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Common Sense and Nutritional Faddism or...
Grandma Was Right!

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I am not the first nor will I be the last to discuss the topic of nutritional faddism. As a child of the Depression and now a well-seasoned professor in the nation’s oldest college of pharmacy, I have witnessed over forty years of change in food and drug use in our society.

More and more lately, I have felt the need to fall back on common sense to understand and help explain today’s headlong rush into the maze of ways to live healthier and longer through exotic diets and modern medicine. First let me quote a few folks on what common sense is. I like Harriet Beecher Stowe’s quote the best: “common sense is the knack of seeing things as they are, and doing things as they ought to be done.” Then there is Robert Green Ingersoll’s definition: “it’s a thousand times better to have common sense without education than have education without common sense.” Finally, Will Rogers succinctly stated “common sense ain’t common” is very much to the point.

As a first generation Armenian-American, growing up in the United States in a loving home with my mother, dad, grandparents, aunts and uncles I learned a lot of folk wisdom. They distilled years of old-world health practices into still useful “modern” approaches to stay well. Wellness and knowing how to avoid illness was common in the 1930s to 1950s in this country, mainly because most immigrants couldn’t afford doctors and modern medicine was in its infancy.

A few examples of common medicines from a half-century ago are peppermint tea for stomachs (known carmi-
native), tincture of iodine as a mild antiseptic (iodine-PVP is still used for this purpose), and yogurt (live cultures of Lactobacillus, etc. now being touted as “probiotics” for various gastrointestinal problems). Most of these “old-timers” have re-emerged as alternative supplements for many of today’s ailments.

The whole “back to nature” or complementary and alternative medicine (CAM) movement has caught on in the last decade — herbal products are used by thirty percent of the North American population. The overall nutraceutical industry is estimated at twenty billion dollars per year. Almost simultaneously, there has been a remarkable decline in what people know anymore about herbs, or food as medicine. This explains the reoccurring food faddism problem in this country, the tendency to consume idiosyncratic diets and take on eating patterns which are supposed and promoted to improve health. Many food fads claim much more than modern nutritional science has substantiated.

Nutritional faddism and fraud flourish today for various reasons. These include the diversity of cultures, the general historical tradition of concern for longer good life and health and simple faith in “natural remedies.” Modern advertising, capitalism, increased pecuniary interests, and the classic profit motive have driven many marketers into this arena. Modern “health food” supplements and remedies abound in the current marketplace.
So what does common sense dictate about this? Basically it involves knowing what’s really needed in a normal diet and consuming these in adequate amounts coupled with moderate exercise, and we need to take the politics out of nutrition. Here are a few simple nutritional facts that have been known for a long time. Eat three to four small meals a day. Drink about eight eight-ounce glasses of water daily. Get adequate fiber from six to eight servings of fruits and vegetables daily. Avoid highly processed foods. Limit the intake of saturated and trans-fats. Eat more of the correct complex carbohydrates. Avoid stress, as this often leads to undereating or overeating problems. Avoid consuming excessive junk or fast foods like sodas, French fries, high sugar cookies, high fat burgers, and avoid alcohol and tobacco. Remember that the best way to assimilate vitamins and minerals is through a balanced diet of the plants that contain them. Avoid too much caffeine (coffee, tea, chocolate, guarana, mate tea) because it can increase heart rate, possibly increase irritability, interfere with sleep patterns, possibly contribute to stomach problems, and can cause anxiety.

Many commercial diets attempt to provide all of these advantages, but at a price, e.g. program enlistment and special products. Currently in vogue: The South Beach Diet, the Atkins Diet, the Sugar Busters Diet, the Weight Watcher’s Diet, and the low carb diets. Many work when you stick to them, follow their restrictions, spend the money, time and effort, follow the rules and exercise sufficiently. Remember that a common sense diet is less expensive, particularly when you cook your own foods from scratch, make them to your own taste, and avoid too much sugar, salt, fat and other processed food ingredients. A healthy diet should be flexible, varied, not too restrictive, and focus on good health and weight.

Other common-sense suggestions are to use good “food pyramid” guidelines, watch for over-sized portions—eat half and take the rest home for another meal. Serve small portions and eat slowly. Remember, taste is in the mouth! Eat a salad or fresh fruit. Use mayonnaise and other special caloric sauces sparingly. Drink water or diet beverages. Unfortunately, many of these simple approaches have been lost in our modern society. Marketeering for profit has brought us too many foods high in sugar and salt, overly convenient, super-sized, and with dubious health benefits.

There is little doubt that much of today’s food advertising is focused towards children and is out of control. These ads through mediums such as television are known and effective ways to generate high profits, while leading to bad food habits in our children. Some make sense, like adding calcium to orange juice, but others are questionable, such as high-fructose corn-syrup-laced low real juice “fruit juices.” There are many good trends in the nutraceutical era as we discover which foods contain the best nutrients and which foods have true medicinal qualities. We also need to teach basic nutrition as early as grammar school so children can learn what’s good or bad for their bodies at an early age. We need to rid educational facilities of high sugar sodas, candy, and such, and return to more widely available fresh fruits, milk, and appropriate bread and related products.

In teaching nutrition to college age students, I have found a tremendous lack of knowledge in all of these basic pointers. Many college students have never eaten rice, fresh tomatoes, plain yogurt, cabbage dishes, olive oil, fresh bread, and more. There is, therefore, a definite need to lend perspective to the younger generation on things learned (and still practiced) from the old country. Fortunately, we are learning some of this from our newer immigrants, such as Asians and Mexicans. This is important because the next generation is quickly moving into a global economy and world market. Those who can speak foreign languages, appreciate other cultures and enjoy the foods of other lands will benefit. We already have the status of an eclectic food market and are just now enjoying the benefits of foods from all over the world in our newer malls. Many feature the foods of China, Korea, Japan, Italy, the Middle East, etc. All we need to do is be sure we know and promote the advantages of eating a wide variety of foods that hopefully will allow us to reach the full genetic potential for life we have inherited from our parents.

An excellent overview and current coverage of obesity in America has been featured in the June 7, 2004 issue of Time Magazine. It goes into detail on why we eat so much, who the anti-fat crusaders are, who the weight loss heroes are, what we should be teaching our children, and includes a guide to diet books. Several excellent nutritional texts provide solid data on sensible diets, for example, Understanding Nutrition, E. Whitney, et al, 10th ed. 2004, Thomson and Wadsworth, Belmont, California. I believe these kinds of texts and articles should be required reading for all our young. After all, we shouldn’t be eating ourselves to death, but rather eating ourselves to better health and longer lives.
Addressing Human Dimensions in Implementing a Nutraceutical Product Trial

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INTRODUCTION

This article is intended to identify pitfalls and challenges that can arise primarily from human dimensions in trials evaluating nutraceuticals for health maintenance and disease prevention. It is based on the author’s experience in spearheading or helping to direct a number of trials of nutraceuticals, such as a nutraceutical supplement for potential cancer prevention, a quality-of-life impact trial using a nutraceutical product for pancreatic cancer patients at a medical center, a trial of a nutraceutical product to alleviate chemotherapy distress, and a trial evaluating a nutraceutical product to assess its value for maintaining and enhancing prostate health. The latter product was directed toward participants with worrisome values for their prostate-specific antigen (PSA) marker (generally considered to reflect prostate health or conversely, the potentiality of a prostate health problem).

Through these experiences, this researcher has identified a number of problematic issues and pitfalls that arise from human dimensions typically engendered by nutraceuticals. It is not the purpose of this paper to identify those issues that are typically part of a traditional and formal research protocol which should guide clinical trial research. The formalistic needs of effective CAM clinical trial research have already been discussed and presented,1 and other strategies to improve clinical research rigor, particularly strategies to address such amorphous concepts as quality-of-life, have also been presented in the literature.2-16 These published, concrete, clinical trial research strategies and formal methods help achieve rigorous trial outcomes. Rather, the purpose of this paper is to identify what one can consider to be the human dimensions that confound nutraceutical clinical trial research — that is, certain attitudes and actions by participants that can bedevil the best-laid research protocol methods and guidelines. Our goal is to identify key experience-based difficulties that are not supposed to happen when a classical research protocol is rigorously followed. By presenting and hence anticipating such real-world problems and needs, we hope other nutraceutical trial researchers may use these insights to more likely accomplish trial goals they have set for themselves.

One may ask why trials involving nutraceuticals should elicit special humanistic challenges that might be different from a formal trial of a pharmaceutical drug, pursued under classical and FDA-approved methodology. There are a number of reasons why such trials differ from the formal model of traditional FDA-based pharmaceutical product trials. Unlike classical drugs, most nutraceutical products are not claiming to eradicate a specific disease or condition. Virtually every nutraceutical product carries the notice that it is not intended to treat, cure, diagnose, or prevent disease.
As a result, there is often a much looser relationship for trial participants between the product and the “payoff” from taking it. The participants are invariably more casual in their approach since the “promise” of its impact cannot be an immediately efficacious outcome that will cure a specific disease, although there may be measurable and meaningfully positive outcomes that can be clearly identified.

Such nutraceutical trials also suffer from the sometimes inaccurate perception that the product being tested is directly analogous to the many, casually-used over-the-counter health care preparations. Trial participants may think they are equally able to evaluate such products in their own, non-formal ways, rather than viewing the nutraceutical to be assessed as an actual “therapeutic” that must be rigorously evaluated as would be any “real” (i.e., pharmaceutical-industry-distributed) prescription drug. As a result, participants’ attitudes create a different climate for nutraceutical trials which in turn yields a change in the participants’ trial compliance. This shift in attitudes produces some of the “humanistic” challenges identified below.

CHALLENGES IN PATIENT MAINTENANCE

Recruitment

Some participants will make trial commitments with solemn oaths and the most serious of purpose, only to discard the trial casually and preemptively for another initiative that suddenly appeals to them. This may be due to their perception that the nutraceutical being tested is akin to perhaps some cold remedy relief product which may be chosen and/or discarded at will. As a result, one may have to over-book the tentative enrollment size of planned participants to allow for a higher drop-out rate.

Since drop-out rates may be greater and may occur at a geographic distance (wherever the participant is located), the trial administrator has the added difficulty of retrieving trial product from drop-outs. Once a participant drops out of the trial, getting their attention is more difficult. Establishing rapport with participants is of enormous value, although costly in trial administration time, when post-trial cooperation is needed.

Reporting

In recruiting participants, each active participant must complete at least some necessary paperwork. After proceeding with the minimum requirements, such as a signed informed consent, there are usually other forms required as the trial proceeds. Participants have shown themselves to be enormously intolerant of completing other simple forms requested, and we have begrudgingly learned that without some regular reinforcement or some “sticks” and “carrots” to hold over their head, time and again many participants will simply not comply with such requests. This reluctance may stem in part from their casual attitude alluded to above, and the fact that some studies may be administered to participants at a distance rather than onsite at a medical center with a health professional interfacing with each participant.

Simple, clear materials

Making all information clear and simple is a mandate for any trial. In the context of nutraceuticals, it is even more imperative since the casual attitude and sometimes perceived lack of “medical seriousness” makes some participants even more oblivious to directions and the trial protocol. Similarly, a trial should be regularly collecting information on side effects and adverse events, but for reasons noted above, such forms must be even more short and simple since the tolerance of the participants is much lower, and typically they must complete and mail in such information rather than have it collected at a specific care center by a trained health professional.

Trial information and communication

In formal pharmaceutically-based trials, where there is an accepted recognition for secrecy, the amount of information about the product may be limited, guarded, and restricted. In the more general nutraceutical environment, participants’ expectations are for more truth-in-packaging. To the extent possible, far more additional information should be available for interested trial participants. Some participants will assuredly seek additional information on the Internet, which can easily be misleading, misguided, or completely irrelevant. Incorrect information may well be easily disseminated to others by trial participants, creating needless difficulties for the trial administrators. Participants can be expected to have numerous questions, raise new issues, and/or acquire hearsay information which may be irrelevant, but in their mind connected, to the trial. Nutraceutical trial design should anticipate this added communication requirement. In a similar vein, a participant in such trials may have more time on his or her hands than does the average person and often carries the expectation of obtaining much more public information about the trial, the product, comparison products, the trial administrators, the product manufacturer, etc. Again, this implies far more contacts, calls, and personal one-on-one communication needed than is typical of other trials. Staffing requirements need to anticipate this.

Mailing problems

Assuming the participants do not come to a health center to obtain their supply of product, one must otherwise mail or deliver it. The mishaps that can arise under this scenario are numerous. Some mailboxes are too small for packages. Some participants obtain their mail at post boxes but do not check them frequently. Over a slightly longer trial, some participants may go on vacation, leaving forwarding addresses that can add substantially to the delivery time. Others will change their address, be in the middle of a move, etc. Be prepared to allocate time for mailing changes throughout the trial.
High-maintenance patients

Nutraceutical trial research can easily attract participants who are extremely high-maintenance types; many lay people fancy themselves nutraceutical experts, and they mandate regular conversation and interaction. Even when trial administrators think they may be able to inhibit such people, they may underestimate the tenacity of these types of participants. These participants may call in alluding to the need for emergency contact, which cannot be safely ignored. One must allocate staffing time for such participants who inevitably will be involved in nutraceutical trials.

Unilateral protocol shifts

One should not underestimate the degree of confusion on product directions or sudden lack of adherence to protocol that will likely arise. The casual and/or diminished lack of attention by some participants will easily yield significant departure from the required protocol. Some participants may suddenly start taking half the amount, or twice the amount, than they had been taking. Reiterating the directions and protocol requirements is wisely done in every mailing and in interim communications. Moreover this pitfall implies that the overall trial protocol must be as simple as possible to minimize protocol departures.

While on the trial protocol, some patients will intentionally change their personal regimens by adding treatment-related products or additional treatment strategies that may clearly confound product evaluation. Since the trial product may be seen by participants as just an over-the-counter substance, they are used to adding other things as a normal practice even when they have been alerted to call or apprise trial administrators before doing so. Their context of everyday experience with over-the-counter products can override the trial protocol format. Ongoing communication and regular input to participants may mitigate these propensities somewhat.

Participants' illnesses

While on trial, participants may become sick due to other illnesses or conditions. This presents a communication challenge since trial implementers need to have established guidelines to determine whether such participants should terminate the trial, seek medical help, and/or continue to report in, when possible, so any adverse events related to the trial product can be identified. When a trial product (typically a pharmaceutical product) is perceived to potentially save participants' lives, they are far more hesitant to reveal adverse events lest they be removed from the trial; participants consuming a nutraceutical product, however, may be more likely to view any headache or upset stomach as a side-effect of the product.

Participants' patience

With what is perceived as an over-the-counter product, some participants bring their own corresponding expectations to bear. Given the "instant" relief so many over-the-counter advertisers promise, and the "immediate" benefit many commercial products proclaim, it is not surprising to hear a participant complain, "Why hasn't this helped me already?" Well-stated protocol time-frames, in which participant change(s) might be expected to occur, may be quickly ignored by some participants, as the natural expectations of the commercial over-the-counter world take over. Similarly, there may be expectations for all the over-the-counter conveniences participants have come to expect. Prescription medicine to cure a deadly disease may be tolerated with an array of unappealing attributes (e.g., taste, capsule size, number of capsules per day required, etc.), but in contrast, the nutraceutical product must be convenient, passably good-tasting, few capsules in number, easy to swallow, etc., to obtain continued maximum participant compliance is desired.

DATA COLLECTION

Obtaining and posting data

Since some participants may have a far more casual idea about reporting in, it can be an ongoing challenge to get participants to send in data, to complete forms, no matter how simple, and to take time to regularly give required information. Trial implementors must be prepared to call recalcitrant participants, repeatedly notify others, and threaten termination to those who do not seem to grasp the trial designers’ needs. By the same token, by tabulating and posting interim data, participants can be kept in the loop and may be motivated to adhere to the protocol. Posting interim data is rarely done in traditional trials, but to aid patient communication and foster a sense of trial partnership, it can serve worthwhile purposes. It may motivate participation when the data is favorable, it may motivate participants to add their own data to the evolving mix, and it underscores the mutual relationship between participant and researcher. By posting or disseminating interim data, participants have more “ownership” of the trial, which can help many of the problems presented above.

Counting data for drop-outs

In collecting data, incorporating any data for drop-outs is particularly challenging since the reasons for their departure from a nutraceutical trial may be more whimsical, may simply arise from their impatience, or may be based on external reason(s) that cannot be easily identified. If their progress has been poor after little time on the trial, are they a bad data point or an irrelevant data point? If they leave the trial mid-way, but have been showing good progress until then, is their good data to be counted positively or ignored? These situations prompt difficult challenges, not all of which are typically anticipated in protocol definition, and may require impromptu protocol amendments to decide how to utilize the data obtained.
SUMMARY OF IMPLICATIONS FOR NUTRACEUTICAL TRIAL PLANNING

As the discussion has indicated, some participants’ casual attitudes about nutraceuticals in contrast to “serious” drugs implies more staff time per patient. On the positive side, added time to address participants’ needs can yield benefits in overall patient compliance and cooperation. The participants’ potentially greater variations and changes in protocol adherence may entail the need to anticipate more active ongoing planning regarding protocol design during the trial than would be anticipated in more traditional drug trials. Strategies such as posting interim trial data may help participants buy into the trial and its ongoing data requirements. Trial administration for nutraceuticals can be expected to be more demanding because of the typically more casual attitudes participants bring to bear, as some of the challenges presented indicate. These additional requirements can ultimately add to the cost of such trials, making their execution more difficult, albeit just as worthwhile as any other trial.17

REFERENCES

17. Evans S, Block JB. Why funding for nutraceutical clinical trial research will remain minimal. JANA 2002;1:12-5.
**Book Review**

**An Excellent Guide for Practitioners and Patients for a Comprehensive Approach to Managing Hypertension — A book by Mark Houston, MD**

By Cristiana Paul, MS, Research Director
Designs For Health
www.DesignsForHealth.com

Dr. Houston’s book is an excellent comprehensive guide for understanding the causes and physiology of hypertension and all available treatment modalities using the synergy of nutraceuticals with dietary and lifestyle management tools instead of or in conjunction with medications.

Dr. Mark Houston, MD, MSc, FACP, FAHA, *JANA* editor-in-chief, is associate clinical professor of medicine at Vanderbilt University School of Medicine. He is director of the Hypertension Institute, Saint Thomas Hospital, in Nashville, Tennessee, where he has successfully treated thousands of patients for the last thirteen years. He has recently received a masters degree in clinical nutrition from the University of Bridgeport, Connecticut, and has published over 130 medical articles and monographs in peer-reviewed journals. He authored two best selling books: *Handbook of Hypertensive Therapy* and *Vascular Biology for the Clinician*.

Dr. Houston’s intention for the book is for practitioners to work together with patients in managing hypertension, counting on the possibility that, in many cases, it can be reversed or reduced in severity while eliminating the need for medications or at least reducing their numbers and dosages. There’s a greater likelihood of positive results with patients who have been educated about their condition and understand the possible causes of their high blood pressure – and who take an active role in their health by choosing as many health-promoting avenues as possible. And finally, this book is useful for practitioners already partially knowledgeable in nutritional applications but who need a review and update. Dr. Houston has researched the subject extensively and synthesized this information for us in this revolutionary guide for treating high blood pressure.

Chapter II starts with a review of the physiological factors that might contribute to elevated blood pressure (BP). Houston’s well chosen metaphors help even lay persons to understand hypertension and the multiple ways to address it.

While reading chapter II, I realized that along with the central BP control factors (hormones, catecholamines), the role of endothelial cells lining the blood vessels is also important. Endothelial cells are sensors of local BP/oxidative stress/inflammation and produce local messengers that implement vasodilation or constriction (through nitric oxide and other signaling molecules).

In light of this fact we see the impact of nutrients traveling through the bloodstream because they can be absorbed directly by endothelial cells. I knew antioxidants
played critical roles in many physiological processes, but I didn’t know they had a direct impact on BP until I read Dr. Houston’s elegant presentation.

Chapter II lists in detail all possible damaging consequences of elevated BP. The most compelling is life expectancy. These alarming statistics may motivate some patients to change their habits:

<table>
<thead>
<tr>
<th>BP</th>
<th>Life Expectancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>120/80</td>
<td>76</td>
</tr>
<tr>
<td>130/90</td>
<td>67</td>
</tr>
<tr>
<td>140/95</td>
<td>62</td>
</tr>
<tr>
<td>150/100</td>
<td>55</td>
</tr>
</tbody>
</table>

The book is based on the philosophy that it is always better to address and correct underlying deficiencies, imbalances, excesses, conditions, and unhealthy lifestyle practices as the first line of action. Research has shown that even when BP was brought into the normal range using medications exclusively, risks predicted by the initial BP value were not reduced to the normal value as many underlying metabolic problems were not addressed.

It is well known that many medications have side effects, including reduced quality of life and increasing other risk factors of disease. A comprehensive guide to applicable meds is found in Chapter IX, along with a simple explanation of the mechanism of action, possible side effects, and contraindications. This information can motivate patients to explore the broader range of treatment possibilities and health promoting approaches presented in the book.

Chapter V and VI are dedicated to dietary advice, Chapter VII to exercise, and Chapter VIII to stress management, all crucial interventions with tremendous health promoting benefits.

The book reviews in detail the role of nutritional supplements at two levels of understanding in different sections:

* Chapter III “The multifaceted solution” reviews the most effective nutrients used in the management of BP at a level accessible to health practitioners as well as to patients eager to educate themselves and take an active role in their treatment and health.

* In the appendix, “a closer look at the studies” is intended specifically for health care practitioners who want to understand the mechanisms of action of some nutrients currently less known or less well accepted by the mainstream medical community: alpha-lipoic acid, coenzyme Q10, vitamin C, taurine, vitamin B6, lycopene, celery extract, garlic, and vitamin E.

I have listed in Table 1 all nutrients covered in Chapter III and the Appendix with dose ranges used in trials and the reduction in systolic and diastolic blood pressure observed in some studies. Note that the impact on BP of some nutrients or lifestyle interventions is comparable to some phar-maceutical drugs, while others have a modest effect on BP but might be necessary for other health purposes.

From looking at this table it appears to be necessary to take numerous nutritional supplements to achieve the equivalent BP drop from only a few or just one medication. But it may be well worth it to go the nutraceutical way, although it may take a lot of education for both patient and clinician to support such a choice.

At the end of Chapter III, under the section called “Mimicking the Medicines,” is a summary of nutrients, grouped per mechanism of action, equivalent to each of the major classes of BP-lowering drugs — very useful for the patient and the clinician.

Note that not all nutrients work for all patients equally, because they act in conjunction with the particular diet, metabolic condition, and genetics, or may be required to compensate for the side effects from drugs. For example, statins lower CoQ10 production, so patients who take statin drugs might require more CoQ10 than patients who do not take these medications.

All of these considerations are explained in detail for each nutrient so doctor and patient can choose the most effective set of supplements.

In the first column of Table 1, I have checked the nutrients Dr. Houston’s found from experience to be most efficacious for his patients. The outline of his entire program is described in Chapter IV, “The Hypertension Institute Program.”

For those of us who have forgotten details about the BP-regulating metabolic pathways from our physiology classes, I have created a one-page review diagram, Figure 1. Along with normal physiological factors involved in the metabolic regulation of BP, drugs and some of the more prominent nutrients are marked, showing where they can be used to reduce it.

In conclusion, I believe that both patients as well as healthcare professionals will find Dr. Houston’s book, *What Your Doctor May Not Tell You About Hypertension*, to be most informative and will serve as a science based guide to those who are looking for non-drug alternatives to control hypertension.
Table 1. Blood Pressure lowering nutrients, doses and effectiveness.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Dosage</th>
<th>BP Reduction*</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Diastolic [mm Hg]</td>
<td>Systolic [mm Hg]</td>
</tr>
<tr>
<td>x Potassium</td>
<td>60-120 mEq or 2400-4800 mg</td>
<td>2.5</td>
<td>4.4 “Our healthy ancestors consumed a potassium-to-sodium ratio of 5:1 while the average American has a potassium-to-sodium ratio of 1:2 or less”</td>
</tr>
<tr>
<td>Calcium</td>
<td>400-800 mg</td>
<td>1.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>200-800 IU</td>
<td>**</td>
<td>** Works in conjunction with calcium, dosage depends on vitamin D stores or deficiency</td>
</tr>
<tr>
<td>Magnesium</td>
<td>500-1000 mg</td>
<td>3.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Whey protein</td>
<td>30 g</td>
<td>2.5</td>
<td>3</td>
</tr>
<tr>
<td>Sardine meat</td>
<td>3 oz</td>
<td>5.3</td>
<td>9.7</td>
</tr>
<tr>
<td>Fish oils</td>
<td>4-7 g</td>
<td>1.6-2.9</td>
<td>Effective dose depends on body stores of EPA/DHA and AA. It is also influenced by the current intake of AA</td>
</tr>
<tr>
<td>GLA</td>
<td>500mg</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Olive oil</td>
<td></td>
<td>6</td>
<td>8 Effect noticed when it replaced sunflower oil</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>500-1000 mg</td>
<td>1-6</td>
<td>5-11 Effect more pronounced in higher-baseline BP</td>
</tr>
<tr>
<td>CoQ10</td>
<td>60-200 mg</td>
<td>10</td>
<td>15 May take 4 weeks to reach peak effect</td>
</tr>
<tr>
<td>Vit E</td>
<td>400-800 IU</td>
<td>1.6</td>
<td>11 All tocopherols and tocotrienols are recommended</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>100-200 mg</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Zinc</td>
<td>25 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celery</td>
<td>4 stalks or 6.5 g</td>
<td>24</td>
<td>Or 3 tbs celery juice three times per day</td>
</tr>
<tr>
<td>Garlic</td>
<td>4g or 4 cloves</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Wakame seaweed</td>
<td>3 g (2 tbs) dried seaweed</td>
<td>5.7-14</td>
<td>Has a better effect in salt-sensitive subjects</td>
</tr>
<tr>
<td>Fiber</td>
<td>5 g</td>
<td>5.5</td>
<td>7.5-9.4 Oat bran and glucomannan (guar gum) fiber was used</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>5-10 g</td>
<td>6.2</td>
<td>6.8 Especially important when not found in the diet</td>
</tr>
<tr>
<td>Hawthorne</td>
<td>160-900 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taurine</td>
<td>6 g</td>
<td>4</td>
<td>9 Especially necessary for vegetarians</td>
</tr>
<tr>
<td>L-carnitine</td>
<td>1000-200 mg</td>
<td>2.5</td>
<td>4 Especially necessary for vegetarians</td>
</tr>
<tr>
<td>NAC</td>
<td>500-1000 mg</td>
<td>**</td>
<td>** NAC=N-acetyl cysteine</td>
</tr>
<tr>
<td>Alpha-lipoic</td>
<td>100-200 mg</td>
<td>**</td>
<td>** Recommended to be taken with 800 mcg of biotin</td>
</tr>
<tr>
<td>Lycopene</td>
<td>10 mg</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Guava fruit</td>
<td>1 g</td>
<td>7.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Dash II Diet</td>
<td></td>
<td>4.5-6.8</td>
<td>8.9-11.5 low sodium, high in fruits, vegetables, whole grains, low-fat dairy and meats</td>
</tr>
<tr>
<td>Weight loss</td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Exercise</td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>no alcohol</td>
<td></td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

* Number listed was taken from results of particular studies and it is not guaranteed with every patient

** Small but significant changes have been found in some studies
**Figure 1. Blood pressure regulating factors**

- **Arterial Blockages**
  - Low blood flow through kidneys
  - Kidneys produce renin which catalyzes the production of Angiotensin I

- **ACE Inhibitors**
  - Natural ACE Inhibitors: Whey protein, Zinc, Sardine, Hawthorne, Seaweed, Celery, Garlic

- **Angiotensin Converting Enzyme**
  - Angiotensin I
  - Angiotensin II

- **Angiotensin II**
  - GLA
  - Lipoic acid, Zinc, CoQ10, Mg, Chromium

- **ACE Inhibitors**
  - EPA
  - Alpha Beta Blockers

- **Aldosterone**
  - Sodium Retention
  - Elevated Insulin

- **Sodium Retention**
  - Crosslinked collagen, Arterial plaque, Endothelial dysfunction

- **Hemorrhoids**
  - Natural Diuretics, Taurine, B6, Celery

- **Diuretics**
  - Vessel Elasticity

- **Vascular Blockages**
  - Natural Vasodilators: EPA/DHA, GLA, Mg, Arginine

- **BLOOD PRESSURE**

Acids: GLA=Gamma Linolenic, EPA=Eicosapentaenoic, DHA=Docosahexaenoic, AA=Arachidonic

**Stimulatory action**

**Inhibitory action**
Evidence-Based Systematic Review of Selenium (Se)

Ethan Basch, MD,1 Catherine Ulbricht, PharmD,2* Tracee Abrams, PharmD,3 Cindy Quach, PharmD,4 Nancy Tannous, PharmD,4

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2. Massachusetts General Hospital  
3. University of Rhode Island  
4. Northeastern University

Edited and peer-reviewed by members of the Natural Standard Research Collaboration  
www.naturalstandard.com

INTRODUCTION

While some complementary and alternative techniques have been studied scientifically, high-quality data regarding safety, effectiveness, and mechanism of action are limited or controversial for most therapies. Whenever possible, it is recommended that practitioners be licensed by a recognized professional organization that adheres to clearly published standards. In addition, before starting a new technique or engaging a practitioner, it is recommended that patients speak with their primary healthcare provider(s). Potential benefits, risks (including financial costs), and alternatives should be carefully considered. This monograph is designed to provide historical background and an overview of clinically-oriented research, and neither advocates for or against the use of a particular therapy.

RELATED TERMS

• Atomic number 34, Na2SeO3, selenium dioxide, seleniumized yeast, L-selenomethionine, Se, Sele-Pak, selenate, selenite, selenious acid, selenocysteine, selenomethionine (Semet), selepen, Se-methylselenocysteine (SeMCYS).

BACKGROUND

• Selenium is a trace mineral found in soil, water, and some foods. It is an essential element in several metabolic pathways, including the glutathione-peroxidase pathway. Selenium appears to promote antioxidant activity in the body via glutathione peroxidase (GPX), a selenium-dependent enzyme.

• Selenium deficiency can occur in areas where selenium is low, and may cause conditions such as Keshan disease and affect thyroid function. Selenium deficiency is also commonly seen in patients on total parenteral nutrition (TPN) as their sole source of nutrition. Gastrointestinal disorders may decrease the absorption of selenium resulting in depletion or deficiency. Selenium may be destroyed when foods are refined or processed.

• Specific dietary sources of selenium include brewer's yeast, wheat germ, butter, garlic, grains, sunflower seeds, Brazil nuts, walnuts, raisins, liver, kidney, shellfish (lobster, oyster, shrimp, scallops), fresh-water and salt-water fish (red snapper, salmon, swordfish, tuna, mackerel, halibut, flounder, herring, smelts). Selenium is also found in alfalfa, burdock root, catnip, fennel seed, ginseng, raspberry leaf, radish, horseradish, onion, chives, medicinal mushrooms (reishi, shiitake), and yarrow.

• The role of selenium in cancer prevention has been the subject of recent study and debate. Initial evidence from the Nutritional Prevention of Cancer (NPC) trial suggests that selenium supplementation reduces the risk of prostate cancer among men with normal baseline PSA (prostate specific antigen) levels, and low selenium blood levels. However, in this study selenium did not reduce the risk of lung, colorectal, or basal cell carcinoma of the skin, and actually increased the risk of squamous cell skin carcinoma. The ongoing Selenium and Vitamin E Cancer Prevention Trial (SELECT) aims to definitively address the role of selenium in prostate cancer prevention.
Uses based on scientific evidence

These uses have been tested in humans or animals. Safety and effectiveness have not always been proven. Some of these conditions are potentially serious, and should be evaluated by a qualified healthcare provider.

<table>
<thead>
<tr>
<th>Uses based on scientific evidence</th>
<th>GRADE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidant</td>
<td>B</td>
</tr>
<tr>
<td>Selenium is a component of glutathione peroxidase, which possesses antioxidant activity, and demonstrates antioxidant properties in humans. Long-term clinical benefits remain controversial.</td>
<td></td>
</tr>
<tr>
<td>Prostate cancer prevention</td>
<td>B</td>
</tr>
<tr>
<td>Initial evidence has suggested that selenium supplementation reduces the risk of developing prostate cancer in men with normal baseline PSA (prostate specific antigen) levels, and low selenium blood levels. This is the subject of large well-designed studies, including the Nutritional Prevention of Cancer Trial (NPC), and the ongoing Selenium and Vitamin E Cancer Prevention Trial (SELECT), as well as prior population and case-control studies. The NPC was conducted in 1312 Americans, and reported that 200mcg of daily selenium reduces the overall incidence of prostate cancer – although these protective effects only occurred in men with baseline PSA levels less than or equal to 4 ng/mL, and those with low baseline blood selenium levels (&lt;123.2 ng/mL). The NPC trial was primarily designed to measure the development of non-melanoma skin cancers, not other types of cancers, and therefore these prostate cancer results cannot be considered definitive. To settle this question, further study is underway: The SELECT trial is in progress, with a goal to include 32,400 men with serum PSA levels less than or equal to 4 ng/mL. SELECT was started in 2001, with results expected in 2013. Laboratory studies have reported several potential mechanisms for selenium’s beneficial effects in prostate cancer, including decrease in androgen receptors and PSA production, antioxidant effects, angiogenesis inhibition, or apoptosis. It is not known if selenium is helpful in men who already have been diagnosed with prostate cancer to prevent progression or recurrence of disease. It does appear that selenium may not be beneficial in those with elevated PSA levels, or with normal/high selenium levels. It remains unclear whether men at risk (or all men) should have their serum selenium values measured; results of the SELECT study may provide additional guidance. There is evidence that low selenium levels are associated with an increased risk of prostate cancer, and several mechanisms for the beneficial effects of selenium supplementation have been suggested. In the NPC trial, no benefits were seen in reducing the risk of colorectal or lung cancers. Although an overall reduction in cancer risk was observed, it is not clear which specific types of cancer besides prostate cancer prevention may benefit.</td>
<td></td>
</tr>
<tr>
<td>Keshan disease</td>
<td>B</td>
</tr>
<tr>
<td>Keshan disease is a cardiomyopathy (heart disease) restricted to areas of China in people having an extremely low selenium status. Prophylactic administration of sodium selenite has been shown to significantly decrease the incidence of this disorder. Selenium is used to treat and prevent selenium deficiency (for example in those with HIV or receiving enteral feedings).</td>
<td></td>
</tr>
</tbody>
</table>
### Asthma
Preliminary research reports that selenium supplementation may help improve asthma symptoms.\(^{45,46}\) Further research is needed to confirm these results.

### Intracranial pressure symptoms
Preliminary research shows a decrease of symptoms of elevated intracranial pressure (headaches, nausea, emesis, vertigo, unsteady gait, speech disorders, and Jacksonian seizures).\(^{47,48}\) More research is needed before a recommendation can be made.

### Burns
Early study results suggest that supplementation with selenium and other trace elements (copper, zinc) may increase the rate of burn wound healing.\(^{49,50}\) Additional research is necessary before a clear recommendation can be made.

### Cancer treatment
Several studies suggest that low levels of selenium (measured in the blood or in tissues such as toenail clippings), may be a risk factor for developing cancer,\(^{18,51-55}\) particularly prostate cancer.\(^{18}\) Population studies suggest that people with cancer are more likely to have low selenium levels than healthy matched individuals, but in most cases it is not clear if the low selenium levels are a cause or merely a consequence of disease. It remains unclear if selenium is beneficial in the treatment of any type of cancer.

### Cardiomyopathy
Low selenium levels have been associated with the development of cardiomyopathy,\(^ {34,43,56-61}\) and selenium supplementation is likely of benefit in such cases (for example in Keshan disease).\(^ {33,34}\) However, most cases of cardiomyopathy are not due to low selenium levels, and therefore selenium may not be helpful.

It has been suggested that low selenium levels may be a risk for coronary heart disease, although this remains unclear.\(^ {62}\)

### Cataracts
Preliminary research reports that selenium supplementation may affect the development of cataracts.\(^ {63,64}\) Further research is needed before a clear conclusion can be drawn.

### Chemotherapy side effects
Study results of selenium supplementation during chemotherapy are mixed.\(^ {64-66}\) General concern has been raised that antioxidants may interfere with radiation therapy or some chemotherapy agents (such as alkylating agents, anthracyclines, or platinum), which themselves can depend on oxidative damage to tumor cells for anti-cancer activity. Therefore, patients undergoing cancer treatment should speak with their oncologist before taking selenium.

### Cystic fibrosis
Preliminary research of selenium supplementation in CF patients yields indeterminate results.\(^ {67,68}\) Further research is needed in this area before a conclusion can be drawn.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dandruff</td>
<td>Studies report that selenium-containing shampoos may help improve dandruff, and selenium is included in some commercially available products.</td>
<td>C</td>
</tr>
<tr>
<td>Dialysis</td>
<td>The benefits of selenium supplementation in dialysis patients remain unclear. Some methods of dialysis may lower plasma selenium levels.</td>
<td>C</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Evidence of benefit is inconclusive in this area.</td>
<td>C</td>
</tr>
<tr>
<td>Malabsorption</td>
<td>Low selenium status has been demonstrated in several malabsorptive syndromes and in some digestive and gastrointestinal allergic conditions. There is some evidence that children with food allergies have a higher risk of selenium deficiency. There is no clear benefit of selenium supplementation as a therapy for malabsorptive syndromes, although vitamin supplementation in general may be warranted.</td>
<td>C</td>
</tr>
<tr>
<td>Liver disease</td>
<td>Selenium supplementation has been studied in various liver disorders, including hepatitis, with mixed results.</td>
<td>C</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>Selenium supplementation has been studied in HIV/AIDS patients, and some reports associate low selenium levels with complications such as cardiomyopathy. It remains unclear if selenium supplementation is beneficial in patients with HIV, particularly during antiretroviral therapy.</td>
<td>C</td>
</tr>
<tr>
<td>Infection prevention</td>
<td>Preliminary research reports that selenium can be beneficial in the prevention of several types of infection, including recurrence of erysipelas (bacterial skin infection associated with lymphedema) or Mycoplasma pneumonia. Further research is needed to confirm these results before a clear recommendation can be made.</td>
<td>C</td>
</tr>
<tr>
<td>Infertility</td>
<td>Selenium supplementation has been studied for male infertility and sperm motility with mixed results. Evidence is lacking regarding potential effects on female infertility.</td>
<td>C</td>
</tr>
<tr>
<td>Low birth weight</td>
<td>Selenium supplementation has been studied in low birth weight infants. Additional evidence is warranted in this area before a clear conclusion can be drawn.</td>
<td>C</td>
</tr>
<tr>
<td>Lymphedema</td>
<td>Preliminary research reports that selenium supplementation may decrease lymphedema. Further research is needed to confirm these results before a clear recommendation can be made.</td>
<td>C</td>
</tr>
<tr>
<td>Condition</td>
<td>Description</td>
<td>Grade</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Myotonic dystrophy</td>
<td>Selenium and vitamin supplementation has been studied with mixed results.</td>
<td>C</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>There is inconclusive evidence regarding the use of selenium in pancreatitis.</td>
<td>C</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>Preliminary study in women with pregnancy-induced hypertension has reported reduced edema, without significant impact on birth outcomes. No clear conclusion can be drawn in the absence of additional well designed research.</td>
<td>C</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>Research is inconclusive in this area.</td>
<td>C</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Selenium supplementation has been studied with mixed results. Additional research is necessary before a clear conclusion can be drawn.</td>
<td>C</td>
</tr>
<tr>
<td>Sepsis</td>
<td>Study results of selenium supplementation in septic patients are mixed.</td>
<td>C</td>
</tr>
<tr>
<td>Sunburn prevention</td>
<td>Photoprotection was initially observed in preliminary research using selenium supplementation and other antioxidants, although there is some evidence of ineffectiveness in preventing light-induced erythema (skin redness).</td>
<td>C</td>
</tr>
<tr>
<td>Thyroid conditions</td>
<td>An early toxic effect of selenium is disruption of endocrine function, including synthesis of thyroid hormones (T3). Selenium has been suggested to improve inflammatory activity in chronic autoimmune thyroiditis or Grave’s disease. Further research is needed before a clear conclusion can be drawn.</td>
<td>C</td>
</tr>
<tr>
<td>Tinea capitis</td>
<td>Commercially available 1% selenium sulfide shampoo has been reported as equivalent to sporicidal therapy in the adjunctive treatment of tinea capitis infection, although further high-quality evidence is warranted.</td>
<td>C</td>
</tr>
<tr>
<td>Tinea versicolor</td>
<td>Preliminary study of topical selenium (selenium sulfide shampoo) is inconclusive.</td>
<td>C</td>
</tr>
</tbody>
</table>
### Colorectal cancer prevention

Evidence from the Nutritional Prevention of Cancer (NPC) trial suggests that selenium supplementation does not significantly reduce the risk of developing colorectal cancer. This randomized study was conducted in 1312 Americans over a 13 year period, and compared the effects of 200mcg of daily selenium versus placebo. Although initial (interim) analysis suggested possible benefits, a later analysis found a lack of statistical significance.

### Kashin-beck osteoarthropathy

Kashin-Beck disease is an osteoarthropathy endemic in selenium- and iodine-deficient areas. Preliminary evidence suggests that selenium supplementation does not significantly improve this disease.\(^{117}\)

### Lung cancer prevention

Evidence from the Nutritional Prevention of Cancer (NPC) trial suggests that selenium supplementation does not significantly reduce the risk of developing lung cancer.\(^{13,21}\) This randomized study was conducted in 1312 Americans over a 13 year period, and compared the effects of 200mcg of daily selenium versus placebo. Although initial (interim) analysis suggested possible benefits, a later analysis found a lack of statistical significance. Other evidence is inconclusive.\(^{118,119}\)

### Muscular dystrophy

Preliminary studies suggest that selenium supplementation is not helpful in muscular dystrophy.\(^{120-122}\)

### Osteoarthritis

Selenium-ACE, a formulation containing selenium with three vitamins, has been promoted for the treatment of arthritis. Research has failed to demonstrate significant benefits, with a possible excess of side-effects compared to placebo.

### Skin cancer (nonmelanoma) prevention

Results from the Nutritional Prevention of Cancer (NPC) trial, conducted among 1312 Americans over a 13 year period, suggest that selenium supplementation (200mcg daily) given to individuals at high risk of nonmelanoma skin cancer is ineffective at preventing basal cell carcinoma, and actually increases the risk of squamous cell carcinoma and total nonmelanoma skin cancer.\(^{11,16}\) Therefore, selenium supplementation should be avoided in individuals at risk or with a history of nonmelanoma skin cancer.

---

### Key to grades:

- **A**: Strong scientific evidence for this use;  
- **B**: Good scientific evidence for this use;  
- **C**: Unclear scientific evidence for this use;  
- **D**: Fair scientific evidence against this use (it may not work);  
- **F**: Strong scientific evidence against this use (it likely does not work).

### Uses based on tradition, theory or limited scientific evidence

The below uses are based on tradition or scientific theories. They often have not been thoroughly tested in humans, and safety and effectiveness have not always been proven. Some of these conditions are potentially serious, and should be evaluated by a qualified healthcare provider.

Abnormal pap smears, acne, alcoholic cirrhosis, alcoholism, allergic rhinitis, altitude sickness, arsenic poisoning, atherosclerosis, chronic bronchitis, cognitive dysfunction; colitis, Cardiac arrhythmia, error! Bookmark not defined. celiac disease, chdepression, dermatitis herpetiformis, diabetes mellitus, Downs Syndrome, Diabetic retinopathy, error! Bookmark not defined,\(^{123}\) gastric cancer prevention,\(^{123}\) gray hair, helminth reinfection, highHepatitis, error! Bookmark not defined. HIV/AIDS, hypothyr cholesterol, hypersensitivity to electricity,
immune disorders, immune stimulation, inflammatory bowel disease, inflammation, lupus, macular degeneration, metabolic enhancement, meMacular degeneration, Error! Bookmark not defined.

**ADULTS (18 years and older):**

- **U.S. Recommended Dietary Allowance (RDA) for adults (oral):** 80-200 mcg. Specifically: 55 mcg for female adults; 70 mcg for male adults; 40-70 mcg for adolescent males, 45-55 mcg for adolescent females; 65 mcg for pregnant females; 75 mcg for breast-feeding females.
- **Prostate cancer prevention (oral):** The dose of selenium associated with reduced risk of prostate cancer in the NPC trial is 200 mcg daily.
- **Maximum Daily Dose (oral):** 400 mcg per day for those older than 14 years old (including adults and the elderly).
- **Intravenous (should only be used when oral therapy is not feasible, and under the direction of a qualified healthcare professional):** For treatment of selenium deficiency in adults, 100 mcg of elemental selenium daily for 24-31 days has been suggested. For prevention of selenium deficiency in adults, 20-40 mcg of elemental selenium daily has been suggested.
- **Other:** The following doses have been reported in research or practice, although efficacy is not necessarily proven. **Asthma:** 100 mcg daily. **Cancer prevention:** 200mcg daily. **Erysipelas infection:** 300-1000mg daily as selenium selenite. **HIV positive status:** 80 mcg daily. **Infertility (male):** 100 mcg daily. **Keshan disease:** 30 mcg daily. **Myocardial infarction (heart attack):** 100 mcg daily. **Rheumatoid arthritis:** 200 mcg daily.

**CHILDREN (younger than 18 years):**

- **U.S. Recommended Dietary Allowance (RDA) for infants and children (oral):** 10 mcg for 0-6 months; 15mcg daily for 6-12 months; 20 mcg for 1-6 years; 30 mcg for 7-10 years; 45 mcg for 11-14 years; 50 mcg for 5-18 years. Adequate Intake for infants up to 6 months old may be 2.1 mcg/kg/day, and for infants 7-12 months may be 2.2 mcg/kg/day.
- **Maximum Daily Dose (oral):** 45 mcg for 0-6 months; 60 mcg for 7-12 months; 90 mcg for 1-3 years; 150 mcg for 4-8 years; 280 mcg for 9-13 years.
- **Intravenous (should only be used when oral therapy is not feasible, and under the direction of a qualified healthcare professional):** 3 mcg of elemental selenium/kg/day intravenously for the treatment or prevention of selenium deficiency has been noted.
- **Other:** To treat selenium deficiency in premature infants, 5 mcg per day of selenized yeast has been given by a nasogastric tube.

**SAFETY**

The U.S. Food and Drug Administration does not strictly regulate herbs and supplements. There is no guarantee of strength, purity or safety of products, and effects may vary. You should always read product labels. If you have a medical condition, or are taking other drugs, herbs, or supplements, you should speak with a qualified healthcare provider before starting a new therapy. Consult a healthcare provider immediately if you experience side effects.

**ALLERGIES**

Selenium is a trace element, and hypersensitivity is unlikely. Avoid if known allergy/hypersensitivity to products containing selenium.

**Side Effects and Warnings**

- **Chronic toxicity:** The level of selenium exposure that will cause chronic toxicity is not known, although doses 4-5 times normal dietary intake have been implicated (1 gram per day for two years has produced signs of toxicity in women). Selenium toxicity may cause gastrointestinal symptoms (nausea, vomiting, abdominal pain, diarrhea, garlic-like breath odor, metallic taste), neuromuscular-psychiatric disturbances (weakness/fatigue, lightheadedness, irritability, hyperreflexia, muscle tenderness, tremor, peripheral neuropathy), dermatologic changes (skin rash/dermatitis/flushing, fingernail loss/thickening/blotching/streaking/paronychia, hair changes/loss), liver dysfunction, kidney dysfunction, thrombocytopenia (low blood platelets), immune alterations (natural killer cell impairment), thyroid dysfunction (decreased T3), reduced sperm motility, or growth retardation. Blood
selenium levels may be used to assess the degree of toxicity, with levels below 1000 mcg/L usually not associated with serious toxicity, and levels above 2000 mcg/L predictive of potential serious toxicity. Chronic selenium toxicity may resemble arsenic toxicity.

- **Acute overdose (selenosis):** Acute selenium poisoning may cause fever, gastrointestinal symptoms (nausea, vomiting, pain, anorexia), liver or kidney functional impairment, respiratory distress, cardiac complications (EKG changes, increased creating kinase levels, heart damage), and even death if levels are high enough. Other symptoms similar to chronic selenium toxicity may also occur.

- **Cardiovascular:** Chronic low selenium levels are associated with the development of cardiomyopathy, and possibly with coronary artery disease.

- **Endocrine:** An early toxic effect of selenium is disruption of endocrine function, including synthesis of thyroid hormones (T3), with unclear effects on growth hormone and insulin-like growth factor. Selenium deficiency may also worsen thyroid disorders related to iodine-deficiency.

- **Renal:** Kidney failure and dialysis are associated with low selenium levels, and kidney transplant appears to correct selenium levels.

- **Genitourinary:** Chronic high selenium levels may decrease sperm motility, although effects on fertility are not known.

- **Oncologic:** Results from the Nutritional Prevention of Cancer (NPC) trial, conducted among 1312 Americans over a 13 year period, suggest that selenium supplementation (200mcg daily) given to individuals at high risk of nonmelanoma skin cancer is ineffective at preventing basal cell carcinoma, and actually increases the risk of squamous cell carcinoma and total nonmelanoma skin cancer. Therefore, selenium supplementation should be avoided in individuals at risk or with a history of nonmelanoma skin cancer.

- **Psychiatric:** Researchers have reported high levels of selenium in children with behavioral problems, although causality has not been established. Chronic selenium toxicity has been associated with irritability or fatigue.

### PREGNANCY AND BREASTFEEDING

- **No pregnancy category has been established for supplemental selenium intake although it is generally believed to be safe during pregnancy when consumed in amounts normally found in foods. Animal research reports that large doses of selenium may contribute to birth defects.**

- **Selenium is excreted in breastmilk, but is generally believed to be safe to consume during lactation in amounts commonly found in foods.**

### INTERACTIONS

Most herbs and supplements have not been thoroughly tested for interactions with other herbs, supplements, drugs, or foods. The interactions listed below are based on reports in scientific publications, laboratory experiments, or traditional use. You should always read product labels. If you have a medical condition, or are taking other drugs, herbs, or supplements, you should speak with a qualified healthcare provider before starting a new therapy.

### INTERACTIONS WITH DRUGS

- **HMG-CoA reductase inhibitors ("Statins"):** Concomitant use of selenium in combination with beta-carotene and vitamins C and E appears to decrease the effectiveness of the combination of simvastatin (Zocor®) and niacin, although long-term effects are not known. This may be due to antioxidant effects associated with selenium use. Theoretically, selenium could reduce the effectiveness of other HMG-CoA reductase inhibitors such as atorvastatin (Lipitor®), fluvastatin (Lescol®), lovastatin (Mevacor®), and pravastatin (Pravachol®).

- **Niacin:** Concomitant use of selenium in combination with beta-carotene, vitamin C, and vitamin E appears to decrease the effectiveness of the combination of niacin and simvastatin (Zocor®). This may be due to antioxidant effects associated with selenium use.

- **Corticosteroids:** High-dose steroid therapy may decrease plasma selenium levels.

- **Chemotherapy/Radiation Therapy:** Concern has been raised that antioxidants may interfere with radiation therapy or some chemotherapy agents (such as alkylating agents, anthracyclines, or platinum), which themselves can depend on oxidative damage to tumor cells for anti-tumor effects. Studies of the effects of antioxidants on cancer therapies yield mixed results, with some reporting antagonistic effects (interference), others noting synergism (benefit), and most suggesting no significant interaction. This remains an area of study and controversy. In particular, selenium may reduce toxic side effects associated with chemotherapy drugs including cisplatin, doxorubicin, or bleomycin. However, until better evidence is available, selenium supplementation is not recommended during chemotherapy or radiation therapy due to potential interference. Patients considering use of selenium during chemotherapy or radiation therapy should discuss this choice with their medical and radiation oncologists.

- **Antacids:** Agents that alter the pH of the stomach may decrease absorption of selenium.

- **Oral contraceptives:** Selenium levels may be decreased in patients taking oral contraceptives.

- **Erythropoietin (EPO):** Selenium has been suggested to increase the effects of erythropoietin in hemodialysis patients.
• **Clozapine**: It has been suggested that cardiac side effects associated with clozapine use may be related to low selenium concentrations. It is not clear if assessment of selenium levels or selenium supplementation should be routine in patients taking this drug.

**INTERACTIONS WITH HERBS AND DIETARY SUPPLEMENTS**

• **Antioxidants**: Selenium is a component of glutathione peroxidase, which possesses antioxidant activity, and demonstrates antioxidant properties in humans. Long-term clinical benefits remain controversial. Selenium may add to the effect of other antioxidants in the body, such as vitamins A, C, and E, lycopene, green tea, soy, grape seed extract, or melatonin.

• **Vitamin C**: There is preliminary evidence that vitamin C may be necessary for maintaining selenium levels in the body.

**ACKNOWLEDGEMENT**

This information is based on a professional level monograph edited and peer-reviewed by contributors to the Natural Standard Research Collaboration (www.naturalstandard.com). The contents of this monograph are protected under copyright of Natural Standard (www.naturalstandard.com).

Natural Standard was founded by clinicians and researchers to provide high quality, evidence-based information about complementary and alternative therapies. This international multidisciplinary collaboration now includes contributors from more than 100 eminent academic institutions.

For each therapy covered by Natural Standard, a research team systematically gathers scientific data and expert opinions. Validated rating scales are used to evaluate the quality of available evidence. Information is incorporated into comprehensive monographs which are designed to facilitate clinical decision making. All monographs undergo blinded editorial and peer review prior to inclusion in Natural Standard databases.

Natural Standard is an impartial service and is not supported by any interest group, professional organization, or pharmaceutical manufacturer. Individual and institutional subscriptions are available.

• **Last updated**: July 2004.

**SELECTED REFERENCES:**

Natural Standard developed the above evidence-based information based on a systematic review of more than 2600 articles. For comprehensive information about alternative and complementary therapies on the professional level, go to www.naturalstandard.com.

16. Clark LC, Combs GF, Jr., Turnbull BW, et al. Effects of selenium supplementation for cancer prevention in patients with...


Chemically they are C₆-C₃-C₆ compounds in which the two C₆ groups are substituted benzene rings, and the C₃ is an aliphatic chain which contains a pyran ring. Flavonoids occur as O- or C-glycosides or in the free state as aglycones with hydroxyl or methoxyl groups present on the aglycone.

The flavonoids may be divided into seven types: flavones, flavonols, flavonones, chalcones, xanthones, isoflavones, and biflavones.

Some of the best food sources of flavonoids are red wine, apples, blueberries, bilberries, onions, soy products, and tea. The average daily intake of flavonoids in the United States is between 150 and 200 mcg. Numerous medicinal plants contain therapeutic amounts of flavonoids, which are used to treat disorders of the peripheral circulation, to lower blood pressure, to improve aquaresis, and as anti-inflammatory, antispasmodic, and anti-allergic agents. The many pharmacological effects of flavonoids are linked to their ability to act as strong antioxidants and free-radical scavengers, to chelate metals, and to interact with enzymes, adenosine receptors, and biomembranes. Some flavonoids also possess antimicrobial activity.

This article will focus on those flavonoids with antibacterial and antiviral activity that are most often encountered in nature and for which an analysis of possible structure-activity relationship exists.
and acylated derivatives, show antibacterial activity.10 Most bacteria can be divided into two groups based on a stain developed by the Danish physician Hans Christian Gram in 1884.11 This procedure is based on the fact that Gram-positive bacteria retain a crystal violet-iodine complex through decolorization with alcohol or acetone and Gram-negative bacteria do not.12 Gram-positive bacteria possess a thick peptidoglycan layer in their cell wall whereas Gram-negative bacteria possess a thin peptidoglycan layer plus a lipopolysaccharide outer membrane.13 The Gram stain is important because Gram-positive and Gram-negative bacteria have differing susceptibilities to a variety of antibiotics.14

Flavonoids

Ali et al reported that chrysin (5,7-dihydroxyflavone), in the amount of 5 mcg inhibited the growth of the Gram-negative bacilli Escherichia coli (E coli) and Pseudomonas aeruginosa (Ps aeruginosa) at a rate comparable to that of streptomycin.15 Baicalein, on the other hand, which has an additional hydroxyl group at C-6, did not inhibit the growth of Gram-negative bacteria and showed a weak effect against the Gram-positive, spore-forming Bacillus subtilis (B subtilis) and the Gram-positive coccus Staphylococcus aureus (S aureus).15

Basile et al reported that apigenin, vitexin, saponarin, apigenin, and lucenin 2-O-glycoside, and luteolin 7-O-glycoside acted selectively only towards certain Gram-negative bacilli: Proteus vulgaris (P vulgaris), Proteus mirabilis (P mirabilis), Ps aeruginosa, E coli, Klebsiella pneumoniae (K pneumoniae), and Enterobacter cloacae (E cloacae); however, they showed no activity against the Gram-positive cocci S aureus and Enterococcus faecalis (E faecalis). The highest activity and the broadest spectrum of activity was noted for saponarin with a minimum inhibitory concentration (MIC) of 4 to 2048 mcg/ml; weaker activity was noted for apigenin, and the remaining flavones were inactive or active only against single strains.16 MIC or minimal inhibitory concentration, is the lowest concentration of an antimicrobial compound that will inhibit the growth of the organism being tested.17

Oksus et al found that the MIC for apigenin against P vulgaris, Ps aeruginosa, E coli, and K pneumoniae ranged from 54 to 219 mcg/ml and that apigenin also proved to be active against the spore-former B subtilis.18

Flavonones

Waage and Hedin reported that among the queretin mono- and diglycosides tested, queretin-3-O-rhamnoside exhibited the strongest activity against Pseudomonas maltophilia (Ps maltophilia) and E cloacae, while the other queretin-O-glycosides (3-O-glucoside, 3-O-galactoside, 3’-O-galactoside, 3-O-rutinoside, 3-O-galactoglucoside, and 7-O-glucoside) exhibited weak activity or no activity against these bacteria.19

Acylated Flavonols

Liu et al in 1999 tested a number of acylated kaempferol-3-O-glucosides. The most effective against Gram-positive bacteria were compounds having one or two cis-p-coumaroyl groups. The MICs for kaempferol-3-O-(3’,6’-di-O-p-coumaroyl)-β-glucopyranoside, kaempferol-3-O-(3’-Z-p-coumaroyl)-(6’-O-p-E-feruloyl)-β-glucopyranoside and chloramphenicol against Bacillus cereus (B cereus) were 4, 16, 2 mcg/ml, respectively, and against coagulase-positive S aureus were 8, 32, 64 mcg/ml, respectively, but against the coagulase-negative Staphylococcus epidermidis (S epidermidis) they were 2, 4, and 4 mcg/ml, respectively.20 The presence of the enzyme coagulase is an indication that a particular species of staphylococci is more virulent.14

Iwagawa et al reported that among the acetylated derivatives of queretin, queretin-3-arabinopyranoside-2’-gal late inhibited the growth of E coli.21

Mitrokosta et al tested tiliroside [kaempferol-3-O-β-D-(6’-E-p-coumaroyl)-glucopyranoside] and plantanoside [kaempferol-3-O-a-L-(2’,3’-di-di-E-p-coumaroyl) rhamnoside] against the Gram-positive cocci S aureus, S epidermidis, and Group B and Group F streptococci. They were also tested against the Gram-negative bacilli E coli, Ps aeruginosa, and K pneumoniae. Plantanoside showed antibacterial activity stronger than that of tiliroside, which proved to be weakly active to inactive.22

Flavonones

Materund et al tested six flavonones for antibacterial activity (which included naringenin, taxifolin, and dihydrokaempferide); only naringenin showed activity against E coli, S aureus, and E faecalis.23

Bремер and Meyer reported that the flavonones pinocembrin and pinocembrin chalcone, 0.1 and 1 mcg respectively, inhibited the growth of S aureus.24

Wachter et al reported that the isomeric compounds 5,7,4’-trihydroxy-6-methyl-8-isoprenylflavonone and 5,7,4’-trihydroxy-8-methyl-6-isoprenylflavonone, by means of the agar diffusion method, were reported to inhibit of growth of S aureus in a concentration of 0.1 mg/ml.25

Rahman and Gray in 2002 reported that 5,3’,4’-dihydroxy-4’-methoxy-6”-dimethylchromo-(7,8,2”,3”)flavanone and 5,7,4’-trihydroxy-6,8-di-(3-methylbut 2-enyl)-flavonone were reported to be active against both Gram-positive and Gram-negative bacteria.26

Isoflavonones

Bojase et al showed that the isoflavonones containing prenyl groups had the highest activity against Gram-positive bacteria such as S aureus and B subtilis. This activity was greatest when the prenyl groups were located at positions C-6 or C-8 in ring A and C-3’ or C-5’ in ring B.27
**Lipophilic Flavonoids**

Mori et al reported that the lipophilic compound 7,8-dihydroxyflavone exhibited weak activity against *S aureus*, and no activity against *P vulgaris*. These results may be related to the high lipid content of the cell wall of *P vulgaris*, which may have trapped the 7,8-dihydroxyflavone. The cell wall of *S aureus*, lacking a lipid layer, could be penetrated. The flavonoids exhibited a stronger effect on DNA synthesis in *P vulgaris*, while exhibiting a stronger effect on RNA synthesis in *S aureus*.\(^{28}\)

Wang et al measured the activity of a number of lipophilic flavonoids against *B cereus*. The study was performed using 13 different 3-O-methyl ethers of flavonoids and 29 different methyl and acetyl derivatives of flavonoids. The results indicated that the presence of hydroxyl groups at positions C-5 and C-7 was very important for activity, whereas the presence of an additional methoxyl group at C-7 or dihydroxy groups at C-3' and C-4' significantly reduced activity. The most active compound was found to be 3,3'-dimethoxy-5,7,4'-trihydroxyflavone. It inhibited the growth of *B cereus* using TLC plates at 0.25 mg. As a point of reference, quercetin is active when present in the amount of 2.5 mcg.\(^{29}\)

Van Puyvelde et al reported that 3-O-methylquercetin showed activity against Gram-positive bacteria, chiefly *S aureus* and *B subtilis*.\(^{30}\)

**Aglycones of Flavonoids**

Mori et al tested the activities of the aglycones of 28 flavones, flavonols, flavanones, and isoflavones against *P vulgaris* and *S aureus*. The aglycones most active against *P vulgaris* were myricetin (MIC = 50 mcg/ml) and morin, quercetagetin, datiscetin, and robinetin (MIC = 100 mcg/ml). Myricetin and robinetin showed the greatest activity against *S aureus* (MIC = 100 mcg/ml). The other flavonoids studied, chrysin, luteolin, apigenin, apigenin 7,4'-dimethylether, kaempferol, quercetagetin, eriodictyol, hesperidin, and taxifolin, did not inhibit the growth of the bacteria mentioned.\(^{28}\)

**Structure-Activity Relationships of Flavonoids**

Mori et al observed a relationship between the structures of the flavonoids and their activity against *P vulgaris* and *S aureus*. Most of the activity was related to the presence of hydroxyl groups 3',4',5' in ring B and at C-3. Epigallocatechin and dihydrodorobinetin exhibited weak activity indicating that the C-2,C-3 double bond was not crucial for antibacterial activity.\(^{28}\)

Zheng et al tested other methylated flavones which exhibited antibacterial activity mainly against Gram-positive bacteria, and showed a relationship between structure and activity. The most active were compounds with hydroxyl groups at C-5 and C-7 and with three substitutions in ring B. For example, the activity of 5,7,4'-trihydroxy-3',5'-dimethoxyflavone against Gram-positive *S aureus* (MIC 500-1000 mcg/ml) was much greater than that of 7,4'-dihydroxy 3', 5'-dimethoxyflavone. However, against the Gram-negative bacillus *E coli*, the former compound was reported to be weakly active, and against other Gram-negative bacilli, *Ps aeruginosa* and *Proteus species*, it was inactive.\(^{31}\)

**Helicobacter pylori (H pylori)**

Bae et al reported that flavonoids were also shown to be active against *H pylori*.\(^{32}\) This bacterium was isolated in 1983 from patients with chronic gastritis. It produces the enzyme urease which hydrolyses urea to carbon dioxide and ammonia and plays a key role in the pathogenesis of gastritis and peptic ulcer.\(^{33}\) *H pylori* is susceptible to a variety of antimicrobial agents, including bismuth salts, amoxicillin, macrolides, nitrofurans, tetracyclines, and aminoglycosides.\(^{34}\) It has been found that aglycones inhibit the growth of *H pylori*, whereas glycosides are inactive. The presence of a methoxyl group at C-4' was also important, and its replacement with a hydroxyl group caused a significant decrease in the activity of the compound. The presence of an additional hydroxyl group in ring B also reduced the activity. The MIC (mcg/ml) against *H pylori* was >100 for hesperidin and 20 for hesperetin, >100 for poncirin and 10 for ponciretin, >100 for naringin and 40 for naringenin, and >100 for diosmin and 80 for diosmetin. The MIC against *H pylori* for ampicillin is 1 mcg/ml. Among the flavonoids and their phenolic metabolites studied, only hesperidin, phloroglucinol, and resorcinol inhibited the production of urease by *H pylori* by 60–70%, while the others caused only a weak decrease in this activity.\(^{32}\)

**ACTIVITY OF FLAVONOIDS AGAINST ANTIBIOTIC-RESISTANT BACTERIA**

The use of antibiotics is often accompanied by side effects and often the development of resistant strains.\(^{35}\) The traditional treatment of antibiotic-resistant strains of bacteria consists of the administration of vancomycin, a glycopeptide antibiotic used both in the treatment of life-threatening Gram-positive infections and infections caused by resistant organisms.\(^{36}\) At present the search for compounds active against antibiotic-resistant strains of bacteria is continuing among the flavonoids, compounds which are non-toxic or have low toxicity.\(^{37}\)

**Methicillin-Resistant Staphylococcus aureus (MRSA)**

Xu and Lee tested 38 flavonoids (flavones, flavonols, and flavanones) for activity against strains of methicillin-resistant *S aureus* (MRSA). The growth of MRSA was inhibited by only the aglycones of the flavonols and flavones tested, and the order of their activity was as follows: flavone > kaempferone > datiscetin > quercetin > luteolin > myricetin. The flavones acacetin, chrysin, and rhoifolin, and flavanones pinocembrin, hesperidin, narin-
genin, and eriodictyol were inactive. The isoflavone puerarin was inactive as well as the biflavonoid amentoflavone.\textsuperscript{38}

Iinuma et al reported the activity of two flavonones, 5, 7, 2', 6'-tetrahydroxy-6-isoprenyl-8-lavandulyl-4'-methoxyflavonane and 5, 7, 2', 6'-tetrahydroxy-8-lavandulyl-4'-methoxyflavonane, against 12 strains of methicillin-resistant \textit{S aureus} (MRSA) and 4 strains of methicillin-sensitive \textit{S aureus} (MSSA). The first flavonane inhibited the growth of both MRSA and MSSA to the same degree, MIC = 1.56–6.25 mcg/ml. It was also active against oxacillin-resistant strains of \textit{S aureus}.\textsuperscript{39} These organisms often accompany hospital-acquired MRSA.\textsuperscript{40} The second compound, however, turned out to be inactive. In contrast to the flavanones tested, the only antibiotic active against MRSA was vancomycin (MIC = 1.56 mcg/ml). Gentamicin was active against only two strains tested, oxacillin was active against four, and methicillin was inactive against all.\textsuperscript{36}

**Vancomycin-Resistant Enterococci (VRE)**

When the 38 flavonoids were tested against Vancomycin-resistant enterococci (VRE), Xu and Lee found that only myricetin, with an MIC of 128 mcg/ml, was active.\textsuperscript{38}

Liu et al in 2001 tested a combination of vancomycin and flavonoids against VRE. The addition of 12.5 mcg/ml of 3,5,7-trihydroxyflavone (galangin) or of 6.2 mcg/ml of 3,7-dihydroxyflavone so affected resistant strains of \textit{E faecium} and \textit{E faecalis} as to result in a decrease of MIC for vancomycin to a level characteristic of those strains sensitive to this antibiotic, eg, from >250 mcg/ml down to <4 mcg/ml.\textsuperscript{41}

**Antibiotic-Resistant Gram-Negative Bacilli**

When the 38 flavonoids were tested against resistant Gram-negative bacilli, Xu and Lee found that myricetin was active against \textit{K pneumoniae} (MIC = 64 mcg/ml), \textit{Burkholderia cepacia} (B cepacia) (MIC = 32 mcg/ml), and \textit{P aeruginosa} (MIC = 256 mcg/ml).\textsuperscript{38}

**Structure-Activity Relationships**

Xu and Lee noted that the wide range of myricetin activity, both against Gram-positive and Gram-negative bacteria, was related to its inhibition of protein synthesis. They reported that only polyhydroxylated derivatives of flavonoids, except for flavone, which contains no hydroxyl groups, were active against MRSA. The presence of at least one hydroxyl group in rings A or B at C-3,5,7 was important for activity. Compounds without hydroxyl groups in ring B (pinocembrin, chrysin, galangin) or compounds in which the hydroxyl was replaced with a methoxy group (kaempferide, tamarixinetin) turned out to be inactive. Substitution of aglycones with glycones, (myricetin glucoside, quercetin glucoside) resulted in a loss of activity against the strains of bacteria tested.\textsuperscript{38}

**ANTIVIRAL ACTIVITY**

**Herpes Simplex Virus (HSV-1, HSV-2)**

**Flavonols**

Almeida et al reported that kaempferol 3-O-\(\alpha\)-L-rhamnopyranoside and quercetin 3-O-\(\alpha\)-D-arabinopyranoside inhibited the replication HSV-1 resistant to acyclovir. Quercetin 3-rhamnoside was characterized by weak activity and higher toxicity.\textsuperscript{42}

**Biflavonoids**

Lin et al in 1999 evaluated seven natural biflavonoids, their methyl ethers, and their acetates for their activity against HSV-1 and HSV-2. Amentoflavone and robustaflavone were active; however, acetylation or methylation of these compounds caused no significant change in their activity.\textsuperscript{43}

Arthan et al reported that torvanol-isoflavonoid, with a sulfate group at C-4, showed activity against HSV-1, IC\(_{50}\) = 9.6 mcg/ml.\textsuperscript{44}

**Human Immunodeficiency Virus (HIV)**

**Flavonols**

Hu et al reported that acacetin-7-O-\(\beta\)-galactopyranoside and chrysin showed rather high anti-HIV-1 activity with relatively low toxicity. Replacement of the methoxyl group at C-4’ with a hydroxyl group significantly decreased the anti-HIV activity of this compound. Addition of a second sugar moiety as in acacetin-7-O-(6’-rhamnopyranosyl)-\(\beta\)-galactopyranoside abolished the anti-HIV activity. Luteolin and its acetate derivatives possess an anti-HIV activity similar to that of acacetin-7-O-\(\beta\)-galactopyranoside; however, they are more toxic. A comparison of luteolin and quercetin shows that the addition of a hydroxyl group at C-3 dramatically decreases their activity against HIV.\textsuperscript{45}

Schinazi et al showed that baicalein inhibited certain viruses in vitro, including the Rauscher murine leukemia virus and the HIV virus, as well as cellular DNA polymerases, and that the inhibition of reverse transcriptase by the flavone baicalein is highly specific.\textsuperscript{46} These facts suggest that the flavone baicalein may be less toxic than the flavonols to the DNA and RNA polymerases in the host cell infected with retroviruses.

**Flavonols**

Kim et al in 1998 reported that quercetin 3-O-(2’,6’-digalloyl)-galactoside and quercetin 3-O-(2’-galloyl)-arabinopyranoside inhibited HIV-1 integrase and affected its penetration into the DNA of the host cell.\textsuperscript{47} It has been shown that inhibition of integrase can prevent the replication of the virus and can be effective in the treatment of AIDS.\textsuperscript{48} These quercetin-galloyl derivatives demonstrated an inhibitory effect against HIV-1 integrase [50% inhibitory concentration (IC\(_{50}\)) = 18.1 mcg/ml], whereas quercetin 3-galactoside, quercetin 3-rhamnoside, kaempferol 3-rhamnoside
and kaempferol 3-arabinoside were inactive.\textsuperscript{47}

Schinazi showed that the flavonols quercetin, myricetin, and quercetagetin inhibited certain viruses in vitro, including the Rauscher murine leukemia virus and the HIV virus.

Among the 17 flavonols tested, only myricetin and the 3-O-glucosides of kaempferol, quercetin, and myricetin caused significant inhibition of HIV-1 at nontoxic concentrations. The flavonols quercetin and quercetagetin were strong inhibitors of DNA polymerase-\(\beta\) and DNA polymerase-I, respectively. The flavonol myricetin was also a potent inhibitor of both DNA polymerase-\(\alpha\) and DNA polymerase I.\textsuperscript{46}

**Flavonones**

Schinazi reported that none of the flavanones selectively inhibited HIV-1 replication. On the contrary, these compounds were generally more cytotoxic than the flavone derivatives studied.\textsuperscript{46}

Lin et al in 1997 reported that naringenin showed a slight inhibition of HIV-1 reverse transcriptase.\textsuperscript{49}

**Prenylated Flavanones**

Meragelman et al reported that the noteworthy prenylated flavanones lonchocarpol-A-5,7,4'-trihydroxy 6,8-diisoprenylflavanone, lonchocarpol-A-3,5,7,4'-tetrahydroxy 6,8-diisoprenylflavanone, and lonchocarpol-A-3,5,7,4'-tetrahydroxy 6,8-diisoprenylflavonol exhibited activity against HIV. The presence of a prenylated group appears to be necessary for antiviral activity, and flavanones without these substitutions were inactive.\textsuperscript{50}

**Flavonoids**

Schinazi noted that the flavonoids baicalein, baicalein methyl ester, scutellarein, myricetin, and gossypetin inhibited viral polymerases and produced an IC\textsubscript{50} between 35 and 200 mcg/ml; however, none of these flavonoids showed any significant effect on DNA polymerase 1. The flavonoids quercetagetin, 6-hydroxyluteolin, pedalitin, and 6-hydroxykaempferol were the most potent viral DNA polymerase inhibitors (IC\textsubscript{50} = <10mcg/ml).\textsuperscript{46}

**Biflavonoids**

Lin et al in 1997 reported that biflavonoids also show antiviral activity. The biflavonoids made of two apigenin units (amentoflavone, agathisflavone, robustaflavone, and hinokiflavone) demonstrated significant activity against HIV-1 reverse transcriptase, IC\textsubscript{50} = 119, 1,000, 65 and 62 mcg/ml, respectively. Biflavonoids made of flavonane and flavone (morelloflavone and volkseniflavone) were moderately to weakly active. Biflavonoids that linked C-3 to C-8 were moderately active, while biflavonanes made of two naringenin units linked through ring A (rhussflavonane and succedaneaflavonane) were inactive. In contrast, apigenin exhibited activity of IC\textsubscript{50} = 443 mcg/ml and naringenin showed a slight inhibition of HIV-1 transcriptase.\textsuperscript{49}

**INHIBITION OF VIRAL ENZYMES**

**Flavone**

Schinazi reported that the inhibition of reverse transcriptase by the flavone baicalein is highly specific, whereas the flavonols quercetin and quercetagetin were strong inhibitors of DNA polymerase-\(\beta\) and DNA polymerase-I, respectively.\textsuperscript{46}

**Flavonols**

Schinazi reported that quercetin, myricetin, and quercetagetin were shown to inhibit cellular DNA polymerase-\(\beta\) and DNA polymerase-I and that quercetin and quercetagetin were strong inhibitors.\textsuperscript{46}

**Flavonoids**

Of the 18 flavonoids tested by Vlietinck et al, scutellarein and quercetin, as well as the biflavonoid amentoflavone, proved to be the most active inhibitors of avian myeloblastosis reverse transcriptase, Rhouss-associated virus-2 reverse transcriptase, and Moloney murine leukemia reverse transcriptase.\textsuperscript{51}

**ANTI-ROTA VIRUS ACTIVITY**

**Flavonoids**

Bae et al in 2000 measured the influence of 20 flavanones, flavones, flavonols, and isoflavones on the MA 104 cell line infected with rotavirus. The greatest inhibitory effects were demonstrated by those flavonoids that contained glucose or rhamnose as the sugar unit; for example, IC\textsubscript{50} values for the flavanones hesperidin = 10 mcg/ml, poncirin, neohesperidin, and naringin = 25 mcg/ml; the flavone diosmin = 10 mcg/ml; the flavonol rutoside = 98 mcg/ml; the monosides and aglycones were inactive. Hesperidin administered before infection with rotavirus prevented the penetration of the virus in the MA 104 cell line. The above compounds would be less effective in vivo because they are metabolized to aglycones in the cecum and large intestine and rotavirus replication takes place in the small intestine.\textsuperscript{52}

**ANTI-INFLUENZA-A, -INFLUENZA-B ACTIVITY**

**Flavonones**

Kim et al in 2001 reported that hesperetin-07-O-(2",6"-di-O-a-rhamnopyranosyl)-\(\beta\)-glucopyranoside from Citrus junos inhibited replication of the influenza A virus at IC\textsubscript{50} = 918.5 \pm 83.2 mcg/ml, whereas naringenin-7-O-(2",6"-di-O-a-rhamnopyranosyl)-\(\beta\)-glucopyranoside, hesperidin, and narirutin were inactive.\textsuperscript{53}

**Flavonoids**

Among the 103 flavonoids reported on by Nagai et al., 5,7,4'-trihydroxy-8-methoxyflavone from the root of Scutellaria baicalensis showed the most potent activity
against influenza virus sialidase. The inhibitors of influenza virus sialidase have the possibility of blocking this viral infection.\textsuperscript{54}

**Biflavonoids**

Lin et al in 1999 evaluated seven natural biflavonoids, their methyl ethers, and their acetates for their activity against influenza-A and influenza-B among others. Amentoflavone, agathisflavone, robustaflavone, volkensiflavone, and succedaneaflavanone showed the highest activity against the influenza-B virus, while amentoflavone, robustaflavone were active against influenza-A virus, whereas rhusflavanone was effective against the influenza-B virus. Acetylation or methylation of the above compounds caused no significant change in their activity.\textsuperscript{43}

**RESPIRATORY SYNCYTIAL VIRUS (RSV)**

**Biflavonoid**

Ma et al reported that amentoflavone showed potent antiviral activity against the respiratory syncytial virus (IC\textsubscript{50} = 5.5 mcg/ml).\textsuperscript{55}

**SUMMARY**

The antibacterial activity of flavonoids against both Gram-positive and Gram-negative bacteria has been reported. Activity against Gram-positive bacteria (\textit{S. aureus}) was demonstrated mainly by compounds that contained hydroxyl groups in ring B. The most active were the 3',4',5'-tri-hydroxyflavonoids, eg, myricetin-3,5,7,3',4',5'-hexahydroxyflavone. Flavanone aglycones (naringenin, pinocembrin) and their C-6 or C-8 prenylated derivatives also turned out to be active. Only those 3-methoxy flavones having additional hydroxyl groups at C-5 and C-7 showed activity against \textit{B. cereus}. Flavones such as apigenin and its C- and O- glycosides were not active.

Activity against Gram-negative bacilli (\textit{E. coli}, \textit{P. aeruginosa}, \textit{P. vulgaris}, and \textit{K. pneumoniae}) was demonstrated by the flavones apigenin, vitexin, and saponarin, while flavonoid compounds having two or three hydroxyl groups in rings A or B were active against Gram-positive bacteria. Glycosides in general were less active, but acetyl derivatives of kaempferol-3-glucoside showed some activity against \textit{S. aureus}, and \textit{B. cereus}. Their activity, however, was directly related to the presence of an acyl substitution in the \textit{cis} form.

Flavonoids were active against antibiotic-resistant bacteria. However, these bacteria were inhibited only by those compounds having hydroxyl groups in ring B (myricetin > luteolin > quercetin > kaempferol) or by prenylated flavanones. These flavanoids expand the list of natural compounds found to be active against antibiotic-resistant bacteria. Studies using the combination of vancomycin and 3,5,7-trihydroxyflavone (galangin) or 3,7-dihydroxyflavone blend against VRE strains of \textit{E. faecalis} and \textit{E. faecium} showed that the use of these combinations resulted in a decrease in the MIC for vancomycin to a level characteristic of VSE. Flavonoids, in particular flavanones (hesperidin), were found to inhibit the growth of \textit{H. pylori}.

Some flavonoids were found to demonstrate antiviral activity against influenza viruses HSV-1, HSV-2, the rotavirus, and even HIV. In HIV, their activity was related to a direct effect on the virus or on the enzymes responsible for its replication (HIV-1 reverse transcriptase or HIV-1 integrase).

It is evident that a structure-activity relationship exists between the various flavonoids and their antimicrobial activity. It is the aim of this paper to promote further study of this phenomenon.

**REFERENCES**


Suppression of Human Cartilage Degradation and Chondrocyte Activation by a Unique Mineral Supplement (SierraSil™) and a Cat’s Claw Extract, Vincaria®

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ABSTRACT
Cartilage degradation, a hallmark of both rheumatoid arthritis and osteoarthritis, contributes to the dysmobility, pain and compromised quality of life associated with these conditions. We investigated the hypothesis that the unique clay-based mineral supplement SierraSil™ alone, and in combination with an extract of cat’s claw, Vincaria, could limit human cartilage degradation-activated chondrocytes. The investigative model used was human cartilage tissue, obtained at the time of knee surgery, studied in vitro. Cartilage explants were used to quantify cartilage matrix degradation, and for obtaining human chondrocytes that were evaluated in cell culture. SierraSil was subjected to neutral, alkali, and acid washes, followed by neutralization before addition to cartilage explants or cultured chondrocytes (0.05, 0.1, and 0.2 µg/ml). Vincaria, an alkaloid depleted aqueous extract of cat’s claw (Uncaria guianensis) was studied in combination with SierraSil (final concentrations of 2.5, 5 and 10 ng/ml). Chondrocytes were activated with the addition of the inflammatory cytokine interleukin-1β (IL-1β, 5 ng/ml). Measured outcomes were media nitrate/nitrite levels as an index of nitric oxide production, and media glycosaminoglycan (GAG) concentrations as an index of matrix breakdown. Following neutral or alkali washes, SierraSil was ineffective in reducing nitric oxide release, although a small reduction in GAG release was observed with neutral extracts (p<0.05). However, the combination of SierraSil + Vincaria significantly reduced both GAG and nitric oxide release under these conditions. Following an acid wash to mimic passage of the material through the stomach, SierraSil alone significantly reduced IL-1β-induced GAG release by 68-73% (p<0.01) and SierraSil + Vincaria by 58-77% (p<0.01). Production of NO by human chondrocytes was also reduced by acid-washed SierraSil alone (p<0.05) and was more pronounced with the SierraSil + Vincaria combination (p<0.01). IL-1β-induced nitric oxide production and GAG release is known to reflect the activation of inducible pathways (inducible nitric oxide synthase and matrix metalloproteases). The attenuation of these events suggests that this herbo-mineral combination limits cartilage destruction by curtailing these transcriptional events in chondrocytes. Results suggest that this nutraceutical-based therapeutic agent may offer a new approach to limiting joint destruction and dysmobility associated with arthritis.

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INTRODUCTION

Rheumatoid and osteoarthritis are inflammatory states that exert catabolic actions on cartilage.\(^1\)\(^2\) Activation of chondrocytes by various inflammatory signals results in the digestion of cartilage matrix and release of the fragments, leading to an overall reduction in cartilage tissue. A primary initiator of cartilage destruction is the cytokine IL-1\(\beta\), which is produced by activated synovium, infiltrating macrophages, and activated chondrocytes.\(^3\)\(^4\) IL-1\(\beta\) is a potent catabolic agent in arthritis, and approaches that can attenuate these catabolic pathways are sought as potential treatments in the management of arthritis and joint dysfunction.\(^5\)\(^6\)

Diverse cascades of events are activated by IL-1\(\beta\) in chondrocytes. One well-described pathway is enhanced NO production, which results in transcriptional activation of inducible nitric oxide synthase (iNOS).\(^7\)\(^8\) Matrix metalloproteases (MMPs)\(^9\)\(^11\) are also transcriptionally regulated and activated by IL-1\(\beta\). MMPs are enzymes that actively digest the cartilage matrix, releasing degraded glycosaminoglycans.

The nutraceuticals glucosamine and chondroitin are also cartilage matrix elements, essentially bricks in the wall that is cartilage. The popularity of glucosamine and chondroitin as therapeutic agents for arthritis reflects the contention that oral ingestion and absorption of these matrix elements will replace that which is lost to the catabolic inflammatory process.\(^12\) However, this replacement process is not well regulated by the simple ingestion of substrate, and is fighting against a tide of catabolic release. It is assumed that ingested elements will be absorbed and transported to the site of destruction and inserted into the cartilage at a rate that exceeds the rate of loss. For this reason, therapeutic approaches with improved response rates, faster onset of benefits, and enhanced overall effectiveness are desirable.\(^12\)\(^14\)

We have shown in a double-blind placebo-controlled trial\(^15\) that Vincaria, an extract of Uncaria guianensis, which is devoid of immune-enhancing alkaloids, is an effective treatment for osteoarthritis. In this study, benefits were significant within a week and were further enhanced with continued administration. This was an extension of a number of pre-clinical studies that demonstrated that cat’s claw extracts were cytoprotective and anti-inflammatory agents.\(^16\)\(^19\) What was clearly evident was that these actions resulted from the ability of cat’s claw to negate redox events, particularly activation of the transcription factor NF-κB.

NF-κB regulates over 30 genes associated with inflammation, including chemokines and adhesion molecules, but those of particular relevance to cartilage are inducible nitric oxide synthase (iNOS) and MMPs. We had previously shown that cat’s claw was an effective inhibitor of iNOS expression and thereby suppresses the elevation of NO production associated with inflammation.\(^20\)\(^22\) We were also the first group to demonstrate that selective inhibition of iNOS could prevent chronic inflammation and associated cell death and tissue destruction.\(^23\)\(^24\) Perhaps even more impressive is the observation that cat’s claw is the most potent natural substance for preventing the activation of tumor necrosis factor a (TNFα),\(^16\)\(^19\) which has a primary role in rheumatoid arthritis and is the focus of antibody-based therapies (Remicade\(^\text{®}\) and Enalabrel\(^\text{®}\)). However, while NF-κB also regulates formation and activity of MMPs, a direct link between cat’s claw and MMPs had not been reported.

The human cartilage explants and chondrocyte culture models were chosen because they (a) accurately reflect the inflammatory processes in the disease setting, (b) could be well controlled, and (c) had a significant background perspective with other botanical-based transcriptionally-active agents like green tea polyphenols.\(^25\)\(^26\) We had shown that the epigallocatechin gallate (EGCG) was a potent inhibitor of IL-1\(\beta\)-induced MMPs activity, nitric oxide production and iNOS induction, cyclo-oxygenase 2 induction (COX2), and various transcription factors in this model.\(^27\) The important ramifications of these observations were then confirmed in animal models of arthritis.\(^25\) This detailed background allowed us to not only directly test the hypothesis but also to frame these results in a manner that would assist in follow-up clinical trials.

NF-κB and related transcription factors are activated by oxidants, and for this reason are deemed to be redox sensitive—the redox state of a molecule reflecting its oxidative state. Minerals, particularly transition metals, are redox active and can both promote and attenuate free radical and oxidant events,\(^28\)\(^29\) depending on how electron transfer is manipulated. Anecdotal evidence suggested that the mineral-rich clay product SierraSil was effective in alleviating the symptoms of arthritis and inflammation. Further, there were suggestions that the onset benefits occurred with some relative rapidity, i.e., days/weeks as opposed to months for glucosamine and chondroitin. These observations suggested that a locus of action was different between these therapeutic approaches. This suggestion, combined with the appreciation that minerals can affect oxidant processes, led us to hypothesize that SierraSil may attenuate redox-based transcription events, which then formed the basis of our working hypothesis.

MATERIALS & METHODS

Reagents

Culture medium and reagents were purchased from either Cellgro (Mediatech, MD, USA) or GibCO BRL (Bethesda, MD, USA). SierraSil\(^\text{TM}\) (SM317) was obtained from the manufacturer, Sierra Mountain Minerals (Vancouver, Canada) and Vincaria\(^\text{®}\) (RN180) cat’s claw extract was supplied by Rainforest Nutritional, Inc. (Phoenix, AZ). Recombinant IL-1\(\beta\) was purchased from R&D Systems (St. Paul, MN.).
Culture of Human Chondrocytes

Human osteoarthritis cartilage samples were procured through the Cooperative Human Tissue Network with prior approval of the Institutional Review Board of University Hospitals of Cleveland. Chondrocytes were prepared by the enzymatic digestion of femoral head cartilage as previously described.30,31 Chondrocytes were plated (1 x 10⁶ cells/ml) in 35 mm culture dishes (Becton-Dickinson, Mountain View, CA, USA) in complete Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal calf serum. These were allowed to grow for 72 hours at 37°C and 5% CO₂ in a tissue culture incubator. Chondrocytes were serum-starved overnight and then treated with IL-1β (5 ng/ml) and different doses of SierraSil (0.05, 0.1 and 0.2 µg/ml) or SierraSil + Vincaria, with final Vincaria concentrations of 2.5, 5, and 10 ng/ml. Media was collected after 12 hr for determination of nitrite and nitrate levels.

Culture of Cartilage Explants

Full thickness slices (20-25 mg) were obtained from human cartilage samples using sterile scalpel blades (Feather Razor Co., Osaka, Japan). Four to five cartilage pieces were transferred to each well of a 24-well, flat-bottomed plate (NUNC A/S, Roskilde, Denmark) containing DMEM supplemented with antibiotics and 10% fetal calf serum. These were repeated 2–3 times. The cartilage explants were treated with IL-1β alone or in combination with SierraSil (± Vincaria) for 72 hours. Controls included the culture of explants without either IL-1β or SierraSil. SierraSil was tested in the absence of IL-1β for any basal activity.

Quantitation of Glycosaminoglycans

At the conclusion of the explant culture period, the culture medium was collected from each group. A 50 µl aliquot was used to estimate total glycosaminoglycan concentration by a colorimetric method employing DMMB as previously described (Farndale et al., 1986). Color intensity was read spectrophotometrically at 535 nm, and values derived from a standard curve derived from a different concentration of chondroitin sulfate. Results are expressed as micrograms of glycosaminoglycan released per mg of cartilage tissue.

**Determination of Nitric Oxide Production**

Media from cultured chondrocytes in the various treatment groups were collected and used to measure nitrite content using a commercially available kit (R&D Systems).

Wash/Extract Procedures

Preparation of acidic extracts of SierraSil ± Vincaria were made by mixing 0.4 g of the material with 0.1 N HCl (0.4% solution) followed by shaking for 30 minutes at room temperature. The pH was then restored to 7.0 by adding 1M NaOH and the final volume adjusted to 100 ml with deionized water.

Alkaline washes were made similarly to the acidic extracts. SierraSil ± Vincaria 0.4 g was mixed with 0.05 M NaOH and shaken for 30 minutes at room temperature. The pH was then adjusted to 7.0 with 1 M HCl. Neutral washes were made with deionized water.

All washes were centrifuged at 10,000 rpm for 1 minute to pellet all insoluble material and then sterilized by passage through a 0.45 micron filter under vacuum.

**Statistical Analysis**

Results were compared by a One-Way Analysis of Variance; when there was a significant variation between groups, then post-hoc evaluations were made using the Dunnett’s Multiple Comparisons test. A significant difference was inferred if the p value was less than 0.05.

**RESULTS**

Neutral Washes

Cartilage explants and cultured chondrocytes were tested under basal and IL-1β-stimulated conditions where SierraSil ± Vincaria (0.05 and 0.1 µg/ml) were solubilized under neutral pH conditions. The results, summarized in Table 1, indicate that SierraSil alone had no effect on GAG release at the 0.05 µg/ml dose and a small but significant reduction at the 0.1 µg/ml dose. In contrast, SierraSil elevated NO release above that evident with IL-1β at both doses (p<0.01). The combination of SierraSil + Vincaria significantly attenuated NO production when compared to SierraSil alone at both concentrations (p<0.01) and was significantly different from IL-1β at the 0.1 µg/ml dose (p<0.01). Basal NO or GAG release was unaffected by SierraSil or SierraSil + Vincaria, at the highest concentration tested (data not shown).

**Table 1. Influence of SierraSil and SierraSil + Vincaria on NO production and GAG release from human cartilage under neutral pH extraction procedures.**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IL-1β</th>
<th>IL-1β + SierraSil</th>
<th>IL-1β + SierraSil</th>
<th>IL-1β + SierraSil + Vincaria</th>
<th>IL-1β + SierraSil + Vincaria</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>0.06 ± 0.03</td>
<td>0.105 ± 0.001</td>
<td>0.122 ± 0.001*</td>
<td>0.156 ± 0.001*</td>
<td>0.107 ± 0.001</td>
<td>0.097 ± 0.001*</td>
</tr>
<tr>
<td>GAG</td>
<td>0.980 ± 0.043</td>
<td>1.400 ± 0.138</td>
<td>1.438 ± 0.053</td>
<td>1.286 ± 0.025*</td>
<td>1.121 ± 0.017*</td>
<td>0.930 ± 0.006*</td>
</tr>
</tbody>
</table>

*p<0.05 vs. IL-1β
was not evident over the range examined (0.05–0.2 Vincaria (p<0.01, Fig. 3). However, a clear dose-dependency by GAG release and a significant suppression was observed at IL-1β affected basal NO release at the highest concentration tested (data not shown).

Because of the minor effects on NO production, an evaluation of alkaline extracts of SierraSil on GAG release was not performed.

Acid Extraction

Acid washes to mimic passage through the stomach resulted in enhanced bioactivity of SierraSil (Fig. 2 and 3). In cultured human chondrocytes, acid washes of SierraSil resulted in a reduction in IL-1β-induced nitric oxide release at all concentrations tested (p<0.01). As observed with the other experimental conditions, the combination of SierraSil with Vincaria resulted in a further suppression of IL-1β-induced NO formation (p<0.01 vs. SierraSil alone and p<0.001 vs. IL-1β).

Cartilage breakdown induced by IL-1β was determined by GAG release and a significant suppression was observed at all concentrations examined for both SierraSil and SierraSil + Vincaria (p<0.01, Fig. 3). However, a clear dose-dependency was not evident over the range examined (0.05–0.2 µg/ml). The protection of cartilage breakdown approximated 68–83% for SierraSil and 58–77% for SierraSil + Vincaria.

DISCUSSION

Arthritic joints have high levels of pro-inflammatory cytokines that contribute to the pathophysiology by promoting cell recruitment, cell activation, and release of destructive matrix metalloproteases, and by preventing chondrocyte proliferation and extracellular matrix synthesis. These processes culminate in a loss of cartilage matrix and a progressive destruction of joint architecture, and together with symptoms (pain, discomfort) contribute to a compromised quality of life. Despite the enormous size of the osteoarthritis problem and pharmaceutical market, estimated at 12.38 billion dollars annually, current approaches provide symptomatic relief and do not arrest the disease process and loss of cartilage matrix. Based on the need for therapeutic alternatives, nutraceuticals are also used for treating osteoarthritis and represent approximately 640 million dollars in sales in the USA annually. The best known of these complimentary medicine approaches is glucosamine and chondroitin.

However, this approach suffers from a slow onset of action, limited efficacy, and a low response rate. Other approaches are less well appreciated by the consumer and health care providers but mechanistically offer greater appeal. For example redox-active botanicals possess the ability to prevent the activation of a litany of genes involved in the inflammatory process.

One of these classes of agents is the green tea polyphenols which affect signal transduction and limit the catabolic pathways described in this study. Several papers extend these observations to various animal models. Cat’s claw is also a redox-sensitive transcription inhibitor, which limits the formation of Th1 cytokines like TNFa and oxidant-induced cell death. Administration of alkaloid-depleted cat’s claw has also been shown by us to be an effective clinical strategy in osteoarthritis, with benefits evident within a week as expected for an intervention that works by suppressing gene expression. Indeed vincaria cat’s claw is the most potent natural product inhibitor of TNFa formation reported to date with a EC50 of approximately 10 ng/ml. This remarkable potency was confirmed in the present conditions where Vincaria cat’s claw was combined with SierraSil over a concentration range of 2.5–10 µg/ml.

Despite this, however, cat’s claw is sometimes erroneously termed an immune stimulant; this misconception may account for its reduced popularity in treating osteoarthritis. There is limited evidence for any immune-activating actions of cat’s claw at therapeutic doses, and this assertion has largely been attributed to the oxindole alkaloid fraction present in Uncaria tomentosa. In this study, we used an alkaloid-depleted cat’s claw extract (Vincaria) to avoid this chemical confusion and to better link the present observations to the previous clinical observations in osteoarthritis.

Matrix metalloproteases and inducible nitric oxide synthase are two pathways that lead to cartilage catabolism that are regulated at the transcriptional level by redox-sensitive transcription factors. We had previously described Vincaria cat’s claw as an effective inhibitor of iNOS gene expression and nitric oxide production, actions confirmed in this study. The present study also confirms that cat’s claw is an effective inhibitor of cartilage degradation via MMPs. This assertion is indirect, although it is well appreciated that IL-1β-induced GAG release results from the formation and activation of MMPs.

SierraSil is a relatively new nutraceutical with little background information on efficacy and mechanisms of action, although there is anecdotal evidence that it is beneficial to arthritic patients. SierraSil is a mineral-rich clay product, similar but not identical to bentonite. Considering the variety and amount of its mineral constituents, we hypothesized that it may be acting via redox-sensitive pathways. The present results confirmed that hypothesis with a
reduction in IL-1β-induced nitric oxide formation and GAG release. It was also evident that the SierraSil’s active components, which currently are unknown, are more readily released into solution under acidic conditions than either alkaline or neutral pH conditions. Vincaria cat’s claw was effective following acidic, neutral, and alkaline washes, although it was slightly less effective following alkaline washes. Clay products are noticeably insoluble in water, and these extraction processes may have released different amounts of the bioactives accounting for the variations in bioactivity. We interpret the current results as being reflective that doses of SierraSil that are used by consumers (2-3 g/day) will provide ample bioavailable bioactives for limiting joint destruction.

We appreciate that the present results were obtained in vitro, requiring that caution be used in extending these observations to the clinical setting. However, Vincaria has been shown to be effective in a double-blind placebo-controlled trial. Both SierraSil and SierraSil + Vincaria were effective in this in-vitro model. Additionally, other interventions that limit these processes have also been effective in the clinical or experimental model setting. Thus, within the constraints of caution, it is reasonable to assume that SierraSil may limit cartilage destruction and chondrocyte activation in clinical osteoarthritis. A clinical trial is currently underway to confirm this assertion, including its combination with Vincaria cat’s claw.

**ACKNOWLEDGEMENT**

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**Figure 1**. Effects of alkaline-extracted SierraSil and SierraSil + Vincaria on the production of nitric oxide from human chondrocytes. The * depicts a significant difference between the group and the IL-1β controls.
Figure 2. Effects of acid extracted SierraSil and SierraSil + Vincaria on the production of nitric oxide from human chondrocytes. The * depicts a significant difference between IL-1 and all groups (p<0.01), and ** a significant difference between SierraSil and SierraSil + Vincaria at comparable concentrations (p<0.01).

REFERENCES
Figure 3. Effects of acid-extracted SierraSil and SierraSil + Vincaria on the release of GAG from human cartilage explants. The * depicts a significant difference between IL-1β and all other groups (p<0.01)


Synergistic Effect of Combination of Lysine, Proline, Arginine, Ascorbic Acid, and Epigallocatechin Gallate on Colon Cancer Cell Line HCT 116

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ABSTRACT

Limitation of current treatment methodologies to control metastasis, as well as the proposed antitumor properties of specific nutrients, prompted us to investigate the effect of a specific formulation (NS) of lysine, proline, arginine, ascorbic acid, and epigallocatechin gallate on human colon cancer cells HCT 116 for viability, MMP expression, invasion, and morphology. Cell proliferation was based on MTT assay and MMP expression in condition media by gelatinase zymography. Invasion through Matrigel was evaluated and morphology was assessed by Hematoxylin and Eosin staining. NS did not demonstrate antiproliferative effect up to 1000 mcg/ml concentration. Cancer cell invasion through Matrigel was significantly reduced at 100 mcg/ml (76%, p=0.0008) and completely inhibited at 500 mcg/ml (p=0.0002). Gelatinase zymography showed dose-dependent inhibition of MMP-9 expression by NS with virtual total inhibition at 100 mcg/ml. Our results suggest that the specific formulation of nutrients tested is an excellent candidate for therapeutic use in the treatment of colon cancer by inhibiting MMP expression and invasion.

KEYWORDS: colon cancer, MMP, nutrient synergy, antitumor effect

INTRODUCTION

Colorectal cancer is the second most deadly cancer in the United States; the American Cancer Society estimates that approximately 56,000 Americans will die from the disease this year. Colon cancer affects both men and women over age 50 with approximately the same frequency. Other than age, several other risk factors are associated with colorectal cancer: family/individual history of colorectal cancer or adenomatous polyps, inflammatory bowel disease, diets rich in saturated fat, and inactivity. However, it is important to note that 75% of colorectal cancer cases occur in individuals with no known predisposing factors.

While colorectal cancer is treatable upon early detection, once the cancer metastasizes to the lymph, liver, or other areas, 5-year survival is less than 10%; most of these fatalities are associated with metastasis. Improved screening methods have been linked to the recent decline in incidence and mortality of colorectal cancer; however, they are underutilized on basis of cost, discomfort, inaccuracy, and risk of complication, approximately 2 perforations for every 1,000 colonoscopies performed.

Early-stage colon cancer is generally successfully treated with surgery (local excision/colon resection) depending on the size of the lesion; however, side effects can range from mild to severe: diarrhea, constipation,
depression, bleeding, and infection, and 15% of all colorectal patients require a permanent colostomy. Standard treatment of Stage II colon cancer and advanced stages may involve both chemotherapy and radiation therapy. As with most chemotherapy approaches, cancer cells are eventually capable of independent growth, invasion, adhesion, angiogenesis, and avoidance of apoptosis, rendering this approach ineffectual. Furthermore, only 10–20% of patients on fluorouracil experience palliation, yet the associated side effects of chemotherapy include nausea, vomiting, hair loss, mouth sores, diarrhea, fatigue, bleeding, infection, and weight loss. Finally, radiotherapy may be used before surgery to shrink tumors or postoperatively to eradicate remaining cancer cells. External radiotherapy focuses on cancer cell destruction, but not metastases, which are the main cause of death in patients with colorectal cancer. These treatment methods have not only been ineffective in providing a cure, but involve the indiscriminate attack of all cells, causing cellular damage and destruction of the body's connective tissue, facilitating cancer metastasis. Clearly, there is a need for safe and effective therapeutic approaches that can be used to control the process of cancer metastasis as well as to prevent of colon cancer.

Cancer cells form tumors and spread by degrading the extracellular matrix (ECM) through various matrix metalloproteinases (MMPs). The activity of these enzymes correlates with the aggressiveness of tumor growth and invasiveness of the cancer. In 1992 Rath and Pauling postulated that nutrients such as lysine and ascorbic acid could act as natural inhibitors of ECM proteolysis and, as such, have the potential to modulate tumor growth and expansion. These nutrients can exercise their anti-tumor effect through inhibition of MMPs and strengthening of connective tissue surrounding cancer cells (tumor-encapsulating effect). In a previous study, we demonstrated the anti-proliferative and anti-invasive potential of lysine, ascorbic acid, proline and epigallocatechin gallate (EGCG) on human breast cancer (MDA-MB 231), colon cell cancer (HCT 116), and melanoma (A2058) cell lines. NS also suppressed the growth of these tumors, with no adverse effects, in nude mice. In the current study, we investigated the anti-tumor potential of NS on human colon cancer cells HCT 116 by measuring: cytotoxicity, secretion of MMP-9, and matrix invasive potential.

**MATERIALS AND METHODS**

**Cell Culture**

Human colon cancer cells HCT 116 were obtained from ATCC (American Type Culture Collection, Rockville, MD) and cultured in MEM (modified Eagle's medium), supplemented with 10% fetal bovine serum, penicillin G sodium (100 mcg/ml), streptomycin (100 mg/ml), and amphotericin (0.25 mcg/ml) in 24-well tissue culture plates (Costar, Cambridge, MA). Cells were incubated with 1 ml of media at 37°C in a tissue culture incubator equilibrated with 95% air and 5% CO₂. At near confluence, the cells were treated with NS (composed of vitamin C 700 mg, L-lysine 1000 mg, L-proline 750 mg, L-arginine 500 mg, N-acetyl-cysteine 200 mg, standardized green tea extract (82% polyphenol, 36% EGCG) 1000 mg, selenium 30 mg, copper 2 mg, and manganese 1 mg), dissolved in media, and tested in triplicate at 0, 10, 50, 100, 500, and 1000 mcg/ml concentrations. The plates were then returned to the incubator. Cell proliferation was evaluated after 24 hrs following incubation with test reagents. Culture media components were purchased from Gibco (Grand Island, New York), and all other chemicals used were purchased from Sigma (St. Louis, MO).

**MTT Assay**

Cell proliferation was evaluated by MTT assay. The MTT assay is a colorimetric assay based on the ability of viable cells to reduce a soluble yellow tetrazolium salt [3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide] (MTT) to a blue formazan crystal by mitochondrial activity and thus of cell viability. After MTT addition (0.5 mg/ml), the plates were covered and returned to the 37°C incubator for 2 hours, the optimal time for formazan product formation. Following incubation, the supernatant was carefully removed from the wells, the formazan product was dissolved in 1 ml of DMSO (dimethyl sulfoxide), and absorbance was measured at 570 nm in a BioSpec 1601 Shimadzu spectrophotometer. The OD₅₇₀ of the DMSO solution in each well was considered proportional to the number of cells. The OD₅₇₀ of the control (treatment without supplement) was considered 100%.

**Gelatinase Zymography**

MMP expression in condition media was determined by gelatinase zymography. Gelatinase zymography was performed in Novex precast SDS-polyacrylamide gel (Invitrogen Corporation) in the presence of 0.1% gelatin. Culture media (20 μl) was loaded and SDS-PAGE was performed with a tris-glycine SDS buffer, as described by the manufacturer (Novex). Following electrophoresis, the gels were washed twice in 2.5% Triton X-100 for 30 minutes at room temperature to remove SDS. The gels were then incubated at 37°C overnight in substrate buffer containing 50 mM Tris-HCl and 10 mM CaCl₂ at pH 8.0 and stained with Coomassie Blue R250 in 50% methanol and 10% glacial acetic acid for 30 minutes and destained. Protein standards were run concurrently and approximate molecular weights were determined by plotting the relative mobilities of known proteins.

**Matrigel Invasion Studies**

Invasion studies were conducted using Matrigel™ (Becton Dickinson) inserts in 24-well plates. Suspended in medium,
colon cancer cells were supplemented with nutrients, as specified in the design of the experiment and seeded on the insert in the well. Thus both the medium on the insert and in the well contained the same supplements. The plates with the inserts were then incubated in a culture incubator equilibrated with 95% air and 5% CO2 for 24 hours. After incubation, the media from the wells were withdrawn. The cells on the upper surface of the inserts were gently scrubbed away with cotton swabs. The cells that had penetrated the Matrigel membrane and migrated onto the lower surface of the Matrigel were stained with Hematoxylin and Eosin and visually counted under the microscope.

**Statistical Analysis**

The results were expressed as means ± SD for the groups. Data was analyzed by independent sample “t” test.

**RESULTS**

**Colon Cancer Cytotoxicity/Proliferation Study**

NS was not toxic to colon cancer cells (HCT 116) even at 1000 µg/ml concentration (Figure 1).

**Gelatinase Zymography Study**

As shown in Figure 2, zymography demonstrated expression of MMP-9 by human colon cancer cells HCT 116; NS inhibited MMP-9 expression in a dose-dependent fashion with virtual total inhibition at 500 µg/ml concentration.

**Invasion Study**

Invasion of colon cancer cells (HCT 116) through Matrigel was significantly (76%, p=0.008) reduced by NS at 100 mg/ml and completely (100%, p=0.0002) inhibited at 500 mg/ml of NS, respectively (Figure 3A). Morphology was observed using Hematoxylin and Eosin staining. H&E stains did not show any alterations in morphology with different doses of NS (Figure 3B).

**DISCUSSION**

Degradation of basement membranes by MMPs is key to the invasive potential of cancer cells. Moreover, research has shown that highly metastic colon cells (LuM1) secrete higher amounts of MMP-9 than do poorly metastic cells, demonstrating that the level of tumoral invasion correlates with MMP-9 expression in colon cancer. While the results of this study showed that NS did not have any cytotoxic effect on the tested colon cancer cell line (HCT 116) even at high concentrations, NS did show substantial inhibition of invasion and MMP-9 expression at 100 µg/ml, clearly demonstrating antimetastatic ability.

Matrix invasion can be controlled by inhibition of MMP expression, as well as by enhancing connective tissue strength and stability. In this study, the dose-dependent inhibitory effect of NS on MMP-9 expression of the colon cancer cells was consistent with its dose-dependent inhibition of matrix invasion. It has been postulated that lysine can act as a natural inhibitor of collagen matrix degradation.
through its inhibitory effect on plasmin. In addition, lysine can compete for enzyme binding sites in MMPs, thereby controlling activation pathways for various types of MMPs. The mechanistic aspects of MMP-9 inhibition by lysine were not in the scope of this study.

In addition, matrix invasion by cancer cells can be modulated by increased stability and strength of the connective tissue, secondary to the activity of the nutrients provided in NS. Optimization of synthesis and structure of collagen fibrils depends upon hydroxylation of hydroxyproline and hydroxylysine residues in collagen fibers. It is well known that ascorbic acid is essential for the hydroxylation of these amino acids, but as it is not produced in the human body, sub-optimal levels are common. Additionally, low levels of ascorbic acid have been reported in cancer patients.

The inhibitory effects of the individual nutrients composing NS have been reported in both clinical and experimental studies. Ascorbic acid has been reported to have cytotoxic and antimetastatic actions on malignant cell lines. In a six-week in vivo experiment, supplementation of EGCG caused a 60% reduction in colonic preneoplastic tumors, suggesting potent anti-inflammatory and chemopreventative properties.

However, individual nutrients are not as powerful as nutrient synergy. Our previous studies demonstrated that the synergistic anticancer effect of ascorbic acid, proline, lysine, and EGCG on several cancer cell lines in tissue culture studies was greater than that of the individual nutrients. In contrast to radiotherapy and chemotherapy, which cause indiscriminate cellular and ECM damage, morphological studies showed that even at the highest concentrations of NS, the colon cancer cells were unaffected, demonstrating that this formulation is safe to cells.

CONCLUSION

While clinical studies are necessary to better determine the efficacy of nutrient therapy in both cancer prevention and treatment, the results of this study suggest the formulation of lysine, proline, arginine, ascorbic acid, and epigallocatechin gallate tested as a valuable and promising candidate for therapeutic use in the treatment of colon cancer, by inhibiting cell proliferation, MMP expression, and invasion.

ACKNOWLEDGEMENT

Funding for this study was provided Matthis Rath Research.

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New clinical research indicates that SierraSil, when used alone or in conjunction with Vincaria® cat’s claw, supports human cartilage integrity.* This study is in this edition of JANA!

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