inhibit P-gp, thereby increasing the concentration of anticancer drugs within cancer cells.

Figure 10 shows two photographs of human MDR breast cancer. In order to test P-gp regulation, cancer cells were incubated with a fluorescent dye. Once inside the cell, this dye emits fluorescent light. Figure 10a shows the control assay, cancer cells that have not been treated with ammarane Sapogenins. Here, little fluorescent light can be seen because P-gp is highly active, pumping the fluorescent dye out of the cell. Figure 10b shows the test assay, cancer cells that have been treated with Dammarane Sapogenins for 15 minutes. The test assay clearly shows the accumulation of fluorescent dye within the cancer cells, due to the blockage of their P-gp. This study indicates Dammarane

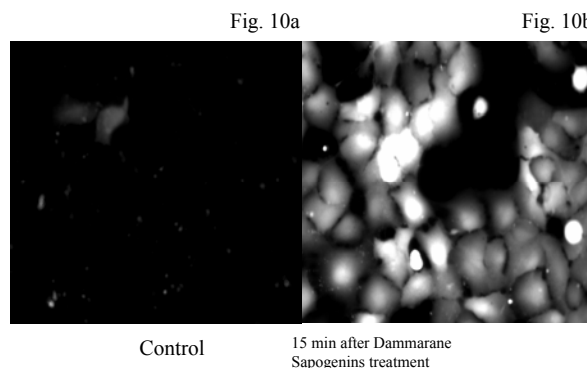


Fig. 10a

Fig. 10b

Control

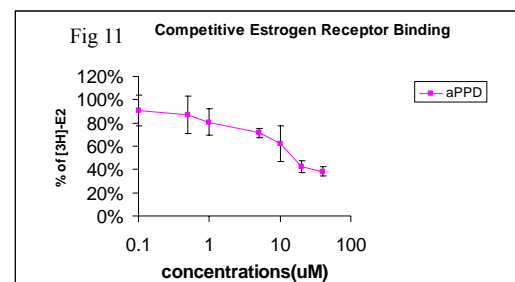
15 min after Dammarane  
Sapogenins treatment

Fig 11 Competitive Estrogen Receptor Binding

### 4. Dammarane Sapogenins Inhibit the Growth of Estrogen-Sensitive Breast Cancer by Blocking Estrogen Receptors

Studies at Dr. William Jia's laboratory at UBC revealed that Dammarane Sapogenins have an affinity for estrogen receptors (Figure 11). Though Dammarane Sapogenins are estrogen analogs, they have very weak estrogen-like actions. In this way, Dammarane Sapogenins competitively inhibit the effects of estrogen, as illustrated in Figure 12. This mechanism has clinical significance for breast cancer prevention, treatment, and lasting remission.

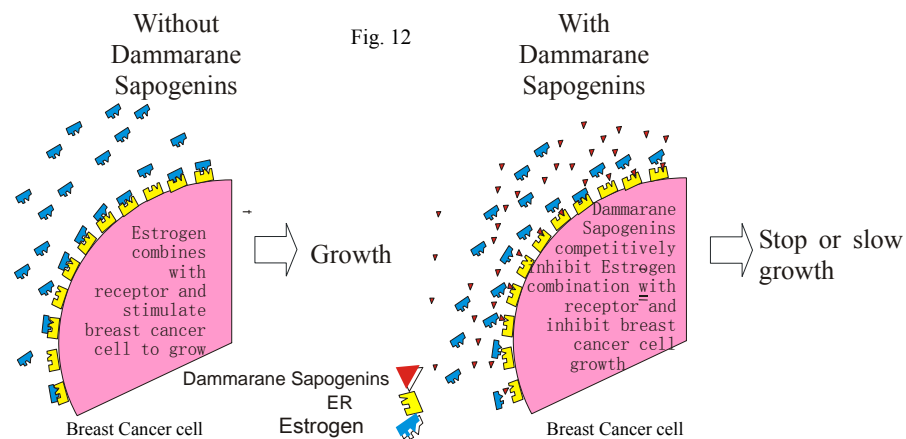


Fig. 12

### 5. Dammarane Sapogenins are Effective in Advanced and Multi-drug Resistant Cancers

The treatment of advanced cancers has yet to be adequately developed. Despite the most innovative standard medical treatment, cancers relapse in the majority cases. Most recurrent cancers are drug resistant. Advanced, multi-drug resistant cancers are difficult to control. However, Dammarane Sapogenins have demonstrated efficacy in the treatment of advanced and multidrug resistant cancers.

P388wt and P388adr are a pair of cancer cell lines with identical genetic backgrounds. The only distinction is that P388adr cells express significantly more P-gp. Therefore, P388wt is sensitive to anticancer drugs while P388adr is multi-drug resistant. Figure 16A shows the chemotherapeutic agent Doxorubicin to be effective in killing P388wt, IC<sub>50</sub> at 0.064  $\mu$ M. Meanwhile, Doxorubicin is far less effective in killing MDR cancer P388adr, with an IC<sub>50</sub> at 20  $\mu$ M, which is over three-hundred times greater than the amount required for P388wt. However, the dose-response curve of Dammarane Sapogenins is different. The dose-response is similar in both drug sensitive P388wt and MDR P388adr. The IC<sub>50</sub> for both cell lines is 15ug/ml (33  $\mu$ M) (Figure 13B). The toxicity of common chemotherapeutic agents makes it difficult to increase their dosages in order to combat MDR cancers. In contrast Dammarane Sapogenins show no significant toxicity. An analysis of its anticancer dose-response curve shows that Dammarane Sapogenins can completely kill cancer cells without toxic effects.

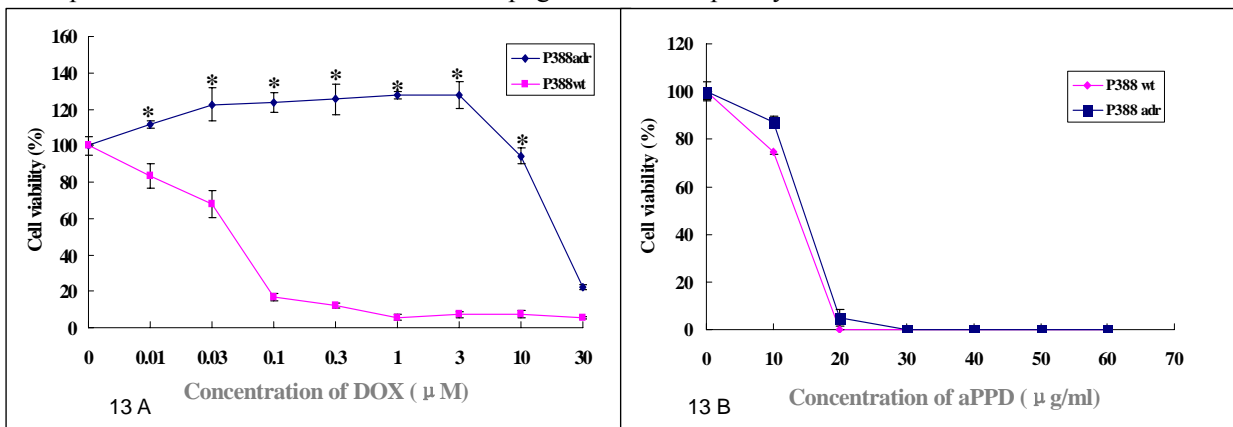


Fig. 13

A. Dose-response curve of regular chemotherapeutic agents in MDR cancer cells

B. Dose-response curve of Dammarane Sapogenins in MDR cancer cells

These results have been confirmed by the clinical application of Dammarane Sapogenins for advanced and MDR cancer patients.

### III. Toxicology Studies of Dammarane Sapogenins

## 1. Toxicology of Orally Administered Dammarane Sapogenins

- Acute Toxicity Studies

Toxicology studies are preliminary for any anticancer drug candidate. Numerous animal studies were conducted on Dammarane Sapogenins. The following is a brief summary:

In acute toxicity studies, mice were fed with Dammarane Sapogenins in oral doses up to 4,000 mg/kg body weight. Some animals exhibited closed eyes, quietness, reduced activity, and drowsiness. After 4 hours these symptoms disappeared; animals regained normal movement, and intake of food and drink. No animal died within 7 days following this course of Dammarane Sapogenins. Pathology examinations showed normal heart, liver, spleen, kidney, stomach, and intestine histology.

- Long-Term Toxicology Studies

In long-term toxicology studies, animals were given high-dose (333.3 mg/kg), medium-dose (131.0 mg/kg), and low-dose (51.5 mg/kg) Dammarane Sapogenins treatment. Dammarane Sapogenins were administered daily for 8 consecutive weeks. Animals were observed for 2 weeks following the regime. The results are summarized as the follows:

Within 24 hours of exposure, few of the animals in the high-dose and medium-dose groups had transient prostatic hypertrophy. Some animals in the high-dose group had transient ovarian hypertrophy. These enlarged organs returned to their normal size within a 2 weeks period following the regime. Routine blood and organ histology revealed no abnormalities.

- Conclusions

Acute toxicology studies demonstrated that Dammarane Sapogenins, at dosages equivalent to 550 times higher than the human dose do not cause any adverse reactions. Long-term toxicology studies demonstrated that the long-term use of Dammarane Sapogenins at dosages equivalent to 50 times higher than the human dose do not cause any changes in heart, liver, kidney and blood counts; nor did these dosages cause damage to the nervous system. No vomiting or hair loss was observed. Dammarane Sapogenins are nontoxic at the dosages used.

## 2. Toxicology of Dammarane Sapogenins in Injectable Dosage Form

- Hemolysis and Pyrogen Tests

Intravenous Dammarane Sapogenins therapy has been in clinical use. The I.V. solution used is 10 mg of Dammarane Sapogenins per milliliter of solution. At this concentration, in vitro 3-hour hemolysis test did not show any hemolysis. This is in compliance with injectable solution standards. In addition, injectable Dammarane Sapogenins met further standards by testing negative to pyrogen tests.

- Blood Vessel Irritation Test

At low dosage, intravenous Dammarane Sapogenins did not show any indication of blood vessel irritation, observed by gross and microscopic examination. High dose exhibited mild irritation of blood vessels in the ears of rabbits.

- Allergy Tests in *Cavia porcellus*

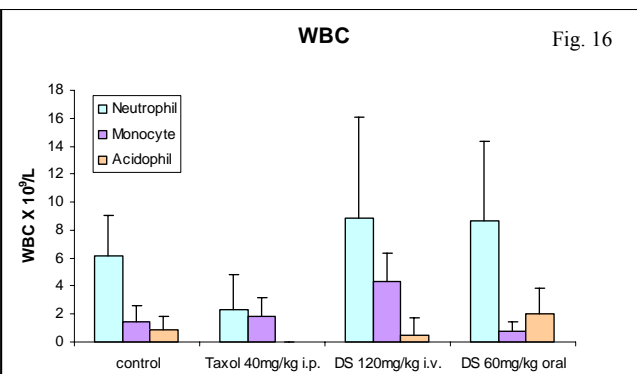
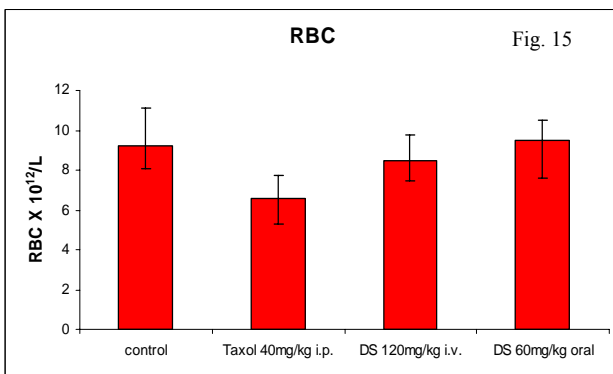
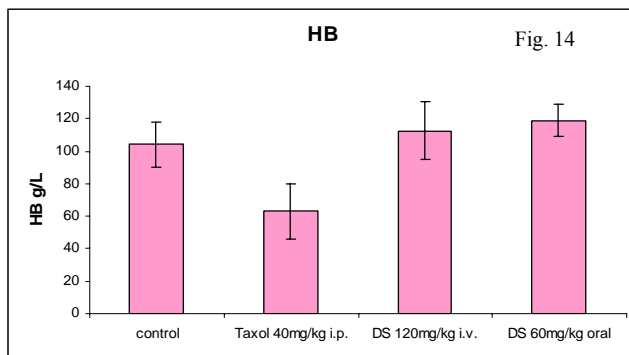
I.V. Dammarane Sapogenins were nonallergenic when tested for local and systemic reactions in *Cavia porcellus*.

● Acute Toxicity Studies of Injectable Dammarane Sapogenin

Acute toxicity studies obtained LD50's for injectable Dammarane Sapogenins. The test found that the LD50 for female mice is 511.28 mg/kg body weight; for male mice it is 494.70 mg/kg. These dosages are 15 times higher than effective clinical doses.

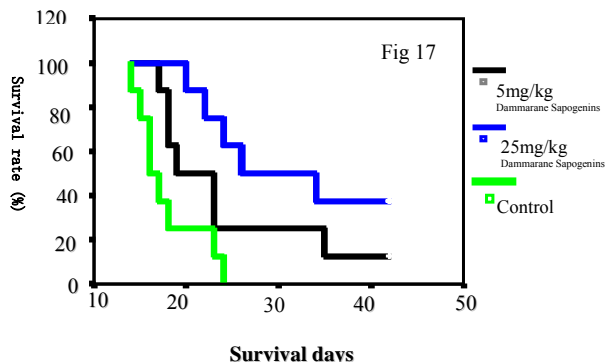
**3. Dammarane Sapogenins VS. Chemotherapeutic Drugs**

Dammarane Sapogenins preparation is considered safe in comparison with most chemotherapeutic drugs. As shown on fig. 13, 14 and 15, Hb, RBC and WBC of animals treated with Taxol all dropped in contrast with control group. However, the RBC count of animal treated with Dammarane Sapogenins was stable, while the Hb and WBC increased. Dammarane sapogenins did not result in bone marrow suppression. (Figures 14, 15, and 16.)



**IV. Pharmacological Actions of Dammarane Sapogenins *In Vivo***

**1. Dammarane Sapogenins Are Effective in Treating Brain Cancers**



Dammarane Sapogenins were studied in brain cancer animal models. Intracranial implantation of gliomas were allowed to develop to a given size before the administration of Dammarane Sapogenins treatment or placebo. The results are expressed in a Kaplan-Meier survival curve. In Figure 17, the vertical-axis

indicates the percentage of animals survived, and horizontal-axis represents the days of survival. As shown, the entire control group died within 24 days. Meanwhile, 40% of animals in the 25 mg/kg group survived for 40 days. The survival was dose dependent; that is, the survival improves if the dose is increased from 5 to 25 mg/kg.

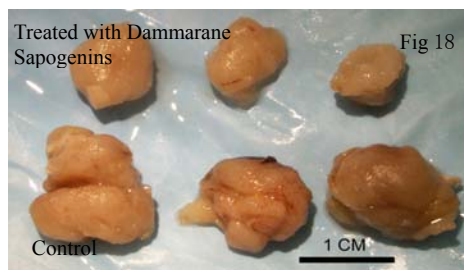
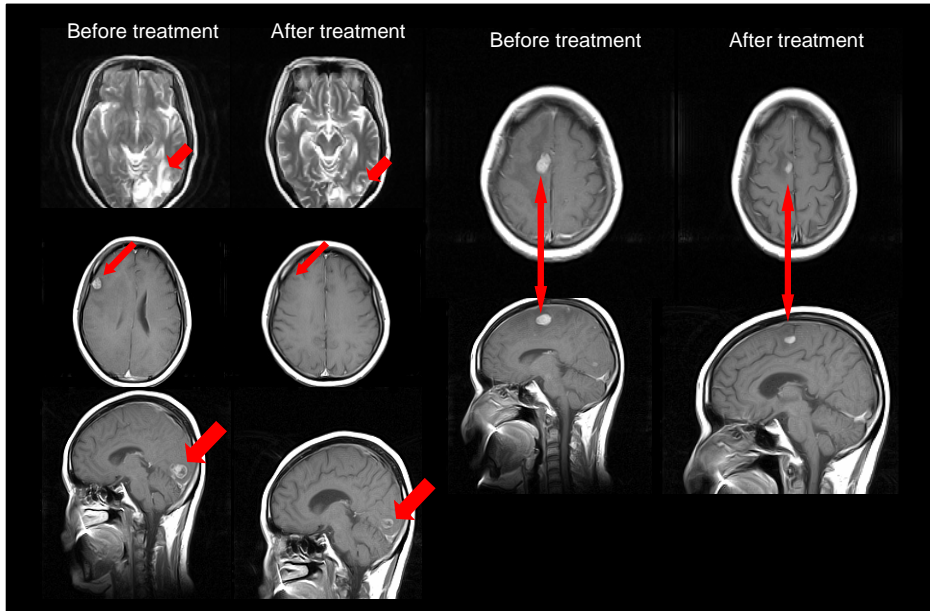


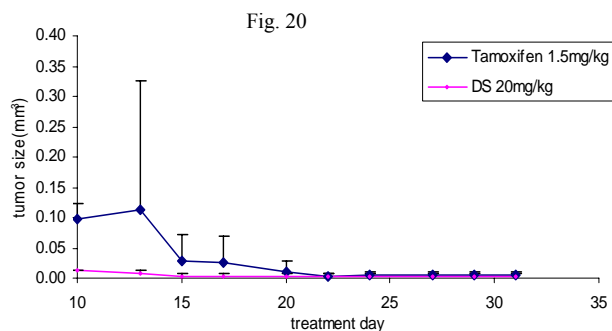
Figure 18 shows Dammarane Sapogenins administration causes a significant reduction in the size of gliomas in a subcutaneous xenograph model. Prostate and pancreatic cancer models also demonstrated similar results.

Clinical studies have also found Dammarane Sapogenins to be effective in the treatment of brain cancers, causing tumors to shrink, with reductions in perilesional edema (Figure 19).

Fig.19 Dammarane Sapogenins in the treatment of non-small cell lung cancer with brain metastases



### 3. Dammarane Sapogenins Inhibit Breast Cancer in Animal Models, and are Tamoxifen Agonists



According to the studies conducted by Dr. William Jia at UBC, oral administration of Dammarane Sapogenins is effective in the treatment and prevention of estrogen-dependent breast cancer. The studies demonstrated that Dammarane Sapogenins have competitive inhibitory effects on estrogen receptor sites, decreasing the estrogen to estrogen receptor binding. Decreased estrogen receptor binding leads to a reduction in the growth of estrogen-dependent breast cancer. In a breast cancer animal model, Dammarane Sapogenins completely arrested the growth of

estrogen-dependent breast cancer in high estrogen exposure. Furthermore, when used in combination, Dammarane Sapogenins can enhance the effects of Tamoxifen. Dammarane Sapogenins have also demonstrated the ability to inhibit the stimulatory effects of estrogen on early stage breast cancers. Tamoxifen does not possess such an effect. Based on these studies, Dammarane Sapogenins therapy has demonstrated the potential to outperform Tamoxifen in the prevention of breast cancer recurrence and metastasis.

As shown in Figure 20, estrogen positive MCF-7 breast cancer cells were implanted in SCID mice subcutaneously. In this xenograph model, Dammarane Sapogenins and Tamoxifen were used to treat the cancer. It is demonstrated that both agents can completely block the growth of breast cancer in 15 days. However, statistical analysis shows Dammarane Sapogenins have a stronger effect than Tamoxifen ( $P < 0.01$ ). It is noted from day 10 to 15 that tumor sizes in the Tamoxifen group were significantly larger than those in the Dammarane Sapogenins treatment group.

These results clearly demonstrate that not only do Dammarane Sapogenins block estrogen-induced breast cancer in high estrogen environments, they also inhibit estrogen-induced growth in early stage breast cancer. In summary, these results indicate that Dammarane Sapogenins are more effective than Tamoxifen in the post-surgical prevention of breast cancer recurrence and metastases.

#### 4. The Pharmacological Actions of Dammarane Sapogenins Intravenous Treatment

Animal studies demonstrated that intravenous administration of Dammarane Sapogenins inhibit the growth murine sarcoma S180, murine melanoma B16, murine Lewis lung cancer, and murine colon cancer colon-26 in a dose dependent manner. The inhibition rate of murine sarcoma S180 and murine melanoma was 36-55% and 32-48% respectively, following 7 consecutive daily intravenous injections of Dammarane Sapogenins at dosages of 15-60 mg/kg. The inhibition rate of murine Lewis lung cancer and murine colon cancer colon-26 was 36-54% and 31-47% respectively, following 10 consecutive daily intravenous injections of Dammarane Sapogenins at dosages ranging from 15-60 mg/kg. In the model of intestine cancer LOVO implanted in nude mice, high dose (60 mg/kg) caused a 60% inhibition rate following a course of 10 consecutive daily intravenous injections. A low dose of 15 mg/kg causes a 30% inhibition.

A dose of 10 mg/kg of intravenous administration of Dammarane Sapogenins was used in combination with adrimycin, cisplatin, and paclitaxel in the treatment of murine sarcoma S180. The results demonstrated that Dammarane Sapogenins enhanced the effects of these drugs.

Intravenous administration of Dammarane Sapogenins has demonstrated an affect on the immune systems in mice bearing Lewis lung cancer. Although low dose did not show obvious evidence of immunomodulation, higher dosages can enhance the growth of the spleen lymph cells, and increase the activation of Natural Killer cells.

In summary, pre-clinical studies demonstrate that intravenous Dammarane Sapogenins therapy has strong anticancer activities in many types of implanted and xenograph animal models. Furthermore, the results indicate a dose dependence. In addition, Dammarane Sapogenins enhance the immune function of the tumor-bearing mice.

### V. The Pharmacokinetics of Dammarane Sapogenins

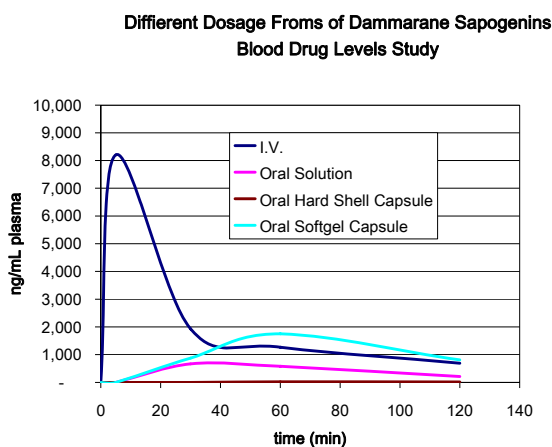
#### 1. Blood Drug Level and Time Distribution Studies

A major challenge to the development of oral Dammarane Sapogenins delivery is overcoming its low bioavailability. Lipid-soluble sapogenins alone cannot be absorbed in the gut since they aggregate and precipitate in the presence of the gastric acid. Pegasus investigated different modes of delivery for Dammarane Sapogenins in order to determine the best means to improve their.

Four different modes of delivery were formulated and their blood drug levels were compared. Blood drug levels were measured at different intervals to show time distribution characteristics. The results are demonstrated in Figure 21. As shown, hard shell capsules with Dammarane Sapogenins powder have the poorest oral absorption.

Oral solution has increased absorption. However, the soft gel capsules resulted in the best overall oral absorption. The blood level peaked between 30 and 40 minutes. This rapid absorption indicates that it is absorbed in the stomach. The blood drug level is even higher than that of I.V., between 40 minutes and 2 hours. Dammarane Sapogenins in soft gel capsule dosage form has very good bioavailability, which yields blood levels in line with our anticancer models.

Fig 21



## 2. Dammarane Sapogenins Cross the Blood Brain Barrier

Figure 21 shows the organ distribution of Dammarane Sapogenins in animals. The vertical-axis represents the index of drug distribution, while the horizontal axes represent the organs and dosages of Dammarane Sapogenins. The chart shows that Dammarane Sapogenins have high distributions in the brain and liver; in fact, the brain has the highest distribution. The distribution is positively correlated with the dose. These findings demonstrate that Dammarane Sapogenins may be useful in the treatment of malignant brain tumors, whether primary or metastatic.

## 3. A New Method of Delivery Yields Maximum Bioavailability of Dammarane Sapogenins

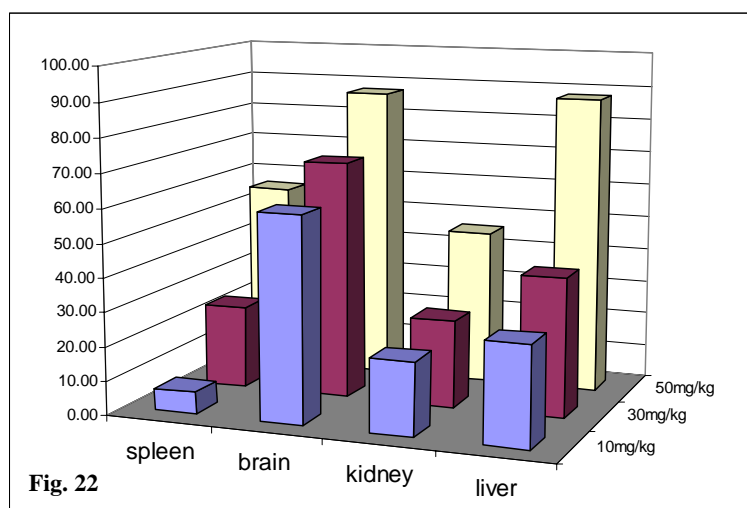


Fig. 22

The patented technology used by Pegasus increases the bioavailability of active hydrophobic botanical compounds by preventing their aggregation and precipitation. This allows the active ingredients to be readily absorbed by the stomach and intestines. Researchers at Pegasus went outside conventional methods of drug delivery, establishing an innovative mode of administration. This innovation has been made possible by Oral Bioavailability Enhancement Technology. This method of delivery allows for sufficient absorption of large dosages of Dammarane Sapogenins. The standard daily oral dose of Dammarane Sapogenins, as scaled from

our anticancer models, is 15-30mg/kg of body weight. Such a dose would not be practically achievable without Oral Bioavailability Enhancement Technology. This technology has made it possible to deliver a therapeutic dose without need of intravenous injection. Oral Bioavailability Enhancement Technology marks a monumental step forward in the clinical applications of Dammarane Sapogenins, as well as other phytopharmaceuticals.

## VI. Clinical Studies of Dammarane Sapogenins

To date, thousands of advanced cancer patients have used Dammarane Sapogenins therapy. Clinical studies at centers worldwide have demonstrated that Dammarane Sapogenins are effective in treating advanced and MDR cancer. The studies have demonstrated that Dammarane Sapogenins can effectively reduce tumor size, stabilize disease condition, improve life quality, prolong survival time, and prevent recurrence and metastases. It is especially noteworthy that Dammarane Sapogenins cross the Blood-Brain Barrier, and have been shown to treat primary and metastatic brain cancers.

### 1. Clinical Studies of Dammarane Sapogenins in the USA

Dr. Jerome Block and Steve Evans conducted a combined phase I/II clinical study at UCLA-Harbor Medical Center. They studied the effectiveness and adverse reactions to oral Dammarane Sapogenins therapy on patients with advanced pancreatic cancer and prostate cancer. In the study, patients had no adverse effects with treatment, and had significant improvement in their quality of life. During the 8-month study period, none of the patients showed any disease progression. With prostate cancer patients, the tumor marker PSA had lowered or stabilized during the study period. The principal investigator, Dr. Block was a renowned oncologist in America. He believed that Dammarane Sapogenins are novel anticancer agents that are safe and effective for the treatment of advanced cancer patients. The study results were published with the American Society of Clinical Oncology.

### 2. Clinical Studies of Dammarane Sapogenins in Canada

Dr. Emma Guns-PhD, from BC Cancer Agency, and Dr. Hal Gunn-MD and Dr. Linda Balneaves-PhD, from the Centre of Integrated Healing in Vancouver, conducted a series of single case studies of various advanced cancer patients using oral Dammarane Sapogenins therapy. The results illustrate the general effectiveness of this regimen. Furthermore, only mild side effects were documented. Slight abdominal discomfort at higher dosages was reported.

Dammarane Sapogenins therapy has been considered as the first line of treatment for advanced cancer. Dr. Jim Chan-ND has specialized in cancer treatment. He has studied over one thousand cases using Dammarane Sapogenins in cancer treatment. His studies indicate that patients who have undergone Dammarane Sapogenins therapy have shown disease stabilization, reductions in tumor size, and in some instances remittance. A study was conducted with advanced, recurrent, metastatic, and multidrug resistant (MDR) cancers. The clinical endpoints evaluated symptoms, quality of life, and overall survival. The result demonstrated that the majority of patients had improved clinical symptoms and quality of life; they also had significantly prolonged survival.

### **3. Clinical Studies of Dammarane Sapogenins in China**

A clinical study was conducted at a major hospital in Fujian, China. Intravenous Dammarane Sapogenins therapy was used alone in the treatment of advanced cancers. It was found to be effective in stabilizing the disease, and in reducing the tumor sizes with MDR cancers. During the study period, no serious adverse effects were observed. All patients tolerated the Dammarane Sapogenins treatment well. The study results were published with American Society of Clinical Oncology.

In 2005 and 2006, another clinical study was conducted in a major hospital in Yichun. High doses Dammarane Sapogenins I.V.(5000mg ~ 7000mg per day) were used in the treatment of advanced cancers. The overall response rate (including complete and partial responses, and stable disease) for primary lung tumor lesions was over 80-percent. Furthermore, 90-percent of patients saw an increase in their WBC count, which demonstrates bone marrow function restoration from damage as a result of conventional chemotherapy. The study also demonstrated that Dammarane Sapogenins was well tolerated by patients, with no severe adverse effects. Dammarane Sapogenins was safe and effective.

### **4. Clinical studies of Dammarane Sapogenins in Japan**

In 2005, a study was conducted in Tokyo Japan to determine the clinical safety of Dammarane Sapogenins. The result showed that Dammarane Sapogenins enhanced humoral immune function. Average values of IgE and IL-6 were decreased. Only light gastrointestinal discomfort was evident in some subjects. Blood tests confirm that Dammarane Sapogenins therapy is safe for human use.

## Reference

- Baek NI, Kim DS, Lee YH, Park JD, Lee CB, Kim SI. Ginsenoside Rh4, a genuine dammarane glycoside from Korean red ginseng. *Planta Med* 1996;62(1):86-7.
- Cao J, Zheng YQ, Liu TP, Feng LZ. Inhibitory effects of ginsenoside Rg1 and Rb1 on rat brain microsomal Na<sup>+</sup>,K<sup>+</sup>-ATPase activity. *Chung Kuo Yao Li Hsueh Pao* 1990;11(1):10-4.
- Chan TW, But PP, Cheng SW, Kwok IM, Lau FW, Xu HX. Differentiation and authentication of *Panax ginseng*, *Panax quinquefolius*, and ginseng products by using HPLC/MS [published erratum appears in *Anal Chem* 2000 May 15;72(10):2329]. *Anal Chem* 2000;72(6):1281-7.
- Chen YJ, Xie H, Pei YP, Xu SX, Yao XS. [Isolation and identification of the anti-tumor constituent, ginsenoside-PAN-11]. *Chung Yao Tung Pao* 1988;13(1):40-2, 64.
- Chen YJ, Zhang SL, Wang ZX, Lu YJ, Xu SX, Yao XS, et al. [Isolation and elucidation of a new minor saponin from the leaves of *Panax ginseng* C.A. Meyer]. *Yao Hsueh Hsueh Pao* 1990;25(5):379-81.
- Cheng YJ, Su SX, Ma QF, Pei YP, Xie H, Yao XS. [Studies on new minor saponins isolated from leaves of *Panax ginseng* C. A. Meyer]. *Yao Hsueh Hsueh Pao* 1987;22(9):685-9.
- Chung E, Lee KY, Lee YJ, Lee YH, Lee SK. Ginsenoside Rg1 down-regulates glucocorticoid receptor and displays synergistic effects with cAMP. *Steroids* 1998;63(7-8):421-4.
- Corthout J, Naessens T, Apers S, Vlietinck AJ. Quantitative determination of ginsenosides from *Panax ginseng* roots and ginseng preparations by thin layer chromatography--densitometry. *J Pharm Biomed Anal* 1999;21(1):187-92.
- Cui JF, Garle M, Bjorkhem I, Eneroth P. Determination of aglycones of ginsenosides in ginseng preparations sold in Sweden and in urine samples from Swedish athletes consuming ginseng. *Scand J Clin Lab Invest* 1996;56(2):151-60.
- Cui M, Song F, Zhou Y, Liu Z, Liu S. Rapid identification of saponins in plant extracts by electrospray ionization multi-stage tandem mass spectrometry and liquid chromatography/tandem mass spectrometry [In Process Citation]. *Rapid Commun Mass Spectrom* 2000;14(14):1280-6.
- Danesi R, Figg WD, Reed E, Myers CE. Paclitaxel (taxol) inhibits protein isoprenylation and induces apoptosis in PC-3 human prostate cancer cells. *Mol Pharmacol* 1995;47(6):1106-11.
- Deng HL, Zhang JT. Anti-lipid peroxidative effect of ginsenoside Rb1 and Rg1. *Chin Med J (Engl)* 1991;104(5):395-8.
- Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, et al. Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey [see comments]. *Jama* 1998;280(18):1569-75.
- Elkin YN, Makhankov VV, Uvarova NL, Bondarenko PV, Zubarev RA, Knysh AN. Combination of HPLC and 252-Cf plasma desorption mass spectrometry for identifying composition of ginseng tinctures. *Chung Kuo Yao Li Hsueh Pao* 1993;14(2):97-100.
- Enomoto Y, Ito K, Kawagoe Y, Morio Y, Yamasaki Y. Positive inotropic action of saponins on isolated atrial and papillary muscles from the guinea-pig. *Br J Pharmacol* 1986;88(1):259-67.
- Fan ZH, Isobe K, Kiuchi K, Nakashima I. Enhancement of nitric oxide production from activated macrophages by a purified form of ginsenoside (Rg1). *Am J Chin Med* 1995;23(3-4):279-87.
- Hasegawa H, Matsumiya S, Murakami C, Kurokawa T, Kasai R, Ishibashi S, et al. Interactions of ginseng extract, ginseng separated fractions, and some triterpenoid saponins with glucose transporters in sheep erythrocytes. *Planta Med* 1994;60(2):153-7.
- Hong M, Jin Y, Mai YQ, Boersma A, Han KK, Vantuyghem MC, et al. The decline of atrial natriuretic peptide (ANP) gene expression in older rats and the effects of ginsenoside on ANP gene expression. *Comp Biochem Physiol [b]* 1992;101(1-2):35-9.
- Huong NT, Matsumoto K, Kasai R, Yamasaki K, Watanabe H. In vitro antioxidant activity of Vietnamese ginseng saponin and its components. *Biol Pharm Bull* 1998;21(9):978-81.
- Iijima M, Higashi T. Effect of ginseng saponins on nuclear ribonucleic acid (RNA) metabolism. II. RNA polymerase activities in rats treated with ginsenoside. *Chem Pharm Bull (Tokyo)* 1979;27(9):2130-6.
- Iishi H, Tatsuta M, Baba M, Uehara H, Nakaizumi A, Shinkai K, et al. Inhibition by ginsenoside Rg3 of bombesin-enhanced peritoneal metastasis of intestinal adenocarcinomas induced by azoxymethane in Wistar rats. *Clin Exp Metastasis* 1997;15(6):603-11.
- Jiang Y, Zhong GG, Chen L, Ma XY. Influences of ginsenosides Rb1, Rb2, and Rb3 on electric and contractile activities of normal and damaged cultured myocardiocytes. *Chung Kuo Yao Li Hsueh Pao* 1992;13(5):403-6.
- Jin YH, Yoo KJ, Lee YH, Lee SK. Caspase 3-mediated Cleavage of p21<sup>WAF1/CIP1</sup> Associated with the Cyclin A/Cdk2 Complex is a Prerequisite for Apoptosis in SK-HEP-1 Cells. *J Biol Chem* 2000.
- Kaku T, Kawashima Y. Isolation and characterization of ginsenoside-Rg2, 20R-prosopogenin, 20S-prosopogenin and delta 20-prosopogenin. Chemical studies on saponins of *Panax ginseng* C. A. Meyer, Third report. *Arzneimittelforschung* 1980;30(6):936-43.
- Kanazawa H, Nagata Y, Matsushima Y, Tomoda M, Takai N. Simultaneous determination of ginsenosides and saikosaponins by high-performance liquid chromatography. *J Chromatogr* 1990;507(327):327-32.
- Kang M, Yoshimatsu H, Oohara A, Kurokawa M, Ogawa R, Sakata T. Ginsenoside Rg1 modulates ingestive behavior and thermal response induced by interleukin-1 beta in rats. *Physiol Behav* 1995;57(2):393-6.
- Kang SY, Lee KY, Lee SK. Ginsenoside-Rg1 regulates the induction of tyrosine aminotransferase gene transcription in rat hepatocyte cultures. *Biochem Biophys Res Commun* 1994;205(3):1696-701.
- Karikura M, Miyase T, Tanizawa H, Takino Y, Taniyama T, Hayashi T. Studies on absorption, distribution, excretion and

- metabolism of ginseng saponins. V. The decomposition products of ginsenoside Rb2 in the large intestine of rats. *Chem Pharm Bull (Tokyo)* 1990;38(10):2859-61.
- Kenarova B, Neychev H, Hadjiivanova C, Petkov VD. Immunomodulating activity of ginsenoside Rg1 from *Panax ginseng*. *Jpn J Pharmacol* 1990;54(4):447-54.
- Kikuchi Y, Sasa H, Kita T, Hirata J, Tode T, Nagata I. Inhibition of human ovarian cancer cell proliferation in vitro by ginsenoside PAN-11 and adjuvant effects to cisplatin in vivo. *Anticancer Drugs* 1991;2(1):63-7.
- Kim HE, Oh JH, Lee SK, Oh YJ. Ginsenoside RH-2 induces apoptotic cell death in rat C6 glioma via a reactive oxygen- and caspase-dependent but Bcl-X(L)-independent pathway. *Life Sci* 1999;65(3):PL33-40.
- Kim HS, Lee JH, Goo YS, Nah SY. Effects of ginsenosides on Ca<sup>2+</sup> channels and membrane capacitance in rat adrenal chromaffin cells. *Brain Res Bull* 1998;46(3):245-51.
- Kim MY, Lee KY, Lee SK. Inductive effect of ginsenoside-Rg1 on tyrosine aminotransferase gene expression in rat primary hepatocyte cultures. *Biochem Mol Biol Int* 1994;34(4):845-51.
- Kim ND, Kang SY, Kim MJ, Park JH, Schini KV. The ginsenoside Rg3 evokes endothelium-independent relaxation in rat aortic rings: role of K<sup>+</sup> channels. *Eur J Pharmacol* 1999;367(1):51-7.
- Kim ND, Kang SY, Park JH, Schini KV. Ginsenoside Rg3 mediates endothelium-dependent relaxation in response to ginsenosides in rat aorta: role of K<sup>+</sup> channels. *Eur J Pharmacol* 1999;367(1):41-9.
- Kim SE, Lee YH, Park JH, Lee SK. Ginsenoside-Rs3, a new diol-type ginseng saponin, selectively elevates protein levels of p53 and p21WAF1 leading to induction of apoptosis in SK-HEP-1 cells. *Anticancer Res* 1999;19(1A):487-91.
- Kim SE, Lee YH, Park JH, Lee SK. Ginsenoside-Rs4, a new type of ginseng saponin concurrently induces apoptosis and selectively elevates protein levels of p53 and p21WAF1 in human hepatoma SK-HEP-1 cells. *Eur J Cancer* 1999;35(3):507-11.
- Kim YS, Jin SH, Lee YH, Kim SI, Park JD. Ginsenoside PAN-11 induces apoptosis independently of Bcl-2, Bcl-xL, or Bax in C6Bu-1 cells. *Arch Pharm Res* 1999;22(5):448-53.
- Kitts D, Hu C. Efficacy and safety of ginseng. *Public Health Nutr* 2000;3(4A):473-85.
- Kitts DD, Wijewickreme AN, Hu C. Antioxidant properties of a North American ginseng extract. *Mol Cell Biochem* 2000;203(1-2):1-10.
- Landes P. Survey indicates increasing herb use. In: 1996: American Botanical Council and Herb Research Foundation; 1996. p. 56.
- Lee KY, Lee SK. Ginsenoside-Rg1 positively regulates cyclin E-dependent kinase activity in human hepatoma SK-HEP-1 cells. *Biochem Mol Biol Int* 1996;39(3):539-46.
- Lee KY, Park JA, Chung E, Lee YH, Kim SI, Lee SK. Ginsenoside-PAN-11 blocks the cell cycle of SK-HEP-1 cells at the G1/S boundary by selectively inducing the protein expression of p27kip1. *Cancer Lett* 1996;110(1-2):193-200.
- Lee YN, Lee HY, Chung HY, Kim SI, Lee SK, Park BC, et al. In vitro induction of differentiation by ginsenosides in F9 teratocarcinoma cells. *Eur J Cancer* 1996:1420-8.
- Li J, Zhang J. Inhibition of apoptosis by ginsenoside Rg1 in cultured cortical neurons. *Chin Med J (Engl)* 1997;110(7):535-9.
- Liu M, Zhang JT. Effects of ginsenoside Rg1 on c-fos gene expression and cAMP levels in rat hippocampus. *Chung Kuo Yao Li Hsueh Pao* 1996;17(2):171-4.
- Liu SR, Luo ZJ, Yang SX, Yu ML. [Dammarane-type saponins in leaves of *Panax* collected in Sichuan]. *Hua Hsi I Ko Ta Hsueh Hsueh Pao* 1989;20(3):331-4.
- Liu WK, Xu SX, Che CT. Anti-proliferative effect of ginseng saponins on human prostate cancer cell line. *Life Sci* 2000;67(11):1297-306.
- Liu WK, Xu SX, Che CT. Anti-proliferative effect of ginseng saponins on human prostate cancer cell line [In Process Citation]. *Life Sci* 2000;67(11):1297-306.
- Mindell E. *Herb Bible*. New York: Fireside Publishers; 1991.
- Minyi C. *Anticancer Medicinal Herbs*. Beijing: Hunan Science and Technology Press; 1992.
- Mogil JS, Shin YH, McCleskey EW, Kim SC, Nah SY. Ginsenoside Rf, a trace component of ginseng root, produces antinociception in mice. *Brain Res* 1998;792(2):218-28.
- Moon J, Yu SJ, Kim HS, Sohn J. Induction of G(1) cell cycle arrest and p27(KIP1) increase by panaxydol isolated from *Panax ginseng*. *Biochem Pharmacol* 2000;59(9):1109-16.
- Nakata H, Kikuchi Y, Tode T, Hirata J, Kita T, Ishii K, et al. Inhibitory effects of ginsenoside PAN-11 on tumor growth in nude mice bearing human ovarian cancer cells. *Jpn J Cancer Res* 1998;89(7):733-40.
- Odashima S, Ohta T, Kohno H, Matsuda T, Kitagawa I, Abe H, et al. Control of phenotypic expression of cultured B16 melanoma cells by plant glycosides. *Cancer Res* 1985;45(6):2781-4.
- Oh M, Choi YH, Choi S, Chung H, Kim K, Kim SI, et al. Anti-proliferating effects of ginsenoside PAN-11 on MCF-7 human breast cancer cells. *Int J Oncol* 1999;14(5):869-75.
- Ong YC, Yong EL. *Panax (ginseng)--panacea or placebo? Molecular and cellular basis of its pharmacological activity*. *Ann Acad Med Singapore* 2000;29(1):42-6.
- Ota T, Maeda M, Odashima S, Ninomiya TJ, Tatsuka M. G1 phase-specific suppression of the Cdk2 activity by ginsenoside PAN-11 in cultured murine cells. *Life Sci* 1997;60(2):PL39-44.
- Oura H, Hiai S, Odaka Y, Yokozawa T. Studies on the biochemical action of ginseng saponin. I. Purification from ginseng extract of the active component stimulating serum protein biosynthesis. *J Biochem (Tokyo)* 1975;77(5):1057-65.
- Pan Z, Ye D, Xie X, Chen H, Lu W. [Antiangiogenesis of ginsenoside Rg3 in severe combined immunodeficient mice with human ovarian carcinoma]. *Zhonghua Fu Chan Ke Za Zhi* 2002;37(4):227-30.

- Park JA, Lee KY, Oh YJ, Kim KW, Lee SK. Activation of caspase-3 protease via a Bcl-2-insensitive pathway during the process of ginsenoside PAN-11-induced apoptosis. *Cancer Lett* 1997;121(1):73-81.
- Popovich DG, Kitts DD. Structure-function relationship exists for ginsenosides in reducing cell proliferation and inducing apoptosis in the human leukemia (THP-1) cell line. *Arch Biochem Biophys* 2002;406(1):1-8.
- Salim KN, McEwen BS, Chao HM. Ginsenoside Rb1 regulates ChAT, NGF and trkA mRNA expression in the rat brain. *Brain Res Mol Brain Res* 1997;47(1-2):177-82.
- Samukawa K, Yamashita H, Matsuda H, Kubo M. [Simultaneous analysis of ginsenosides of various ginseng radix by HPLC]. *Yakugaku Zasshi* 1995;115(3):241-9.
- Sato T, Miyata G. The nutraceutical benefit, part II: ginseng [published erratum appears in *Nutrition* 2000 Jun;16(6):472]. *Nutrition* 2000;16(5):391-2.
- Shao CJ. [Chemical constituents of flower-buds of *Panax ginseng*--isolation and identification of ginsenoside-Rb3 and ginsenoside-Rc]. *Chung Yao Tung Pao* 1984;9(4):172-3.
- Shibata S, Ando T, Tanaka O. Chemical studies on the oriental plant drugs. XVII. The prosapogenin of the ginseng saponins (ginsenosides-Rb1, -Rb2, and -Rc). *Chem Pharm Bull (Tokyo)* 1966;14(10):1157-61.
- Shibata S, Ando T, Tanaka O, Meguro Y, Soma K, Iida Y. [Saponins and sapogenins of *Panax ginseng* C.A. Meyer and some other *Panax* spp.]. *Yakugaku Zasshi* 1965;85(8):753-5.
- Shibata S, Tanaka O, Ando T, Sado M, Tsushima S, Ohsawa T. Chemical studies on oriental plant drugs. XIV. Protopanaxadiol, a genuine sapogenin of ginseng saponins. *Chem Pharm Bull (Tokyo)* 1966;14(6):595-600.
- Shinkai K, Akedo H, Mukai M, Imamura F, Isoai A, Kobayashi M, et al. Inhibition of in vitro tumor cell invasion by ginsenoside Rg3. *Jpn J Cancer Res* 1996;87(4):357-62.
- Tachikawa E, Kudo K, Kashimoto T, Takahashi E. Ginseng saponins reduce acetylcholine-evoked Na<sup>+</sup> influx and catecholamine secretion in bovine adrenal chromaffin cells. *J Pharmacol Exp Ther* 1995;273(2):629-36.
- Takino Y, Odani T, Tanizawa H, Hayashi T. Studies on the absorption, distribution, excretion and metabolism of ginseng saponins. I. Quantitative analysis of ginsenoside Rg1 in rats. *Chem Pharm Bull (Tokyo)* 1982;30(6):2196-201.
- Tanaka O, Nagai M, Shibata S. Chemical studies on the oriental plant drugs. XVI. The stereochemistry of protopanaxadiol, a genuine sapogenin of ginseng. *Chem Pharm Bull (Tokyo)* 1966;14(10):1150-6.
- Tao H, Yao M, Zou S, Zhao D, Qiu H. Effect of angiogenesis inhibitor Rg3 on the growth and metastasis of gastric cancer in SCID mice. *Zhonghua Wai Ke Za Zhi* 2002;40(8):606-8.
- Thatte U, Bagadey S, Dahanukar S. Modulation of programmed cell death by medicinal plants. *Cell Mol Biol (Noisy-le-grand)* 2000;46(1):199-214.
- Tong LS, Chao CY. Effects of ginsenoside Rg1 of *Panax ginseng* on mitosis in human blood lymphocytes in vitro. *Am J Chin Med* 1980;8(3):254-67.
- Verhoef MJ, Sutherland LR. Alternative medicine and general practitioners. Opinions and behaviour. *Can Fam Physician* 1995;41:1005-11.
- Verhoef MJ, Sutherland LR. General practitioners' assessment of and interest in alternative medicine in Canada. *Soc Sci Med* 1995;41(4):511-5.
- Wang X, Sakuma T, Asafu-Adjaye E, Shiu GK. Determination of ginsenosides in plant extracts from *Panax ginseng* and *Panax quinquefolium* L. by LC/MS/MS. *Anal Chem* 1999;71(8):1579-84.
- Xia LJ, Han R. [Differentiation of B16 melanoma cells induced by ginsenoside PAN-11]. *Yao Hsueh Hsueh Pao* 1996;31(10):742-5.
- Yang Z, Xu JD. [Isolation and identification of saponin IV, V, VI and VII in ginseng stems]. *Chung Yao Tung Pao* 1987;12(8):32-6,63.
- Yip TT, Lau CN, But PP, Kong YC. Quantitative analysis of ginsenosides in fresh *Panax ginseng*. *Am J Chin Med* 1985;13(1-4):77-88.
- Yokozawa T, Yasui T, Oura H. Molecular biological analysis of the effects of ginsenoside-Rb2 on albumin mRNA in streptozotocin-induced diabetic rats. *J Pharm Pharmacol* 1996;48(7):763-7.
- Yoshikawa M, Murakami T, Yashiro K, Yamahara J, Matsuda H, Saijoh R, et al. Bioactive saponins and glycosides. XI. Structures of new dammarane-type triterpene oligoglycosides, quinquenosides I, II, III, IV, and V, from American ginseng, the roots of *Panax quinquefolium* L. *Chem Pharm Bull (Tokyo)* 1998;46(4):647-54.
- Zhang SL, Chen YJ, Cui CB, He GX, Xu SX, Pei YP, et al. [A new minor saponin from the leaves of *Panax ginseng* C. A. Meyer]. *Yao Hsueh Hsueh Pao* 1989;24(11):877-9.
- Zhao Y, Yuan C, Lu H. [Isolation and identification of 20(R)-ginsenoside-PAN-11 (an anti-cancer constituent) from the fruits of *Panax ginseng*. C.A. Meyer]. *Chung Kuo Chung Yao Tsai Chih* 1991;16(11):678-9, 704.
- Zhao YQ, Yuan CL. [Chemical constituents of the fruit of *Panax ginseng* C. A. Meyer]. *Chung Kuo Chung Yao Tsai Chih* 1993;18(5):296-7, 319.