Anticancer Plant Extracts
&
Anticancer Green Teas

John Hall, PhD

Senior Scientific Advisor

The Beljanski Foundation

Disclosure Statement of Financial Interest

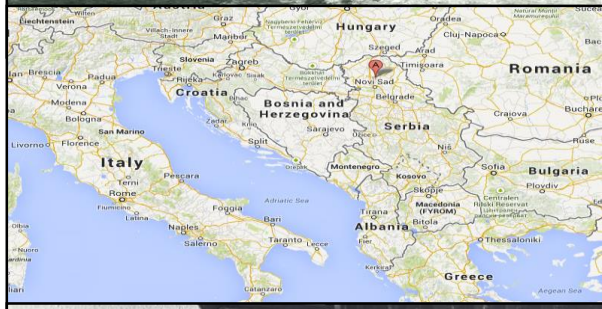
I, John Hall, PhD do have the following relationship or affiliation with a commercial vendor or manufacturer of medically-related products or service:

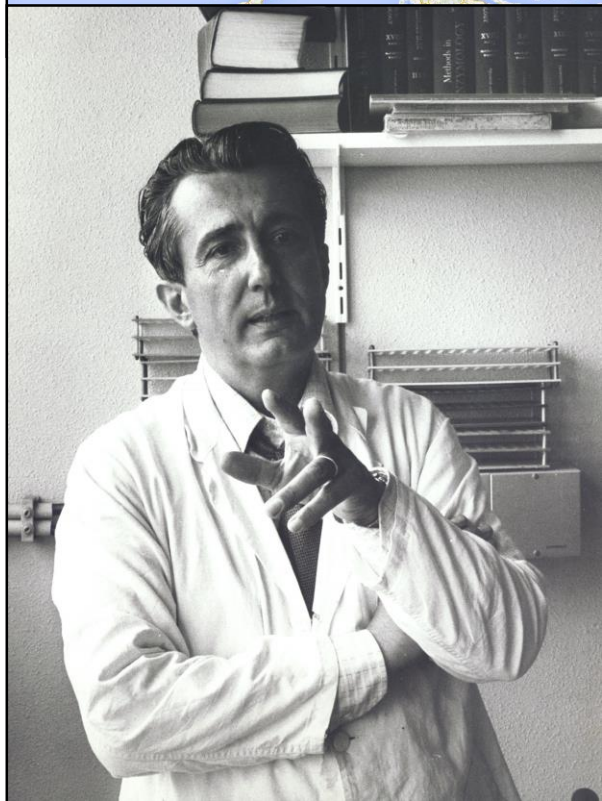
Affiliation/Financial Interest:	**Name of Organization**
Employer	Natural Source International, Ltd.
NO off-label uses will be discussed	

All recommendations involving *clinical* medicine given during my presentation are based upon evidence that is accepted within the profession of medicine for the care of patients.

I will not present recommendations, treatments or manners of practicing medicine that are not within the definition of CME, or are known to have risks or dangers that outweigh the benefits, or are known to be ineffective in the treatment of patients.

Mirko Beljanski, PhD



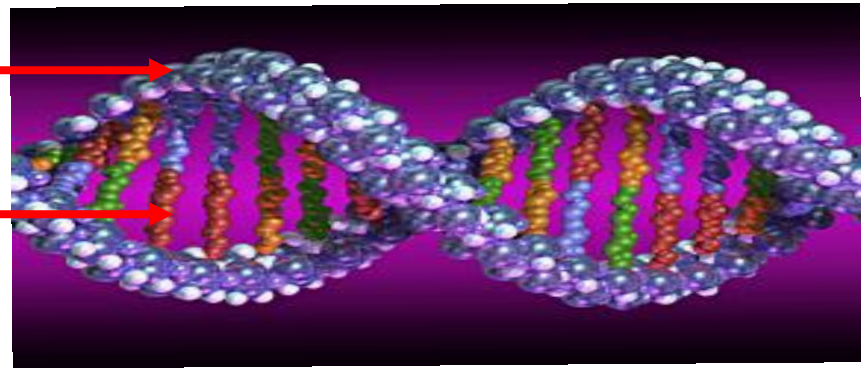


French/Serbian National 1923 - 1998

1947	Bachelor of Science, University of Paris
1951	PhD, University of Paris
1948-1978	Pasteur Institute, Paris, France
1951	Research Attaché for the C.N.R.S.*
1955	Research Manager for the C.N.R.S.
1956-1958	Fellow of New York University, USA
1960	Charles Leopold Mayer Prize
1960	Master of Research for the C.N.R.S.
1978-1988	Pharmaceutical University, Chatenay/France
1985	Director of Research for the C.N.R.S.
1988	Scientific Director of CERIBOL

*CNRS (National Center for Scientific Research) is the French equivalent of the NIH.

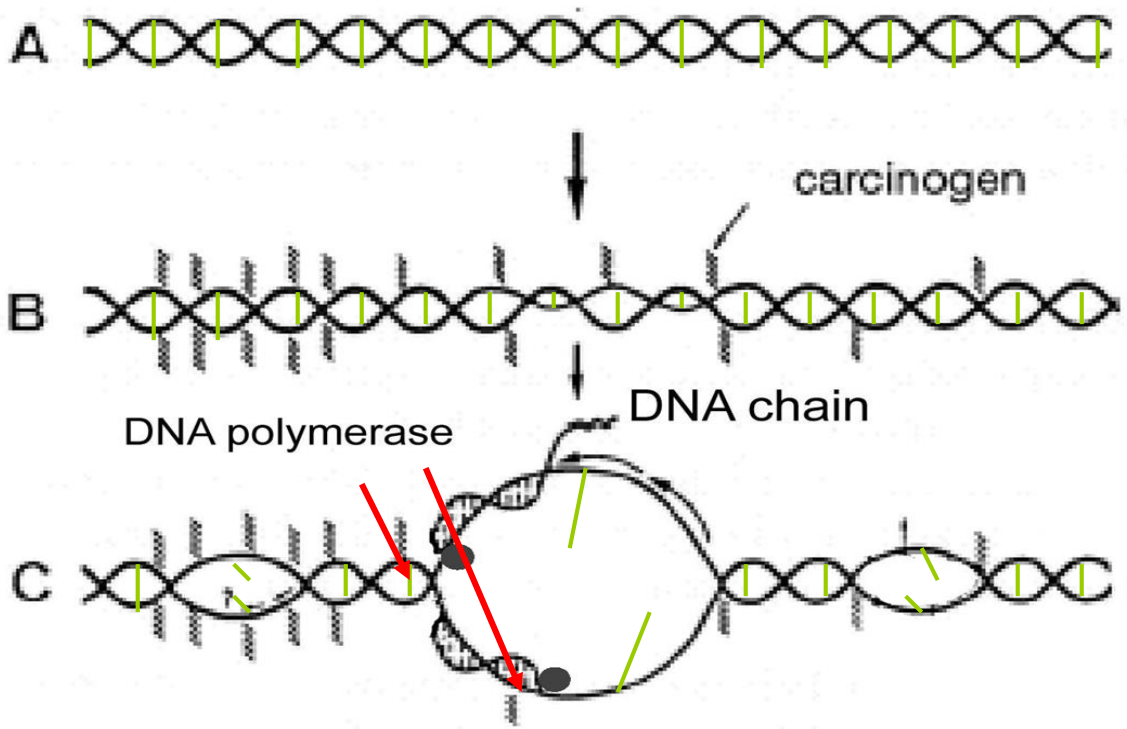
Beljanski's Theory of Cancer



Mutations = modifications in one or more nucleotides

Beljanskis theory is that *cancer DNA differs from normal DNA in its secondary structure*, rather than only its primary structure

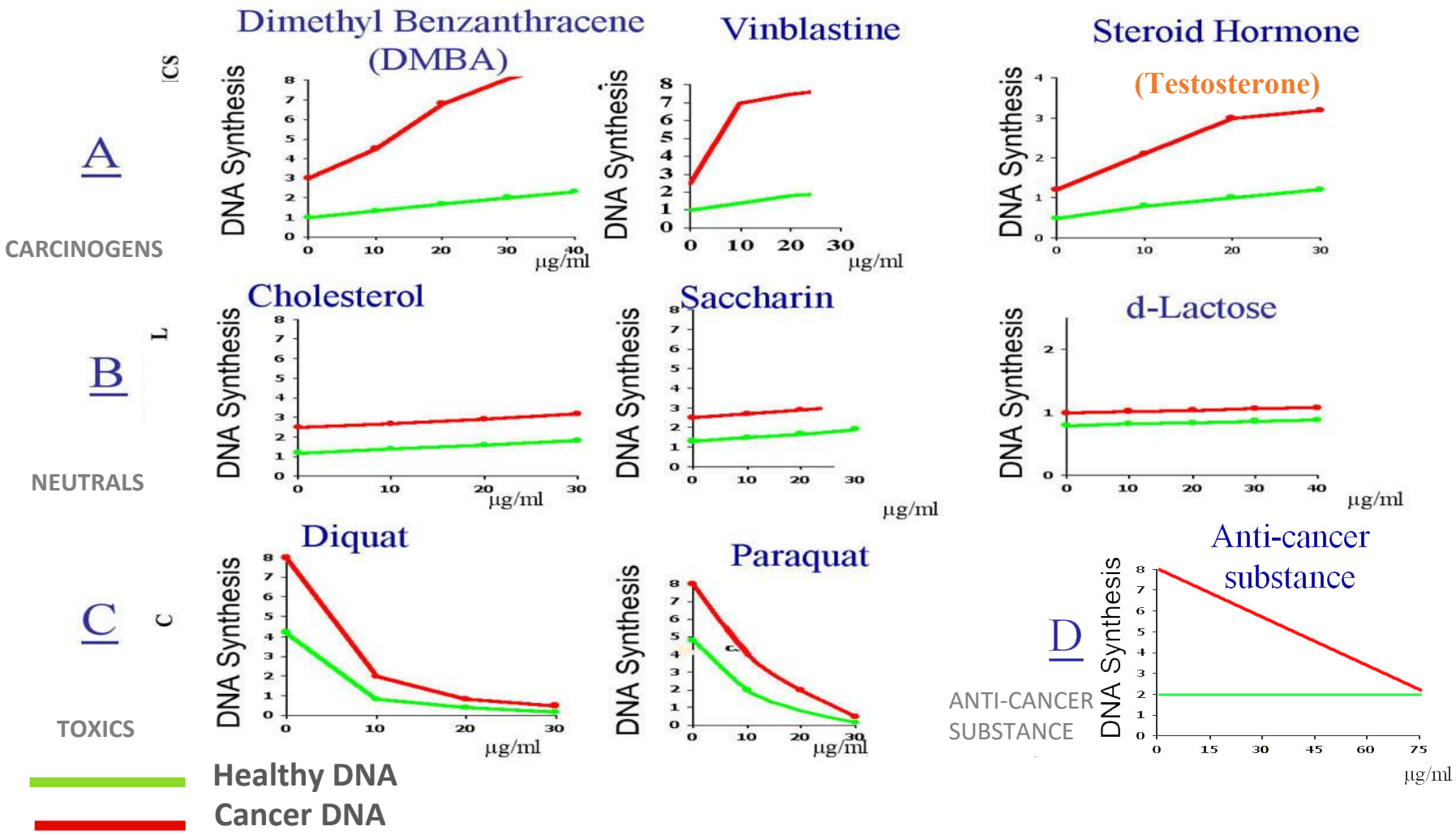
What Happens in the Presence of a Carcinogen



Once the strands are separated, enzymes for DNA replication have increased access to the duplication sites located inside the double helix and duplication can become abnormally accelerated.

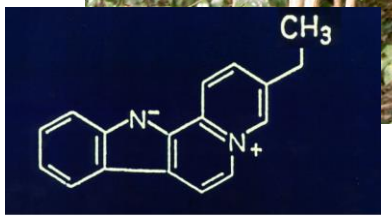
Mirko Beljanski thought that if Nature created carcinogens, Nature had also created anti-carcinogenic molecules, that would prevent the duplication process of destabilized DNA

The Oncotest



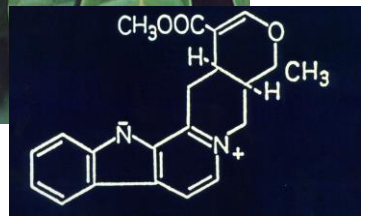
Two Plant Extracts with Anti-cancer Properties

Pao pereira



Flavopereirine

Rauwolfia vomitoria



Alstonine

Pao and *Rauwolfia* Extracts: Anti-cancer Activity

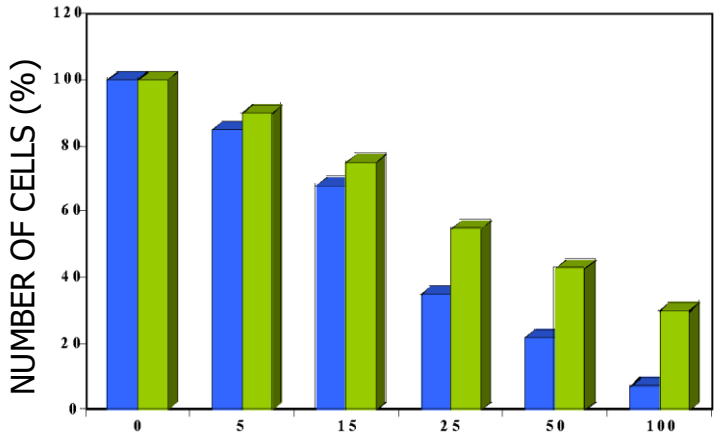
Pasteur Institute, Paris

Pao

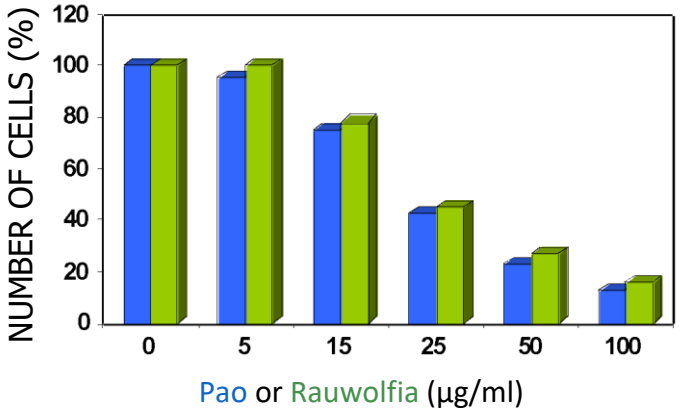
Rauwolfia

Effect of *Pao* and *Rauwolfia* Extracts on Human Liver

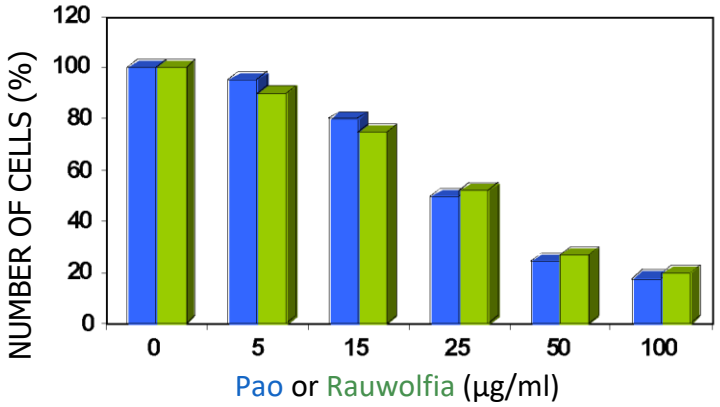
ADENOCARCINOMA CELL LINE (SK-HEP1)



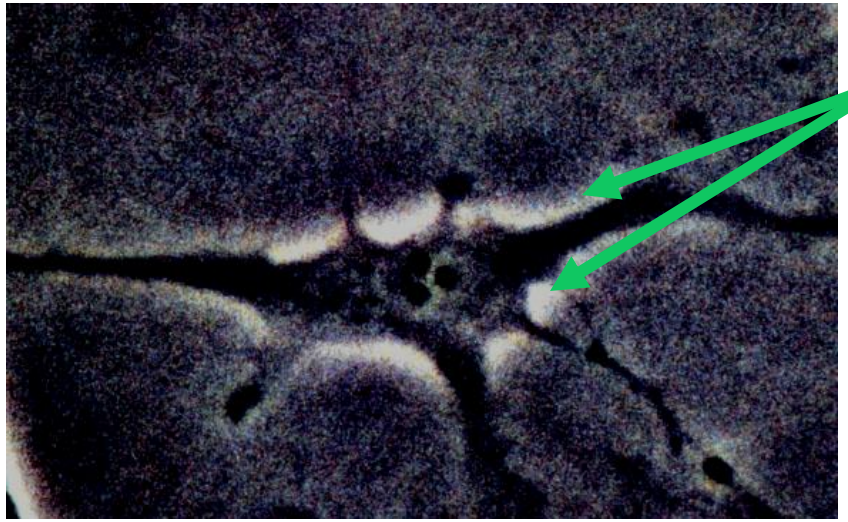
Effect of *Pao* and *Rauwolfia* Extracts on Human Thyroid Carcinoma Cell Line



Effect of Pao and Rauwolfia Extracts on Human Breast Cell Line (ZR-75-1)

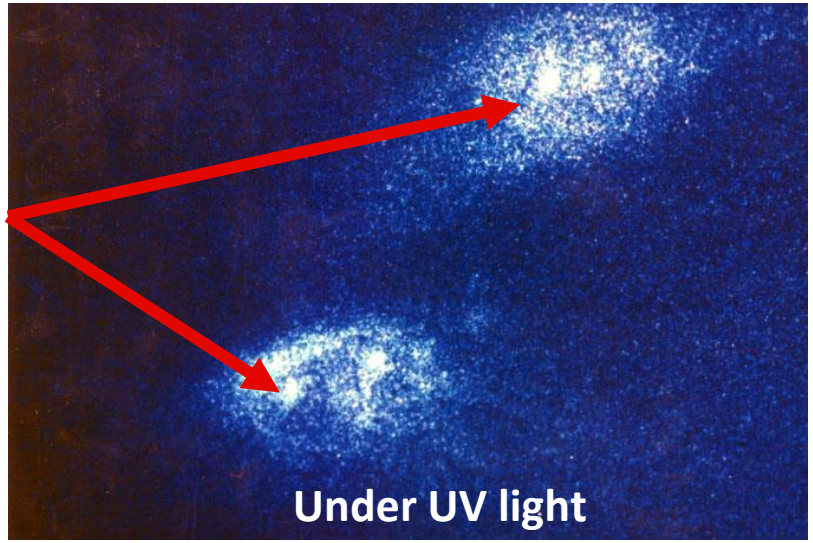


Selectivity of Action



Naturally fluorescent, *Pao pereira* can be seen outside a healthy cell (astrocyte), unable to penetrate its non-porous membrane

The *Pao pereira* extract can be seen penetrating the cancerous cell (glioblastoma)



Pao and *Rauwolfia* Extracts: Anti-Prostate Cancer Activity

Columbia University Medical Center

Aaron E. Katz M.D.

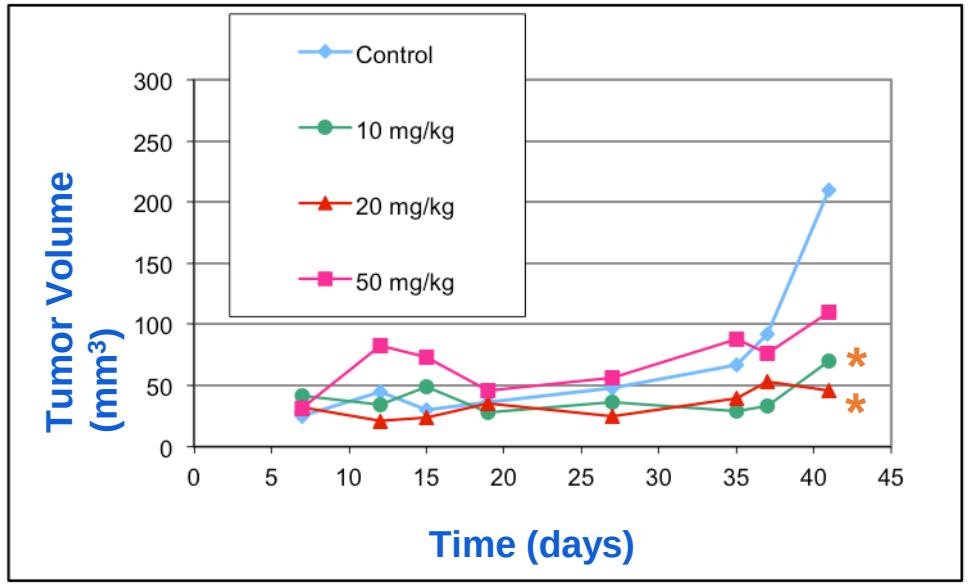
Director, Center for Holistic Urology,
Columbia University

In vivo Analysis of *Pao* and *Rauwolfia* Extracts on a Mouse Model of Prostate Cancer

Rauwolfia and Pao Suppress LNCaP Tumor Xenograft Growth

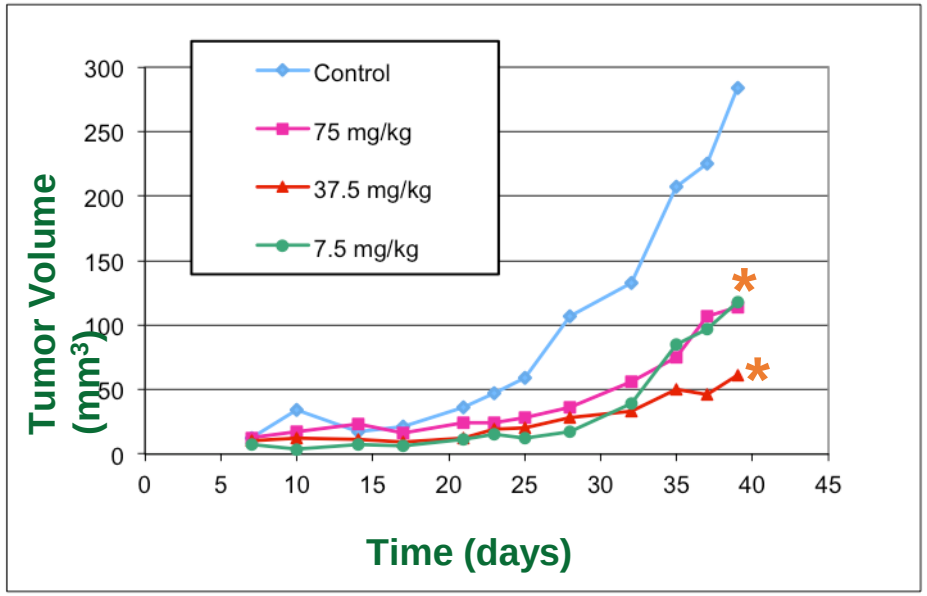
Center for Holistic Urology, Columbia University Medical Center

Pao



* P < 0.001, Kruskal Wallis test

Rauwolfia



P < 0.05, Kruskal Wallis test

- LNCaP Xenograft implants into immunodeficient mice
- Daily feeding of extract at various doses for 5.5 weeks
- Analyze effect of extracts on tumor growth
- Immunohistochemical analysis of tumor sections (TUNEL and BrdU)

Publications from Columbia University

INTERNATIONAL JOURNAL OF ONCOLOGY 29: 1065-1073, 2006

Anti-prostate cancer activity of a ß-carboline alkaloid enriched extract from *Rauwolfia vomitoria*

D.L. BEMIS[1], J.L. CAPODICE[1], P. GORROOCHURN[2], A.E. KATZ[1] and R. BUTTYAN[1]

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Abstract. The tropical shrub, *Rauwolfia vomitoria*, is a medicinal plant used traditionally to treat a variety of ailments. A bioactive ß-carboline alkaloid, alstonine, present in this extract was previously shown to have anti-cancer activity against cancer cell lines. This study considers the potential anti-prostate cancer activity of this extract *in vitro* and *in vivo*. *Rauwolfia vomitoria* extract standardized for ß-carboline alkaloids was tested for ability to influence the growth and survival of the human LNCaP prostate cancer cell line. A WST-1 assay was used to measure cell growth, and cell cycle analyses were conducted with flow cytometry. Western blot detection of PARP cleavage and accumulation of cells containing sub-genomic DNA indicated induction of apoptosis. Pathway specific microarray analyses were utilized to identify the effect of *Rauwolfia* extract on the expression of 225 genes. Mice xenografted with LNCaP cells were treated with the extract or placebo control, and tumor growth was measured for 5 weeks. The effects of the extract on xenografted tumor cell proliferation and apoptosis were measured by *in situ* BrdU incorporation and TUNEL staining. *Rauwolfia* extract decreased *in vitro* cell growth in a dose-dependent manner ($p<0.001$) and induced the accumulation of G1 phase cells. PARP cleavage demonstrated that apoptosis was induced only at the highest concentration tested (500 µg/ml) which was confirmed by detection of cells containing sub-genomic DNA. The expression of genes associated with DNA damage signaling pathway was up-regulated by *Rauwolfia* treatment, including that of GADD153 and MDG. The expression of a few cell cycle genes (p21, cyclin D1 and E2F1) was also modulated. These alterations were confirmed by RT-PCR. Tumor volumes were decreased by 60%, 70% and 58% in the groups fed the 75, 37.5 or 7.5 mg/kg *Rauwolfia*, respectively (Kruskal-Wallis test, $p<0.001$). The *Rauwolfia vomitoria* extract significantly suppressed the growth and cell cycle progression of LNCaP cells, *in vitro* and *in vivo*.

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E-mail: dlb2004@columbia.edu

Key words: *Rauwolfia*, prostate cancer, DNA damage

Introduction

Prostate cancer is predicted to be the third leading cause of cancer-related deaths among men in the USA in 2006 (1). Traditionally, chemotherapy and radiotherapy have not proven to provide significant survival benefits to patients with advanced prostate cancer and most treatment options available are only palliative. Recent studies on taxane derivatives alone and in combination with other chemotherapeutic agents have demonstrated some limited benefit (2), but a need for more effective and less toxic means to target and/or prevent this disease clearly exists.

Natural products have long proven to be a bountiful resource for identification of bioactive compounds used in the treatment of a variety of ailments and diseases, including cancer. The taxane derivatives currently being used for the treatment of hormone-independent prostate cancer are but one example among many of the importance of this resource. However, systematic characterization of natural product and herbal therapies and identification of their mechanism(s) of action are crucial for the development of safe and efficacious therapies for prostate cancer prevention and treatment. Regarding this, we have begun to study a unique extract derived from the root bark of a plant found in the tropical secondary forests of Africa, *Rauwolfia vomitoria* (family: Apocynaceae) to determine whether it might have activity against prostate cancer. Various parts of this plant have been used as a traditional medicine for centuries to treat a variety of ailments including fever, general weakness, intestinal diseases, liver problems and mental disorders (3,4). Extracts from the root bark of this plant are enriched for compounds of the ß-carboline alkaloid family of which the main constituent is alstonine. This compound has been previously reported to reduce tumor cell growth in mice inoculated with YC8 lymphoma cells or Ehrlich ascitic cells (5). The data presented herein suggest that this plant extract has anti-prostate cancer activity in both *in vitro* and *in vivo* model systems which, based upon our analyses of gene expression patterns of treated prostate cancer cells, may be modulated by its effects on DNA damage and cell cycle control signaling pathways.

ARTICLE

β-Carboline Alkaloid–Enriched Extract from the Amazonian Rain Forest Tree Pao Pereira Suppresses Prostate Cancer Cells

Debra L. Bemis, PhD, Jillian L. Capodice, LAc, MS, Manisha Desai, PhD, Aaron E. Katz, MD, Ralph Buttyan, PhD

Bark extracts from the Amazonian rain forest tree *Geissospermum vellosii* (pao pereira), enriched in α-carboline alkaloids have significant anticancer activities in certain preclinical models. Because of the predominance of prostate cancer as a cause of cancer-related morbidity and mortality for men of Western countries, we preclinically tested the in vitro and in vivo effects of a pao pereira extract against a prototypical human prostate cancer cell line, LNCaP. When added to cultured LNCaP cells, pao pereira extract significantly suppressed cell growth in a dose-dependent fashion and induced apoptosis. Immunodeficient mice heterotopically xenografted with LNCaP cells were gavaged daily with pao pereira extract or vehicle control over 6 weeks. Tumor growth was suppressed by up to 80% in some groups compared with tumors in vehicle-treated mice. However, we observed a striking U-shaped dose-response curve in which the highest dose tested (50 mg/kg/d) was much less effective in inducing tumor cell apoptosis and in reducing tumor cell proliferation and xenograft growth compared with lower doses (10 or 20 mg/kg/d). Although this study supports the idea that a pao pereira bark extract has activity against human prostate cancer, our in vivo results suggest that its potential effectiveness in prostate cancer treatment may be limited to a narrow dose range.

Key words: *pao pereira, preclinical, prostate cancer*

Numerous chemotherapeutic agents used in the treatment of cancer were originally derived from plants. Such agents include the vinka alkaloids, extracted from the Madagascar periwinkle; taxanes, extracted from Pacific yew tree bark; etoposide, extracted from the may-apple plant; and irinotecan and topotecan, extracted from *Camptotheca acuminata*. In a similar manner, Beljanski and Crochet proposed that extracts of the bark of an Amazonian rain forest tree, *Geissospermum vellosii* Allemão (familiarly known as pao pereira), used medicinally by South American indian tribes, might have activity against human tumors.[1-3] In preliminary investigations, Beljanski and Crochet demonstrated that pao pereira bark extract suppressed the in vitro growth of several human cancer cell lines, including ones derived from melanoma and glioblastoma.[1,2] Pao pereira bark extract is enriched for alkaloids of the β-carboline family. These types of alkaloids have been shown to be cytotoxic for cancer cells, and their mechanism of action may involve the targeting of cyclin-dependent kinases (CDKs).[3-5] None of the previous studies on pao pereira bark extracts involved human prostate cancer cell lines, so here we report our preclinical studies to test whether a standardized extract of pao pereira bark might affect the in vitro or in vivo growth of a prototypic human prostate cancer cell line, LNCaP.

Our focus on prostate cancer derives from the predominance of this cancer as a health concern for men in Western countries. Prostate cancer is the most frequently diagnosed malignancy in males and a leading cause of cancer deaths in men.[6] Given the relatively high frequency with which prostate cancer occurs, prevention offers the most likely means to reduce the health risk to men posed by the disease. If pao pereira bark extract has tumor-suppressing activity for prostate cancer without overt toxicity, one can consider the possibility that it might be used as a preventive agent as a dietary supplement. Moreover, there is a great need for better therapeutic agents to treat advanced (metastatic) prostate cancer. Although hormone therapy is the standard for men with this stage of disease, it is mainly a palliative treatment that loses effectiveness over time. Once prostate cancer progresses to

Debra L. Bemis, Jillian L. Capodice, and Aaron E. Katz, Department of Urology, College of Physicians and Surgeons, Columbia University Medical Center, New York, NY; Manisha Desai, Department of Biostatistics, Mailman School of Public Health, Columbia University Medical Center, New York, NY; Ralph Buttyan, The Ordway Research Institute, Albany, NY

Reprint requests: Debra L. Bemis, PhD, Urology Institute for Cancer Research, 405 Third Avenue, New York, NY 10156; e-mail: dlbemis@hotmail.com

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DECKER

Journal of the Society for Integrative Oncology, Vol 7, No 2 (Spring), 2009: pp 59–65

In Combination *Pao* and *Rauwolfia* Extracts are Synergistic

Pao and *Rauwolfia* extracts kill prostate cancer cells and shrink prostate tumors.

Pao induces apoptosis in prostate cancer cells.

Rauwolfia induces cell cycle arrest in prostate cancer cells.

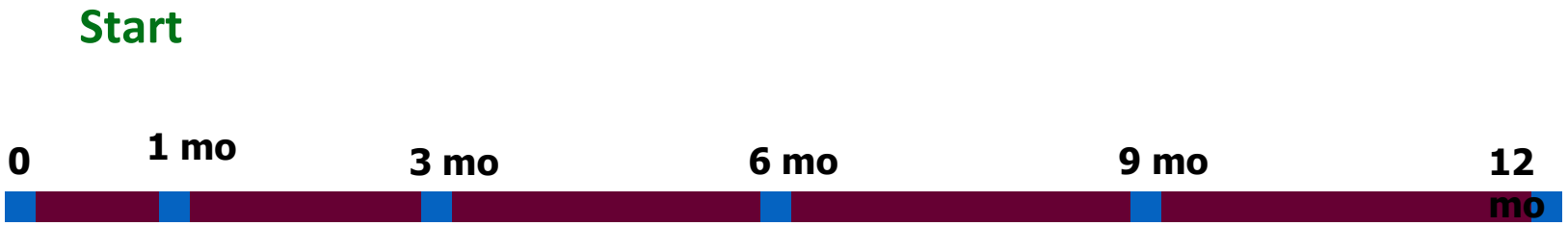
The combination of the two extracts in a single product combines both mechanisms of action and yielding synergy of action.

Combination of *Pao* and *Rauwolfia* in Men

Phase I Trial to Assess the Safety and Tolerability of
Pao/Rauwolfia Extract Combination
for Men with an Elevated PSA

Columbia University Medical Center
Aaron Katz MD
Director of Holistic Urology

Study Procedure



Enrollment (3 subjects per Regimen)

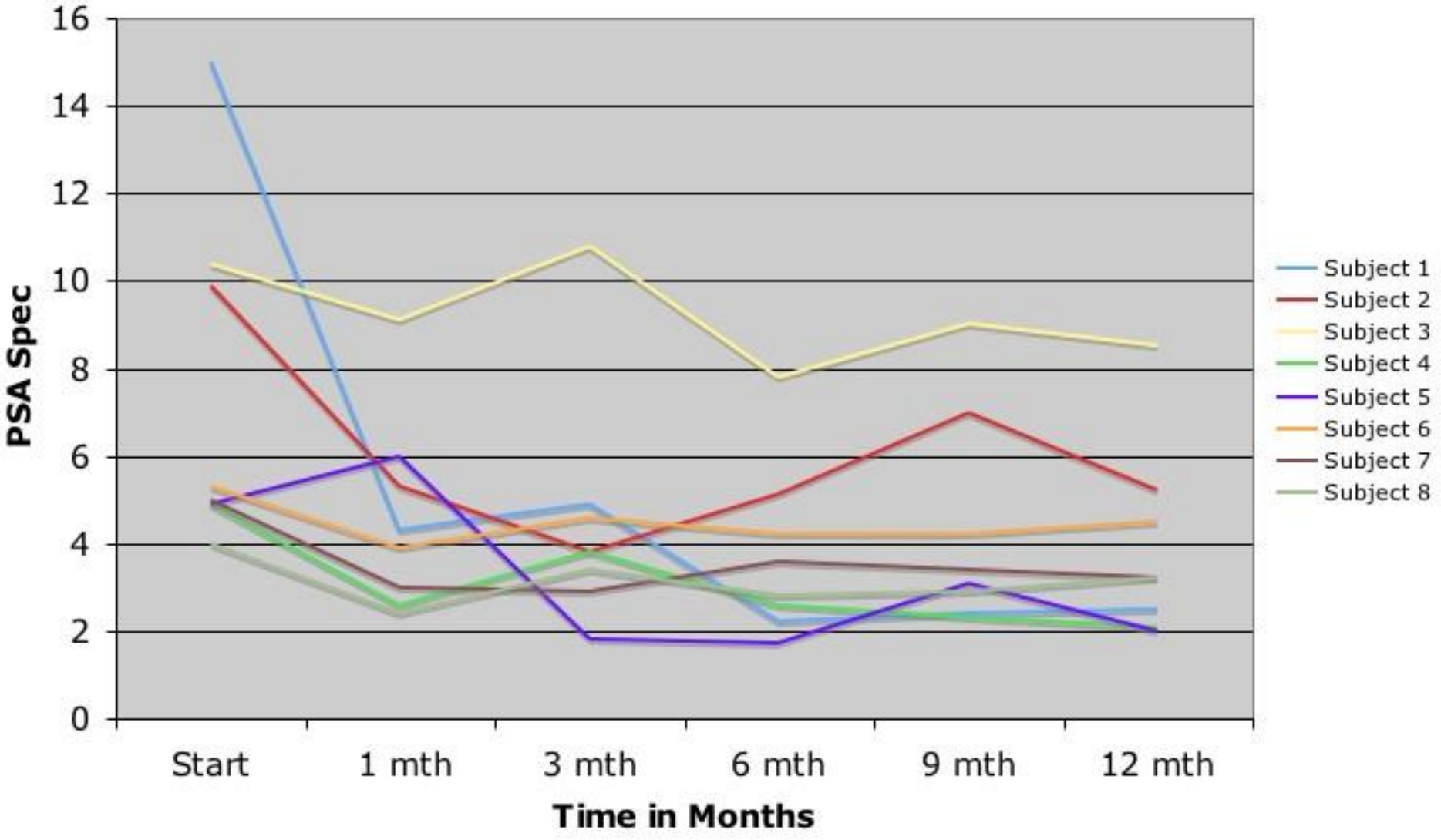
- **Regimen I (R1) 2 caps/day**
- **R2 3 caps/day**
- **R3 4 caps/day**
- **R4 5 caps/day**
- **R5 6 caps/day**
- **R6 7 caps/day**
- **R7 8 caps/day**

Monitor…

- **Toxicity using National Cancer Institute common toxicity criteria**
- **PSA increase exceeding 50% of the previous value**
- **Compliance level of less than 80%**

Biopsy is taken within 1 month prior to completing the study to assess for change from the initial biopsy

PSA Values for Men Taking a Mix of *Pao pereira* and *Rauwolfia vomitoria* in Clinical Trial at Columbia University Medical Center



PROSTABEL REDUCES Men's PSA Counts

"Initially, I learned about Prostabel from my patients who were taking it for prostate conditions and using it for effectively lowering their PSAs," says Dr. Aaron Katz, the nationally recognized urology surgeon, researcher, author, and director of the Columbia University Center for Holistic Urology.

To understand why we think so highly of Prostabel, you should know why we think so highly of the physician Dr. Katz and also the work of Dr. Mirko Beljanski.

Aaron Katz, M.D., is probably the most important clinician today when it comes to the fast-growing field of complementary medicine and men's health. Katz is also a national leader in cryosurgery, particularly focused on cryoablation. That his research is coming out of Columbia University, whose hospital and teaching schools are considered to be among in the world, adds even greater cre also author of *Dr. Katz's Guide to Pr* (Freedom Press, 2005).

In the case of Prostabel, Dr. K Sylvie Beljanski of Natural Source Inter Ms. Beljanski is the daughter of the la ian and scientist Dr. Mirko Beljanski French Pasteur Institute and Monic who was also a notable researcher an the United States.

The two had several meetings York City offices in which they disc Beljanski's work. Sylvie shared with father's many scientific articles a results, especiall research after Pasteur Institute.

Dr. Katz reca home a lot of re rial!" Yet, bringin to Dr. Beljansk seemed to work. science was exce initely many deca his time. He was first to open up t of structural DN alone his vision of the secret wholly unique and powerful."

With more than 130 peer-re cations in his lifetime, Dr. Belj ered the secrets of the structu the same time that others w decipher the genetic code ble helix. Although gene became the buzzword for some four or f

these were largely one-dimensional ways of looking at the secret of life and could not account for earlier damage that occurred to the DNA before the presence of genetic mutations.

Beljanski's work is now becoming the basis for a whole new branch of research into the code of life. And it doesn't hurt that this research is showing promise in a major clinical trial at Columbia University.

STUDIED LIKE A PHARMACEUTICAL

Since that first meeting with Ms. Beljanski and her team, Dr. Katz, together with Columbia University,

Dr. Katz:

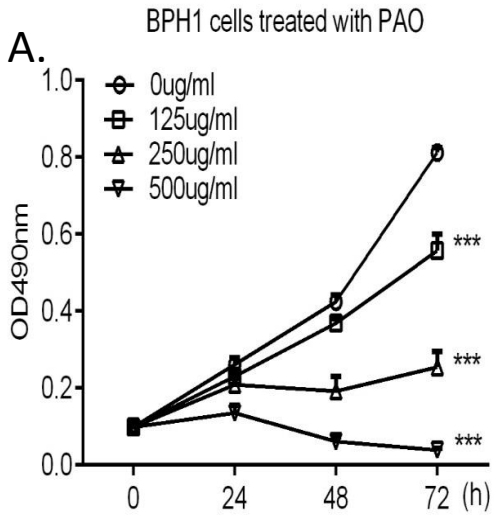
"We now know that the combination of Pao and Rauwolfia extracts can significantly lower PSAs in a 12-month period… and we have found a number of patients who have had a dramatic improvement in their urinary symptoms.

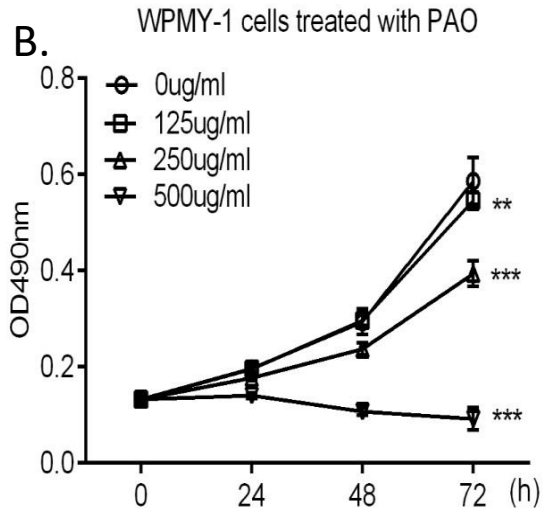
Men are clearly having less frequency, better streams, and better flow rates. They are not getting up at night as often."

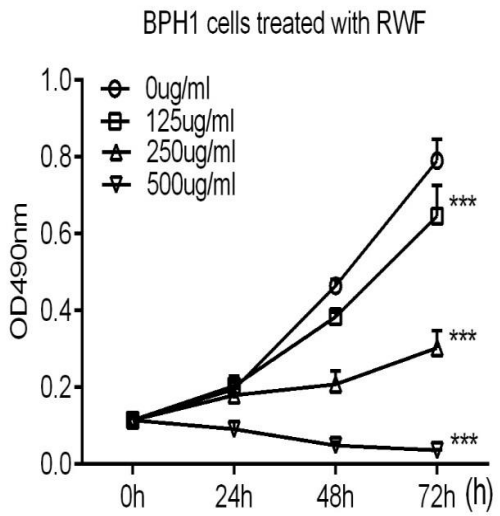
Nanjing University

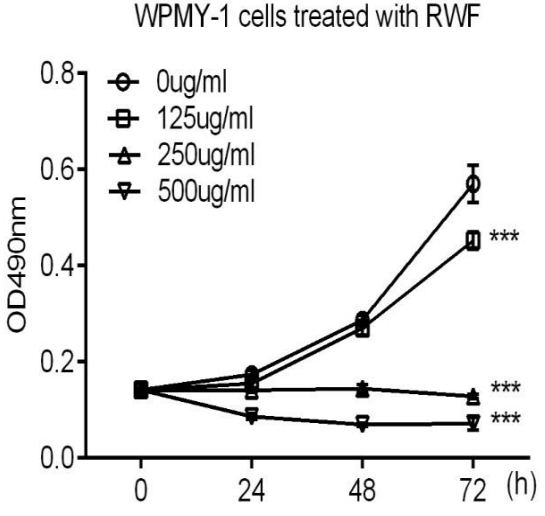
Dr. Jun Yan

Pao and *Rauwolfia* Suppress Proliferation of Human BPH1 and WPMY-1 Cells



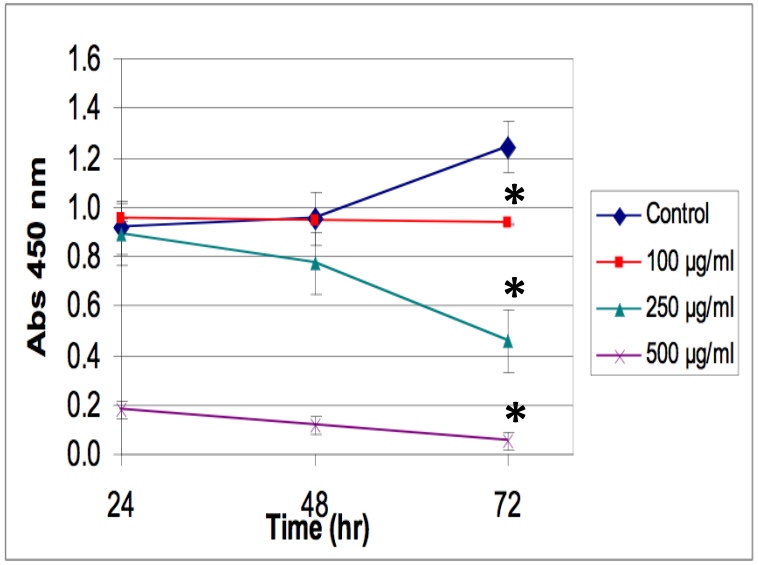




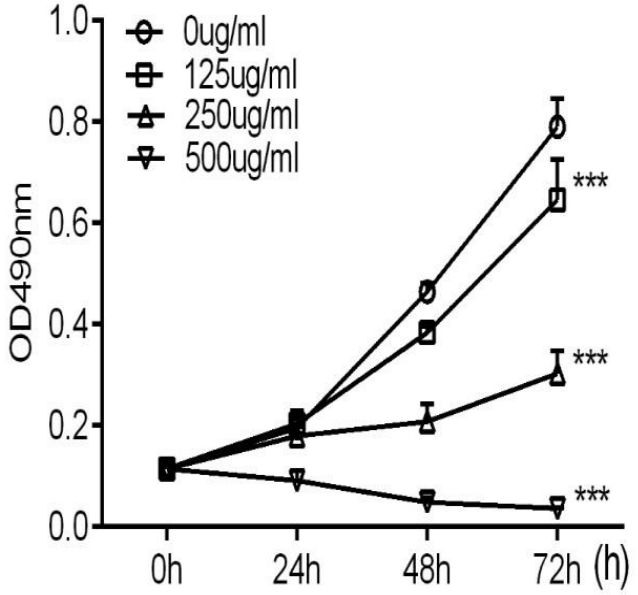


Compare Activity of *Rauwolfia*: Anti-cancer (prostate); Anti-inflammation BPH (prostate)

LNCaP treated with RWF



BPH1 cells treated with RWF



Publication Nanjing University

Original Article

Pao Pereira Extract Suppresses Castration-Resistant Prostate Cancer Cell Growth, Survival, and Invasion Through Inhibition of NFκB Signaling

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201X, Vol XX(X) 1–10

DOI: 10.1177/1534735413510557
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Cunjie Chang, BS[1], Wei Zhao, MS[1], Bingxian Xie, BS[1], Yongming Deng, BS[2,3], Tao Han, BM[2,3], Yangyan Cui, BS[1], Yundong Dai, BS[4], Zhen Zhang, BS[4], Jimin Gao, MD, PhD[4], Hongqian Guo, MD, PhD[2,3], and Jun Yan, PhD[1,4]

Abstract

Pao extract, derived from bark of Amazonian tree Pao Pereira, is commonly used in South American medicine. A recent study showed that Pao extract repressed androgen-dependent LNCaP prostate cancer cell growth. We hypothesize that Pao extract asserts its anticancer effects on metastatic castration-resistant prostate cancer (CRPC) cells. Pao extract suppressed CRPC PC3 cell growth in a dose- and time-dependent manner, through induction of apoptosis and cell cycle arrest. Pao extract treatment induced cell cycle inhibitors, p21 and p27, and repressed PCNA, Cyclin A and Cyclin D1. Furthermore, Pao extract also induced the upregulation of pro-apoptotic Bax, reduction of anti-apoptotic Bcl-2, Bcl-x_L, and XIAP expression, which were associated with the cleavage of PARP protein. Moreover, Pao extract treatment blocked PC3 cell migration and invasion. Mechanistically, Pao extract suppressed phosphorylation levels of AKT and NFκB/p65, NFκB DNA binding activity, and luciferase reporter activity. Pao inhibited TNFα-induced relocation of NFκB/p65 to the nucleus, NFκB/p65 transcription activity, and MMP9 activity as shown by zymography. Consistently, NFκB/p65 downstream targets involved in proliferation (Cyclin D1), survival (Bcl-2, Bcl-x_L, and XIAP), and metastasis (VEGFa, MMP9, and GROα/CXCL1) were also downregulated by Pao extract. Finally, forced expression of NFκB/p65 reversed the growth inhibitory effect of Pao extract. Overall, Pao extract induced cell growth arrest, apoptosis, partially through inhibiting NFκB activation in prostate cancer cells. These data suggest that Pao extract may be beneficial for protection against CRPC.

Keywords

Pao extract, herbal medicine, castration-resistant prostate cancer, cell growth arrest, apoptosis, NFκB signal pathway

Introduction

Prostate cancer is one of the leading causes of deaths in men, with the estimation that more than 258 000 men will die from this disease worldwide in 2011.[1] Most deaths from prostate cancer are due to metastases, and usually these lesions become resistant to androgen ablation therapy.[2] Only about 30% of the patients with distant prostate cancer survive 5 years after diagnosis, compared to almost 100% 5-year relative survival rates among patients with localized or regional prostate cancer.[3] Unfortunately, no curative treatment exists for those patients at this stage. As drugs currently used have significant adverse effects, herbal extracts, as well as phytochemicals derived from them, are considered as attractive alternatives.

Pao extract is the extract of the bark of a tree that grows in the Amazon rain forest, *Geissospermum vellosii* Allemão (familiarly known as Pao pereira), which has been used as a medicine by South American Indian tribes. It is reported that Pao extract has anticancer effects against melanoma and glioblastoma cells in vitro.[4-6] Moreover, Pao extract suppresses cell growth and induces apoptosis of androgen-dependent prostate cancer LNCaP cells in vitro and in vivo.[7] These data suggest that Pao extract is a promising agent against cancer. For men with metastatic disease, it is important to determine whether Pao extract also possesses anticancer effects against devastating castration-resistant prostate cancer (CRPC), which may appear following androgen ablation therapy.

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Summary of Research on Prostate Cancer and Prostate Inflammation

Pao and *Rauwolfia* extracts are effective against prostate cancer cells and prostate tumors as shown by in vitro and in vivo studies.

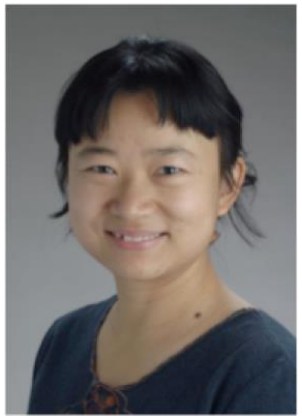
The extracts are selective and safe—they kill cancer cells and shrink tumors—but they do not affect healthy animals.

Pao and *Rauwolfia* are effective against inflammation of the prostate.

The extracts act against prostate cells that are benign, but hyperplasic (BPH).

The *Pao* extract has also been shown to be effective against advanced prostate cancers that no longer respond to hormone treatment.

KU MEDICAL CENTER
The University of Kansas



Jeanne Drisko, MD
Director, KU Integrative Medicine
Riordan Endowed Professor of Orthomolecular Medicine

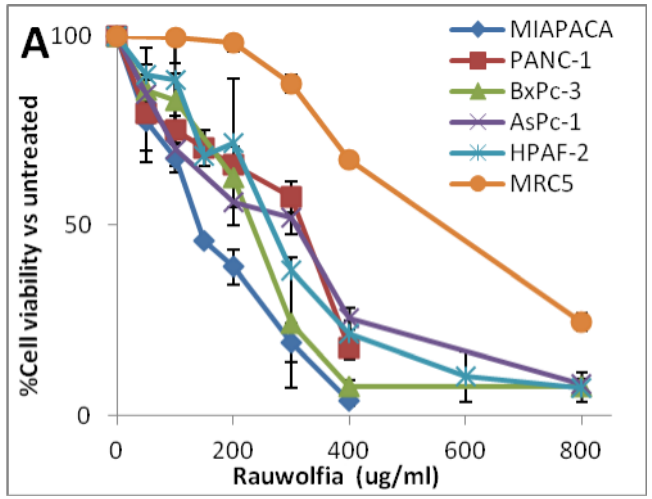
Dr. Qi Chen
Assistant Professor

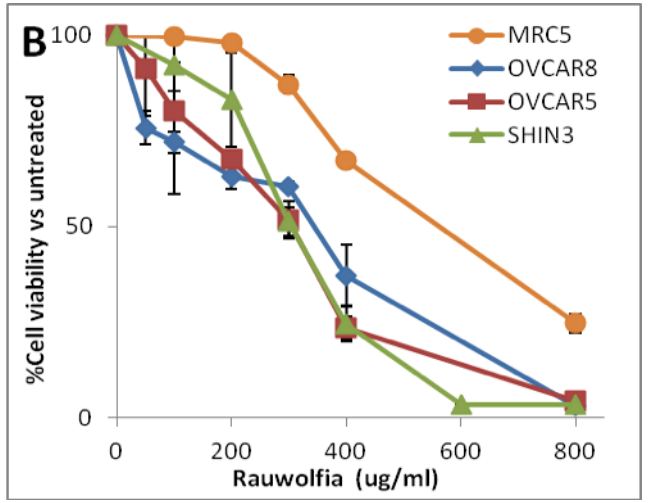
Pao and *Rauwolfia* Selectively Kill Pancreatic and Ovarian Cancer Cells

Pancreatic Cancer

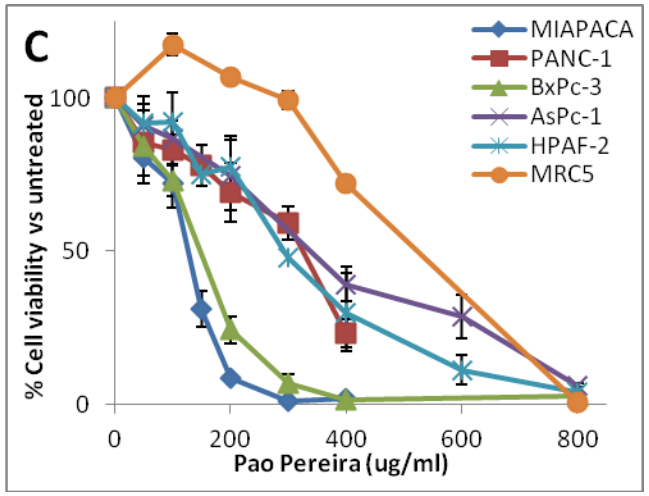
Ovarian Cancer

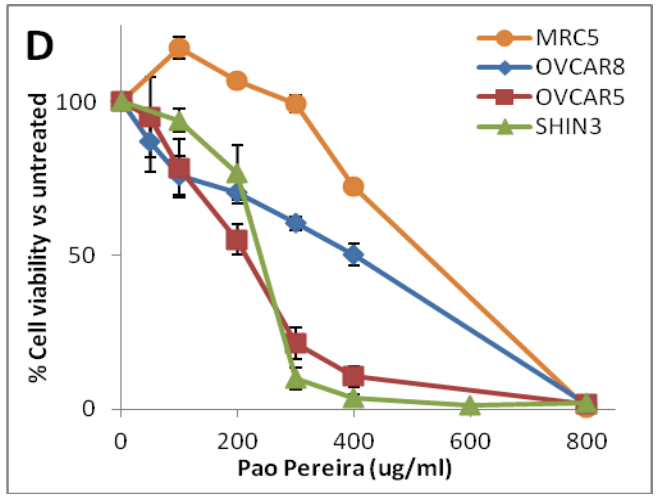
Rau



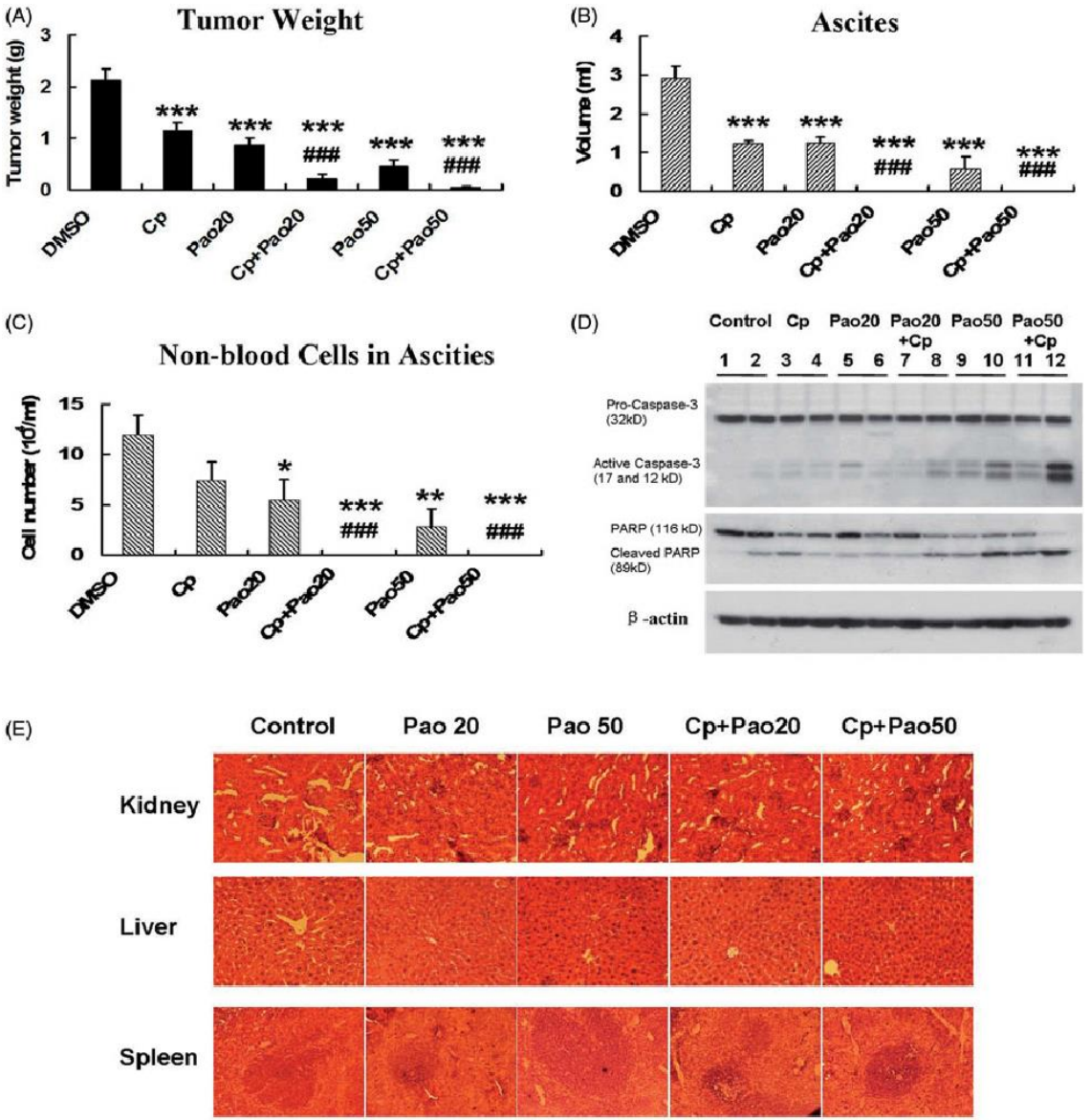


Pao

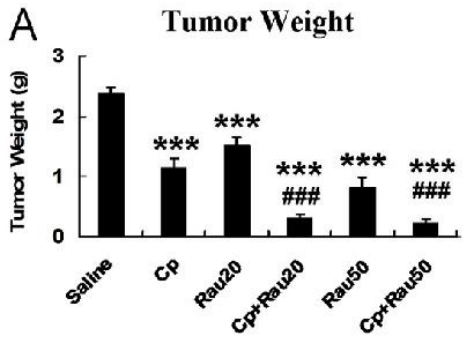


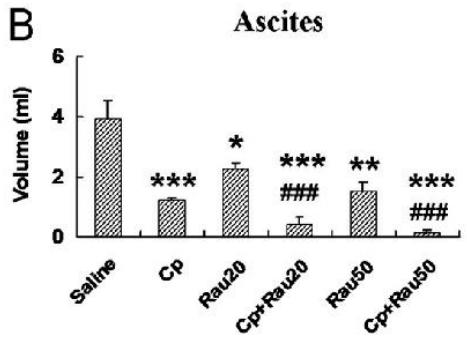


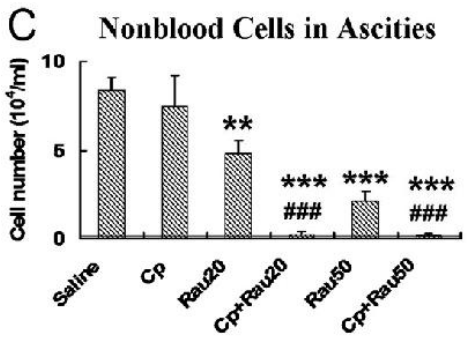
Pao Significantly Reduces Ovarian Cancers by Inducing Apoptosis

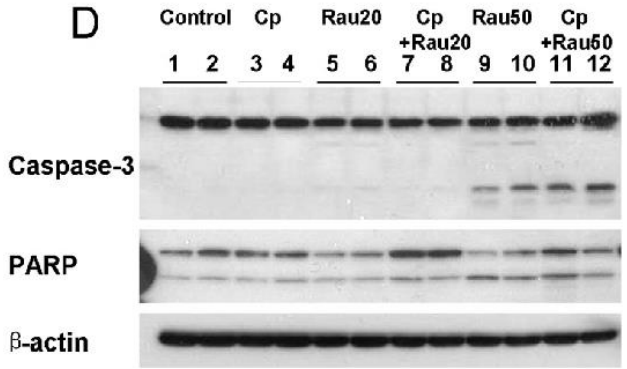


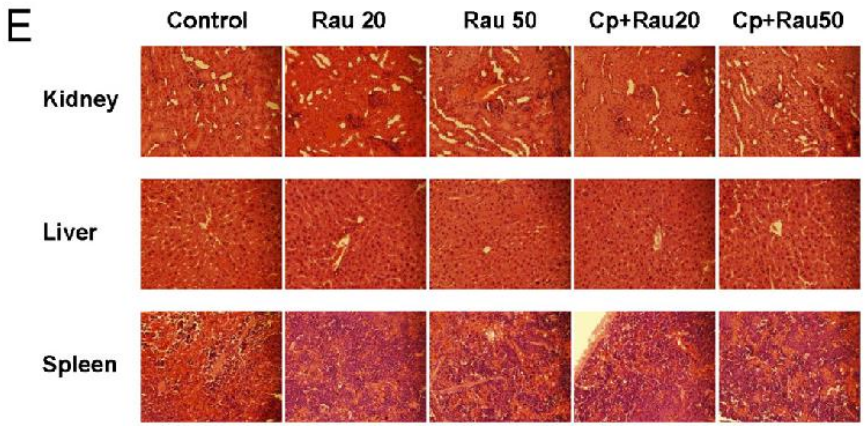
Rauwolfia Significantly Reduces Ovarian Cancers by Inducing Apoptosis











ONCOLOGY REPORTS

Inhibition of pancreatic cancer and potentiation of gemcitabine effects by the extract of Pao Pereira

JUN YU[1,2], JEANNE DRISKO[2] and QI CHEN[1,2]

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Pao treatment possessed low toxicity as no toxic effect was observed associated with the treatments. At the end of the experiments, major organs (kidney, liver and spleen) were subjected to haematoxylin and eosin (H&E) staining and histological analysis. No tissue damage was detected in any of the groups.

The extract of *Pao Pereira* (*Pao*) exhibited strong inhibition in PANC - 1 tumors throughout the course of the experiment, reaching >70% inhibition even when tumors did not respond to Gem anymore. Consistent with the *in vitro* dose the reduction effect for Gem, the combination of Pao and Gem had a better effect than Gem *in vivo.*

In addition, by the dose-reduction effect, *Pao* allowed for lower concentrations of Gem while achieving an equivalent cytotoxicity in cancer cells with higher Gem concentrations alone. This may decrease the toxicity associated with chemotherapy.

Article

Antitumor Activities of *Rauwolfia vomitoria* Extract and Potentiation of Gemcitabine Effects Against Pancreatic Cancer

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SAGE

Jun Yu, PhD[1] and Qi Chen, PhD[1]

Abstract

Pancreatic cancer is one of the most lethal malignancies with very limited treatment option. In the effort of enhancing the effect of the conventional chemotherapeutic drug gemcitabine against pancreatic cancer, we investigated *in vitro* and *in vivo* the anticancer effect of a β-carboline-enriched extract from the plant *Rauwolfia vomitoria* (Rau), either alone or in combination with gemcitabine, in preclinical pancreatic cancer models. Rau induced apoptosis in pancreatic cancer cells in a concentration-dependent manner, and completely inhibited colony formation of PANC-1 cells in soft agar.

The combination of Rau and gemcitabine had synergistic effect in inhibiting cell growth with dose reduction effect for gemcitabine. In an orthotopic pancreatic cancer mouse model, PANC-1 tumor growth was significantly suppressed by Rau treatment.

Metastasis was inhibited by Rau. Adding Rau to gemcitabine treatment reduced tumor burden and metastatic potential in the gemcitabine non-responsive tumor.

These data suggest that Rau possesses anti–pancreatic cancer activity and could improve effect of gemcitabine.

line therapy of metastatic pancreatic cancer. Gemcitabine provides benefit at early stages of the disease; however, it has little impact on median overall survival for patients with locally advanced or metastatic disease, who comprise the majority of cases.[6-8] Recent clinical trials adding agents to gemcitabine had statistical significance, but are not really meaningful for patients.[9-15] A new gemcitabine-free regimen

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healthcare

ORIGINAL ARTICLE

The plant extract of *Pao pereira* potentiates carboplatin effects against ovarian cancer

Jun Yu and Qi Chen

Department of Pharmacology, Toxicology and Therapeutics, KU Integrative Medicine, University of Kansas Medical Center, Kansas City, KS, USA

Abstract

Context: Herbal preparation of *Pao pereira* [*Geissospermum vellosii* Allem (Apocynaceae)] has long been used by oncologic patients and Integrative Medicine practitioners in South America. However, its anticancer activities have not been systematically studied.

Objective: To investigate the anticancer effects of β-carboline alkaloids-enriched extract from *Pao pereira* (Pao), either alone or in combination with carboplatin, in preclinical ovarian cancer models.

Materials and methods: Cytotoxicity of Pao (0–800 μg/ml) against different ovarian cancer cell lines and an immortalized epithelial cell line was detected by flow cytometry, MTT assay and colony formation in soft agar. Combination of Pao and carboplatin, a primary chemotherapeutic drug for ovarian cancer, was evaluated using Chou-Talalay's methods. Mice bearing intraperitoneally spread ovarian cancer were treated with 20 or 50 mg/kg/day Pao by i.p.

Keywords

Anticancer, combination therapy, natural product, synergy

History

Received 4 February 2013
Revised 4 April 2013
Accepted 21 May 2013
Published online 13 September 2013

In vivo, Pao alone suppressed tumor growth by 79% and decreased volume of ascites by 55%. When Pao was combined with carboplatin, tumor inhibition reached 97% and ascites was completely eradicated.

Discussion and conclusion: Pao possess potent antitumor activity and could enhance carboplatin effect, and therefore holds therapeutic potential in the treatment of ovarian cancer.

the female reproductive system. Due to lack of sufficiently accurate screening approaches in the early detection of ovarian cancer, the majority of cases (63%) are diagnosed at advanced and distant stage (Beller et al., 2006; Buys et al., 2005; Chen et al., 2011). These patients suffer from a dismal prognosis and severely impaired quality of life. Though primary therapy has improved 5-year survival, it has not increased the overall rate of cure (Bast, 2011), because more than 70% of ovarian cancer patients relapse and develop resistance to platinum- and taxane-based treatment (Beller et al., 2006; Monk & Coleman, 2009). Malignant ascites resistant to conventional chemotherapy affects 28% of ovarian cancer patients in their last period of life (Bellati et al., 2010). There is an urgent need for novel and effective treatment options for advanced ovarian cancer.

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incorporating newer cytotoxic agents. Natural products have long been proven a bountiful resource for bioactive anticancer agents. Combination of natural compounds to standard chemotherapeutic drugs may exert additive or synergistic effects in killing cancer cells, therefore would achieve better therapeutic effect or allow lower and safer drug doses to be applied. One of such examples is the success of taxol as a chemotherapeutic agent, which was first isolated from the bark of the Pacific yew tree Taxaceae *Taxus brevifolia* Nutt. The platinum–taxol combined chemotherapy had achieved much better clinical outcomes in ovarian cancer patients than either drug alone and has become a standard regimen in treating ovarian cancer (Donaldson et al., 1994; du Bois et al., 1997; Goldberg et al., 1996; McGuire et al., 1996; Milross et al., 1995; Ozols, 1995; Pujade-Lauraine et al., 1997). In recent decades, numerous experimental and clinical works have been done investigating the anticancer effects of plant extracts, especially those used as folk medicines.

Pao pereira [*Geissospermum vellosii* Allem (Apocynaceae)] (Pao) extract, an herbal preparation of the bark of the Amazonian tree Pao, has been used traditionally as

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Antitumor Activities of *Rauwolfia vomitoria* Extract and Potentiation of Carboplatin Effects Against Ovarian Cancer☆

Jun Yu, PhD[1,2], Yan|Ma, PhD[1,2], Jeanne Drisko, MD[2], Qi Chen, PhD[1,2,*]

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ARTICLE INFO

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ABSTRACT

Background: Tumor resistance to platinum-based drugs has been an obstacle to the treatment of ovarian cancer. Extract of the plant *Rauwolfia vomitoria* has long been used by cancer patients. However, there have not been systematic studies of its anticancer activity.

Tumor growth in mice was significantly suppressed by 36% or 66% with Rau treatment alone at a low (20 mg/kg) or a high dose (50 mg/kg), respectively, an effect comparable to that of Cp alone. The volume of ascitic fluid and the number of nonblood cells in ascites were also significantly decreased. Combining Rau with Cp remarkably enhanced the effect of Cp and reduced tumor burden by 87% to 90% and ascites volume by 89% to 97%.

Conclusions: Rau has potent antitumor activity and in combination significantly enhances the effect of Cp against ovarian cancer.

biomarkers for early detection, ovarian cancer is usually diagnosed in patients at a late stage of the disease.[2–4] As a result, these patients have a poor prognosis and severely impaired quality of life. Although current primary therapy can improve the 5-year survival

Numerous studies have attempted to improve the efficacy of standard platinum-based therapy by incorporating newer cytotoxic agents. A promising strategy is to use natural products with anticancer effects in combination with platinum-based drugs. One of the advantages of some natural products is their low toxicity compared with conventional chemotherapy drugs. Combinations of natural compounds with standard chemotherapy drugs may exert additive or synergistic effects on killing cancer cells, thereby allowing lower and safer doses of the more toxic drug to be used.

Herbal preparations of *Rauwolfia vomitoria*, a tropical shrub in the family of Apocynaceae, have been used in traditional folk medicine in Africa to treat a variety of ailments including fever, general weakness, gastrointestinal diseases, liver diseases,

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0011-393X/$ - see front matter
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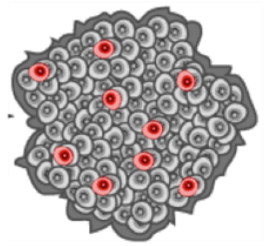
Synergy with Multiple Chemotherapy Drugs

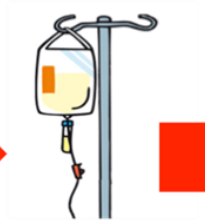
- Lomustine is an alkylating agent, one of a family of chemotherapy drugs that attaches alkyl groups to DNA thus preventing DNA replication and cell division.
- Docetaxel is an anti-mitotic agent, one of a family of chemotherapy drugs that prevents cells from dividing by disrupting microtubule function.
- Carboplatin is one of the platinum-based drugs that bind to DNA and interfere with DNA repair.
- Gemcitabine is a nucleoside analog resembling cytidine that is incorporated into DNA and that blocks further DNA synthesis.
- When applied together to lung cancer cell lines, gemcitabine and docataxel were antagonistic with one another and thus cannot be taken together.
- These data suggest that the *Pao* and *Rauwolfia* extracts will work together with a broad spectrum of anti-tumor drugs.

Source: Theodossiou C, Cook JA, Fisher J, et al. Interaction of gemcitabine with paclitaxel and cisplatin in human tumor cell lines. *International Journal of Oncology*, 1998, 12, pp. 825-32.

Cancer Stem Cells

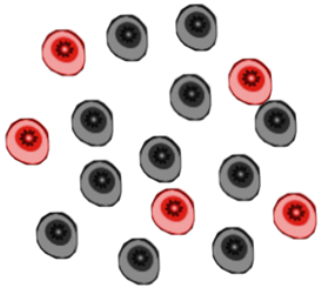


Malignant Tumor
BEFORE
Chemotherapy

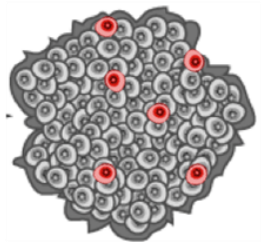


Chemotherapy

Tumor shrinks but remaining cells are drug resistant and have similar characteristics to normal stem cells



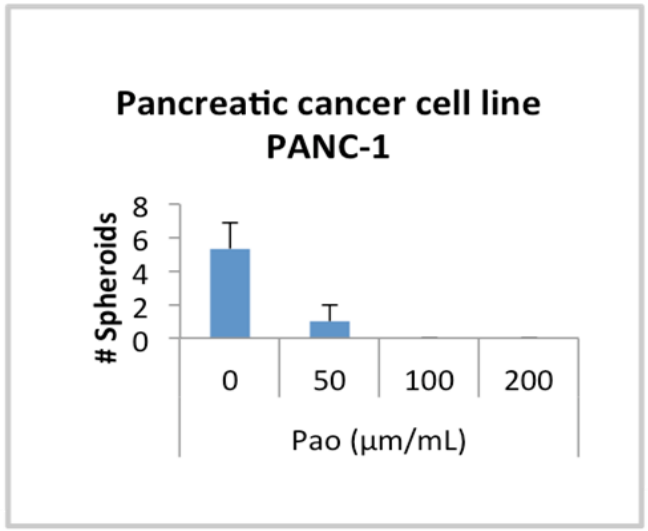
Cancer Stem Cell proliferation
& differentiation 'Spheroids '



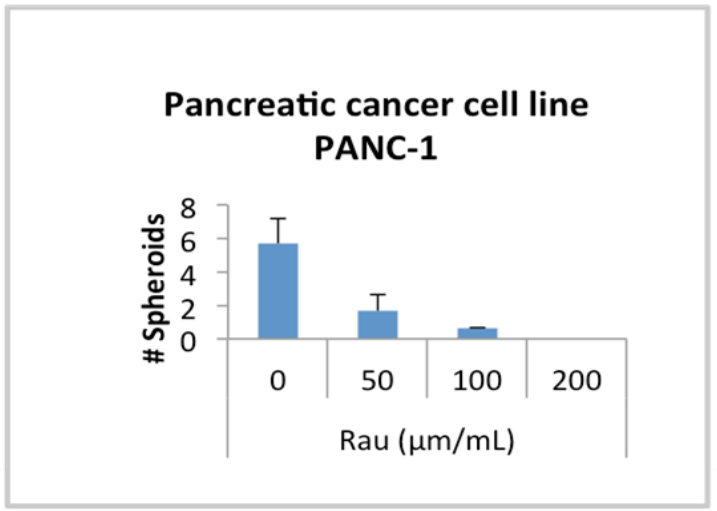
Tumor relapse

Cancer cell that can initiate new tumors - Stem Cell (Spheroid)
Cancer cell that cannot initiate new tumors

In vitro Studies of the Activity of *Pao pereira* and *Rauwolfia vomitoria* on Pancreatic Cancer Stem Cells PANC-1 at Doses ≤ 200 µg/ml

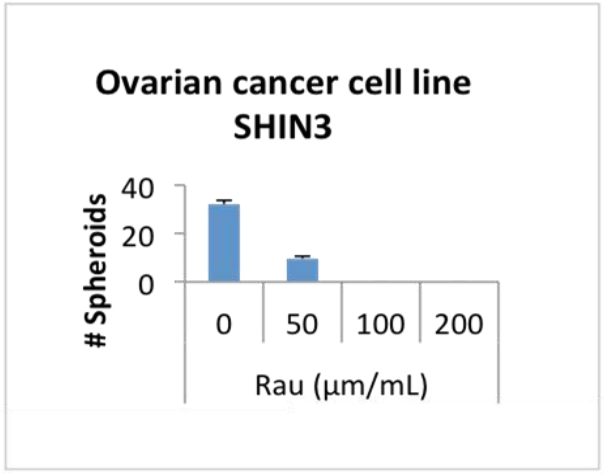


Pao	% Primary Spheroids formation
Ctrl	1.07
50 µg/ml	0.20
100 µg/ml	0
200 µg/ml	0

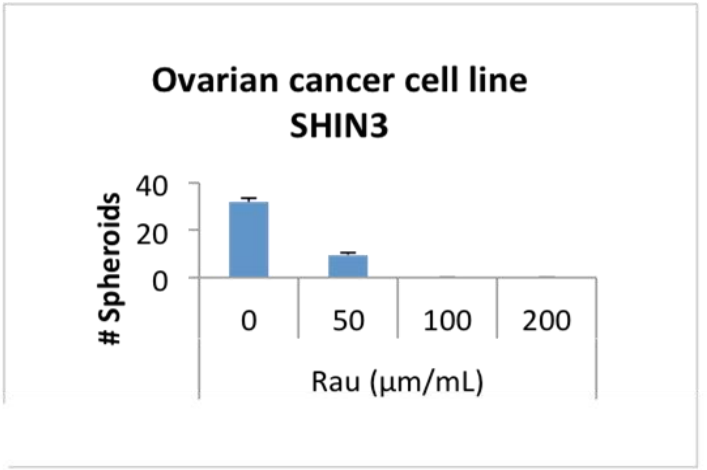


Rau	% Primary Spheroids formation
Ctrl	1.13
50 µg/ml	0.33
100 µg/ml	0.13
200 µg/ml	0

In vitro Studies of the Activity of *Pao pereira* and *Rauwolfia vomitoria* on Ovarian Cancer Stem Cells SHIN3 at Doses ≤ 200 µg/ml



Pao	% Primary Spheroids formation
Ctrl	6.2
50 µg/ml	1.0
100 µg/ml	0
200 µg/ml	0



Rau	% Primary Spheroids formation
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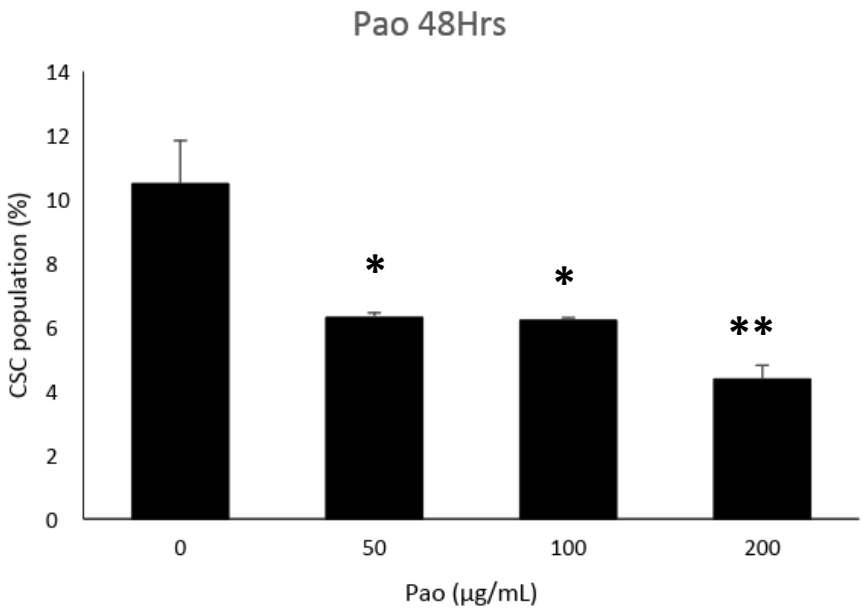
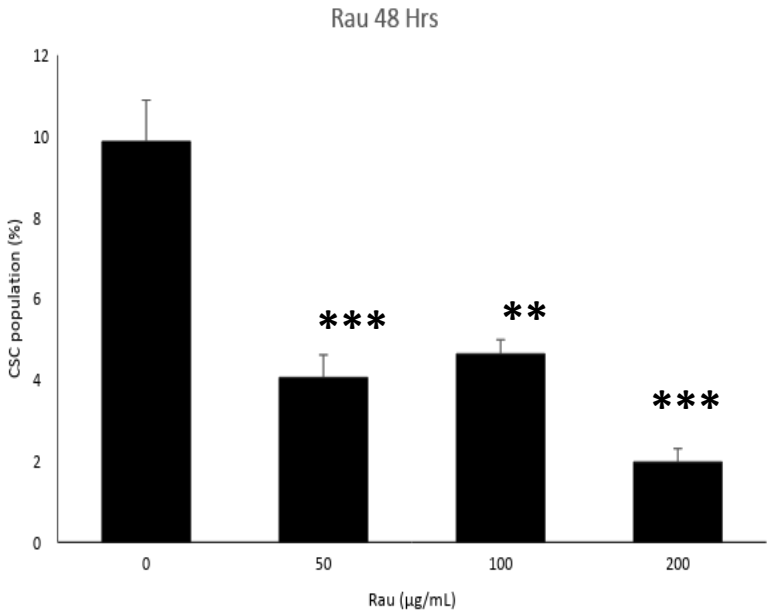
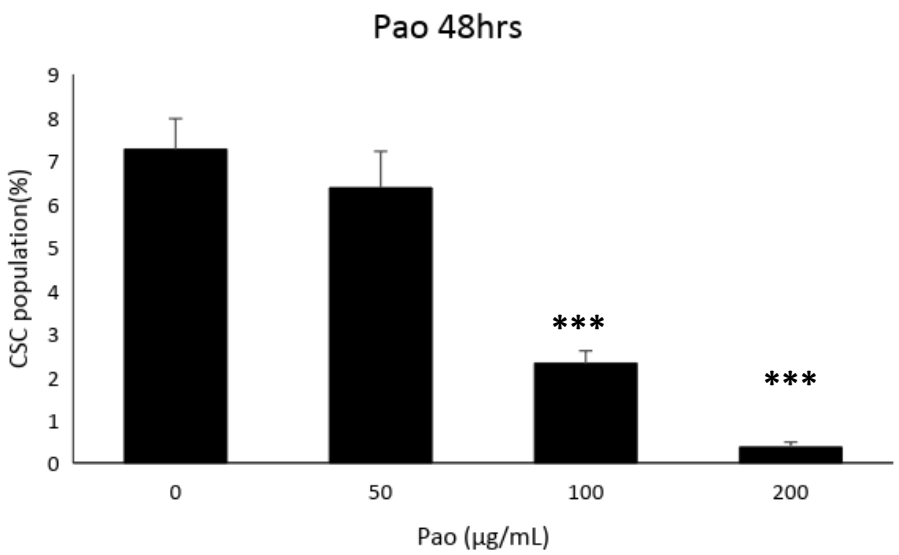
100 µg/ml	0
200 µg/ml	0

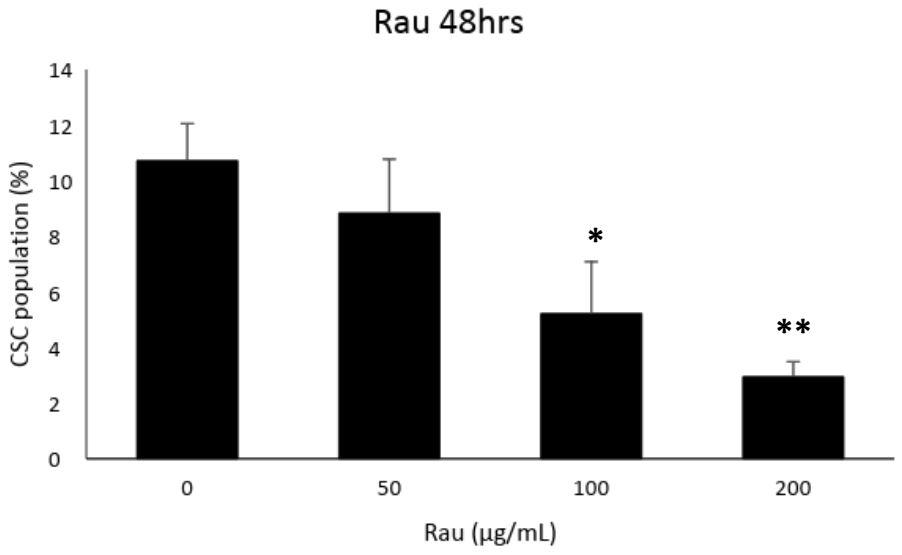
Effect of Extracts on CD44+CD117 Ovarian Cancer Stem Cells



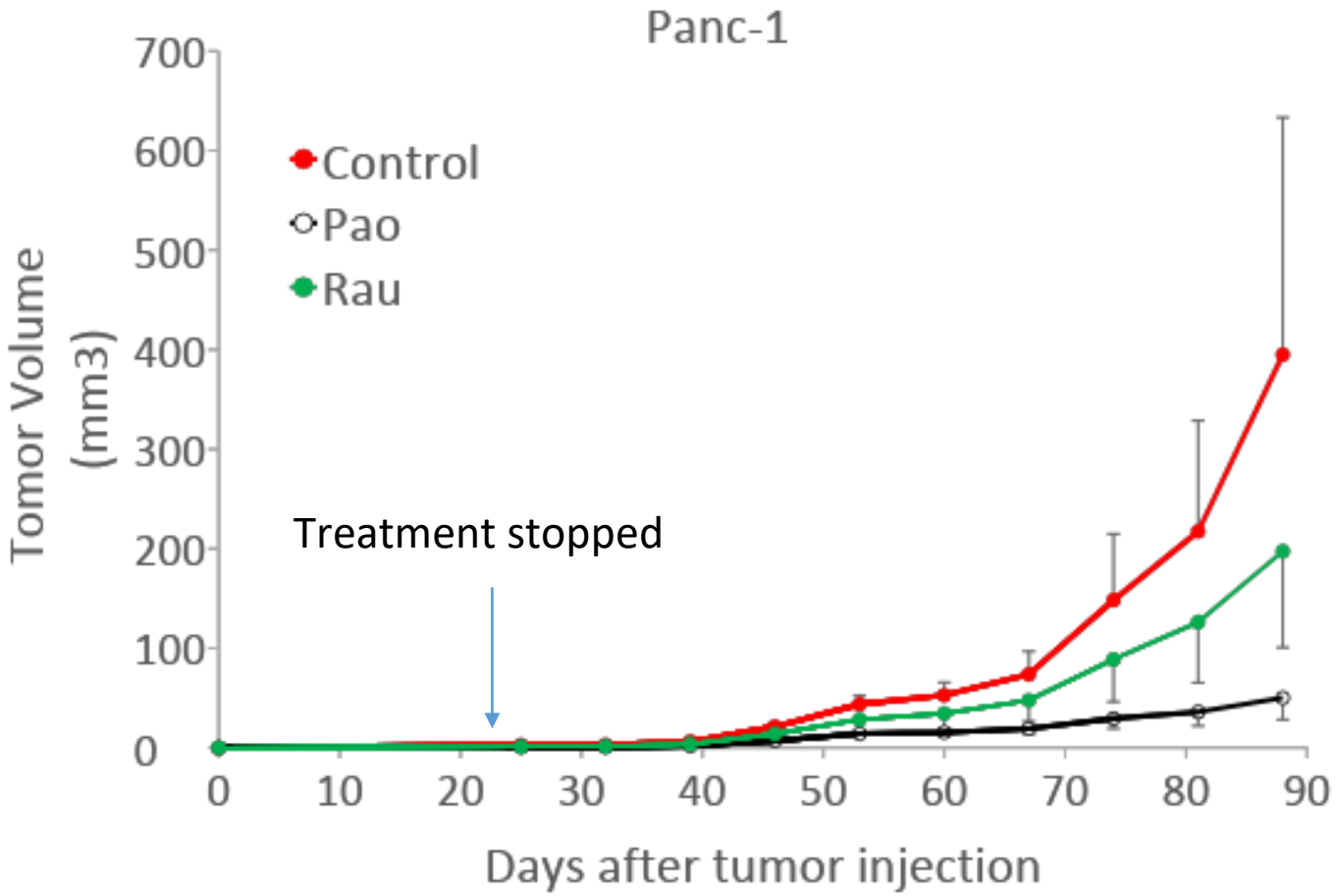


Effect of Extracts on CD24+CD44+EpCam+ Pancreatic Cancer Stem Cells





Effect of Extracts on Tumors Initiated by Pancreatic Cancer Stem Cells



R.G.C.C. – RESEARCH GENETIC CANCER CENTER

- A laboratory in Greece called RGCC has developed a revolutionary test that is changing our perception of cancer and advancing our methods of treatment.
- RGCC is a genetic research laboratory that has developed a patented membrane that is able to capture malignant cells from the blood of cancer patients.
- The circulating cancer cells are then tested to determine which treatments work best against the individual's cancer. The RGCC test allows oncologists to be specific with their chemotherapy treatments and also allows other practitioners as well as the patient to know what may be the best natural approach for their cancer therapy as well.

R.G.C.C. – RESEARCH GENETIC CANCER CENTER

- A test to detect in vitro the death of specific cancer cells
- Tests on patients with the following cancers:

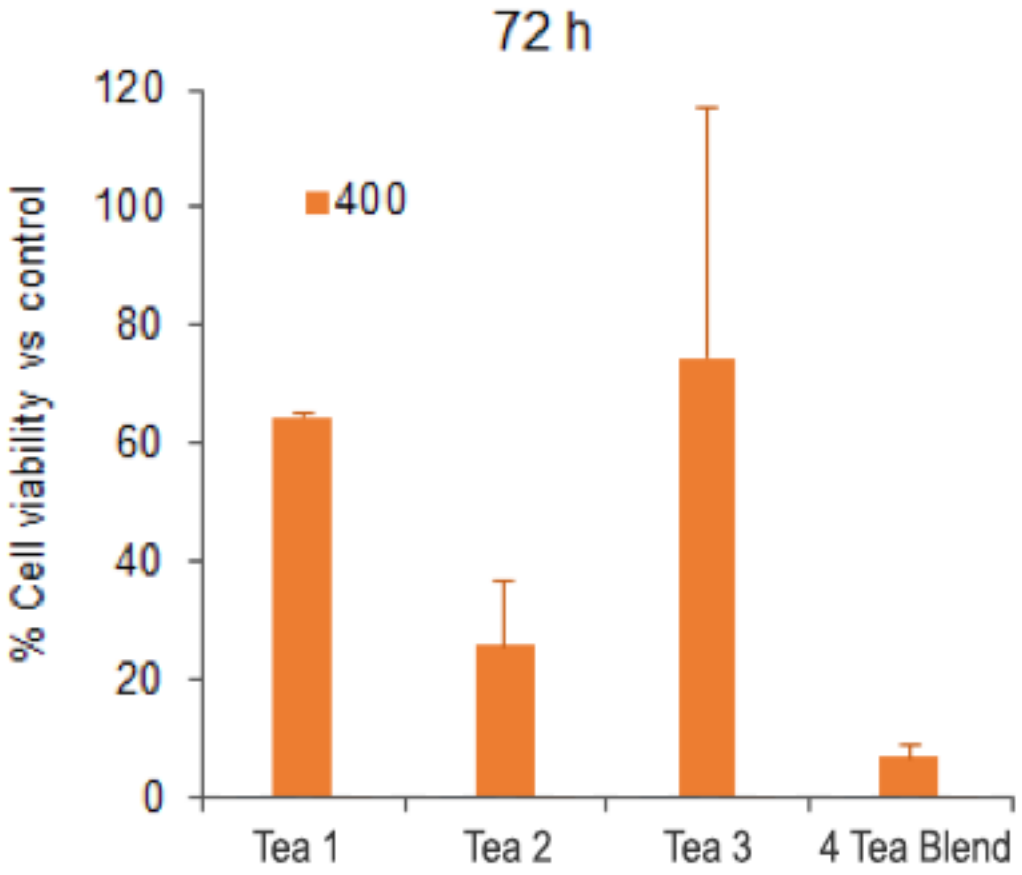
  - **ovarian** stage 2 and 4
  - **pancreatic**
  - **stomach** stage 4
  - **lung**
  - **uterus**
  - **breast**
  - **parotid gland** stage 4
  - **colon** stage 3
  - **small intestine** stage 4

- Results:

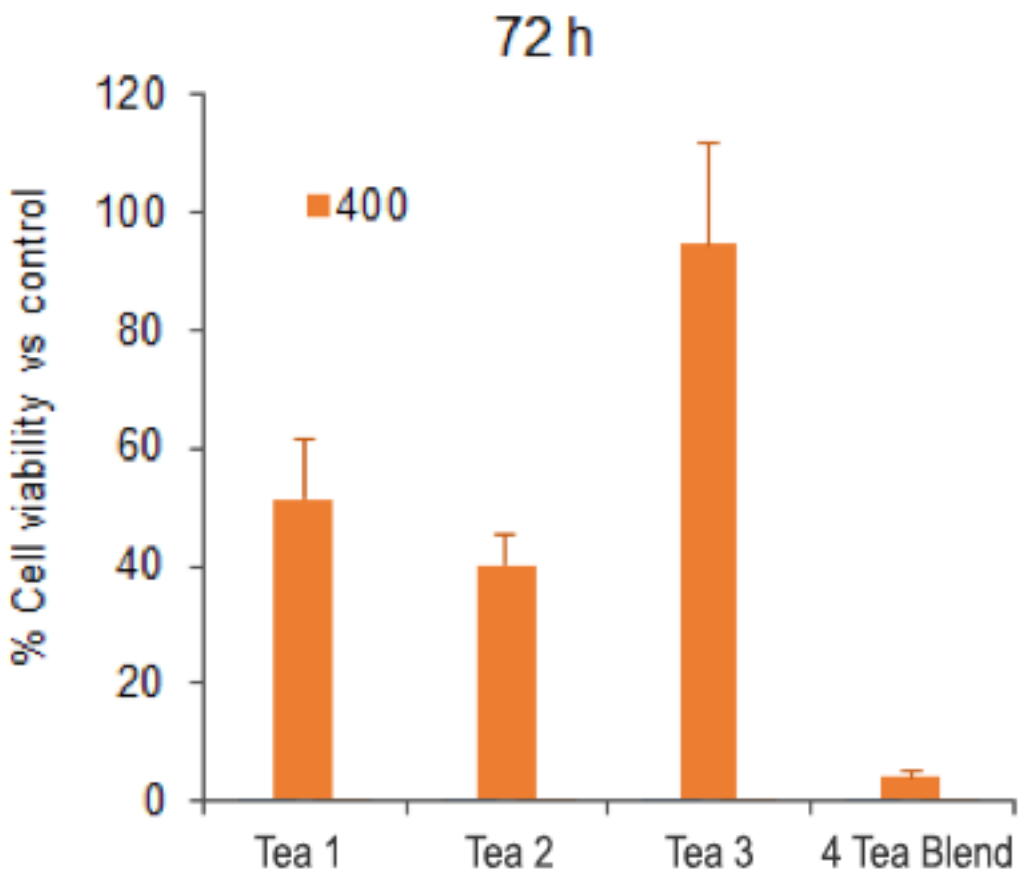
  - Selectivity of action confirmed on cancer cells
  - No apoptosis in health cells triggered by the *Pao pereira* or *Rauwolfia vomitoria*

Activity of Tea Extracts on Breast Cancer Cells

MCF-7 Breast Cancer



MDA-MB-231 Metastatic Breast Cancer



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Thank You

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