L-CITRULLINE
Pharmacy Compounding Advisory Committee
November 20-21, 2017

A.J. Day, PharmD
Director of Clinical Services, PCCA
L-Citrulline Source

- FDA-registered & inspected cGMP facility
- Fermentation pathway

Common Dose Range
- 500-650 mg
- Weight-based, so dose will vary

Handful of pharmacies across the USA specialize in these disorders
- Relevance for MOU
THANK YOU

Questions from the Committee?
PREGNENOLONE

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Director of Clinical Services, PCCA
I. INTRODUCTION

Pregnenolone has been nominated for inclusion on the list of bulk drug substances for use in compounding under section 503A of the Federal Food, Drug, and Cosmetic Act (FD&C Act) for use in the treatment of rheumatoid arthritis, hypercholesterolemia, as an adjunctive therapy in patients with schizophrenia, and as an adjunctive therapy in the treatment of bipolar disease.¹

The routes of administration and doses proposed in the nomination included: oral capsules (5-200 mg), oral suspension (10-200 mg/mL), topical cream and gel (1-20%), as well as injection suspension (0.1 – 5 mg/mL).
Thank you for contacting me regarding PCCA's submitted nomination for pregnenolone for consideration to the 503A bulk drug substances list. The below information is intended to clarify our nomination, and it is consistent with the nomination intent from three other nominating entities: IACP, NCPA, and Fagron. Representatives from those groups are copied on this email.

Sincerely,
A.J. Day, PharmD, RPh
Director of Clinical Services, PCCA

**Proposed use & route of administration**

Oral formulations of pregnenolone are compounded as adjunctive therapy for positive and negative symptoms in patients with schizophrenia, and adjunctive therapy for manic and depressive symptoms in depressed patients with and without a history of substance abuse. Commonly compounded doses range from 5 – 200 mg. Below are clinical references.

References:

FAERS and CAERS data

January 1, 2000 - June 7, 2017. The search resulted in seven distinct cases where pregnenolone was taken orally or topically in combination with various drugs and/or supplements. In the three cases where the dose was reported, oral dosing was either 10 or 50 mg per day and topical dosing was 0.5% topical solution (combined with multiple other ingredients) applied to the scalp twice a day. No serious adverse events (AEs) were reported. AEs included recurrence of menopausal symptoms, dizziness, nausea, and excessive growth of hair. None of these AEs can be directly linked to the exclusive use of pregnenolone since in all of the reported cases other concomitant drugs/supplements were taken.

(CAERS). A search of CAERS was conducted for adverse events associated with the use of pregnenolone on June 23, 2017, and retrieved 30 reports of adverse events. Of these, serious adverse events were reported in 18 cases, including one death and 17 hospitalizations or other serious medical events. Medical interventions were reported in 11 out of 18 cases of serious adverse events. The dose of pregnenolone was reported in only 3 cases, and all 3 cases reported a daily dose of pregnenolone 50 mg capsule. The most commonly reported symptoms in the serious adverse events included increase in heart rate (n=5), increase in blood pressure (n=3), dizziness (n=3), headache (n=4), pain (n=3), hypersensitivity (n=3), dyspnea (n=3), tremor (n=4), anxiety (n=3), and fatigue (n=3). However, all cases were confounded with the use of multiple products and/or the use of products containing multiple ingredients. Adverse events in the multi-ingredient reports could not be attributed to pregnenolone.
Ritsner et al. (2010) reported a small double-blind, randomized, placebo-controlled trial of oral pregnenolone as adjunctive therapy in 58 patients with chronic schizophrenia or schizoaffective disorder. Treatment groups were: pregnenolone 30 mg/day (n=14), pregnenolone 200 mg/day (n=6), DHEA 400 mg/day (n=13), and placebo (n=11). Forty-four patients completed the trial. Six scales\textsuperscript{13} and their subscales were used as outcome measures and analyses were exploratory. All patients were taking concomitant medication(s) for their disease. The article states that no significant adverse events were observed. Compared with patients taking placebo, patients in the pregnenolone 30 mg/day arm showed improvement trends in the PANSS positive symptom subscale score and the attention and memory subtasks of the CANTAB; they had a positive trend in extrapyramidal side effects score. Of interest, the higher-dose (pregnenolone 200 mg) group did not show any improvement, which adds to uncertainty about efficacy results. The authors concluded that further studies are needed.
FDA – Concerns of Downstream Effects

• Marx et al. (2009)

Treatment with pregnenolone resulted in fourfold elevations in serum levels of pregnenolone (paired t-test \( p = 0.017, t = 3.11, \text{df} = 7 \)), tripled serum pregnenolone sulfate levels (paired t-test \( p < 0.0001, t = 10.44, \text{df} = 8 \)), and increased the GABAergic neurosteroid allopregnanolone fivefold (paired t-test \( p = 0.009, t = 3.59, \text{df} = 7 \)) (Table 6). Pregnenolone administration also increased serum progesterone over fourfold and DHEAS levels by approximately 16% (Table 6). **Treatment with pregnenolone did not increase serum testosterone, free testosterone, cortisol, DHEA, estradiol, or androstenedione levels** (Table 6).

• Dr. Marx has not seen any adverse events in any of her studies, ongoing and published
Despite the availability of pregnenolone as a dietary supplement in the United States, very few clinical trials have investigated its use. Studies conducted in the 1940s and 1950s showed that pregnenolone 25–500 mg/day was safe and well tolerated in humans (Davison et al., 1950; Freeman et al., 1950a; Guest et al., 1950; Henderson et al., 1950; McGavack et al., 1951; Pincus and Hoagland, 1944, 1945a,b). A number of these earlier studies treated patients with inflammatory diseases, such as rheumatoid arthritis, and several reported improvements in symptoms and overall functioning following pregnenolone. According to these earlier reports, pregnenolone was very well tolerated with minimal side effects, and did not affect weight, heart rate, blood pressure (even in patients with hypertension), menstrual cycle, or glucose levels (either in diabetics or non-diabetics). In a more recent investigation, pregnenolone at low doses (15–30 mg/day) was generally well tolerated in healthy volunteers with no significant side effects compared with placebo (Meieran et al., 2004). Evidence to date thus suggests that pregnenolone has a favorable safety profile, although controlled trials remain very limited.
METHODS

This pilot investigation was a placebo-controlled, double-blind, parallel group, randomized trial of adjunctive pregnenolone for the treatment of cognitive and negative symptoms in patients with schizophrenia or schizoaffective disorder. Following a single-blind 2-week placebo lead-in phase (all patients), subjects were randomized to 8 weeks of treatment with adjunctive pregnenolone or placebo. Patients received a total of six study visits, which took place every 2 weeks. In addition, subjects received staggered telephone check-in calls to assess potential side effects every 2 weeks (i.e., during alternate weeks when a study visit did not take place). The trial was conducted at a single site, the Durham Veterans Affairs Medical Center in Durham, North Carolina. The protocol was approved by the local institutional review board and conducted under FDA Investigational New Drug (IND) no. 71768. The ClinicalTrials.gov number for this study was NCT00560937.
FDA Briefing – Safety Data

• Marx et al. (2011), historical studies

Henderson et al. (1950) reported on clinical studies where no adverse effects were observed following dosing of pregnenolone: orally at 25-75 mg (males, number not given), intramuscularly at 2-20 mg (males, number not given), intramuscularly at 50-150 mg (sex unspecified, 8 patients), orally 100 mg/day for 75 days, or orally 100-300 mg in 10 patients for 5 weeks. The only AE reported among these subjects was erythema in one male following an oral dose of 50 mg/day (duration of treatment was not specified). The authors concluded that pregnenolone is not associated with toxic actions in humans following parenteral administration. However, they stated that the possibility of side effects due to the prolonged parenteral administration of pregnenolone in large doses cannot be excluded.

No significant safety signals were reported from trials in schizophrenic or bipolar patients where pregnenolone was used as an adjunctive therapy to FDA-approved drugs (Marx et al. 2011).
FDA Briefing – Safety Information

- Osuji et al. (2010) – 8 weeks
- Brown et al. (2014) – 12 weeks
- Marx et al. (2014) – 8 weeks
- Kashani et al. (2017) – 8 weeks

Marx et al. (2014) reported a randomized, placebo-controlled trial of pregnenolone as adjunctive therapy in schizophrenia. Patients were treated for 8 weeks with either ascending oral doses of pregnenolone (2 weeks at 100 mg daily, 2 weeks at 300 mg daily, and 4 weeks at 500 mg daily) or matching placebo. The co-primary outcomes were a change in cognition\textsuperscript{14} and a change in functional capacity.\textsuperscript{15} Levels of pregnenolone and other steroids were measured. Safety data were collected at each visit.
FDA Approval of NDA for Ziprasidone

https://www.fda.gov/ohrms/dockets/ac/00/backgrd/3619b1a.pdf

- 3 out of 4 short-term (4 to 6 weeks), fixed-dose, placebo-controlled trials showed superior efficacy of ziprasidone over placebo
- 1 study was 52 weeks (no active control, just placebo)
These studies are summarized in Table 6. The results show that:

- Ziprasidone is effective in the treatment of patients experiencing an acute exacerbation of schizophrenia or schizoaffective disorder. Three out of the four STFDPC trials (Studies 106, 114, and 115) confirmed the superior efficacy of ziprasidone (80 mg, 120 mg, and 160 mg daily) over placebo, with statistically significant improvements in the intent-to-treat analysis performed for all primary outcome measures in those studies (except for the BPRS Core Items Score in Study 106, p = 0.059).

### Table 6. Oral Ziprasidone Placebo-Controlled Studies: Summary

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FDA Approval of NDA for Quetiapine

14 CLINICAL STUDIES
14.1 Schizophrenia

Adults

The efficacy of SEROQUEL in the treatment of schizophrenia was established in 3 short-term (6-week) controlled trials of inpatients with schizophrenia who met DSM III-R criteria for schizophrenia. Although a single fixed dose haloperidol arm was included as a comparative treatment in one of the three trials, this single haloperidol dose group was inadequate to provide a reliable and valid comparison of SEROQUEL and haloperidol.

Adolescents (ages 13-17)

The efficacy of SEROQUEL in the treatment of schizophrenia in adolescents (13–17 years of age) was demonstrated in a 6-week, double-blind, placebo-controlled trial. Patients who met DSM-IV diagnostic criteria for schizophrenia were randomized into one of three treatment groups: SEROQUEL 400 mg/day (n = 73), SEROQUEL 800 mg/day (n = 74), or placebo (n = 75). Study medication was initiated at 50 mg/day and
“There are multiple FDA-approved products indicated to treat the conditions proposed…”


Antipsychotic safety and efficacy concerns.

Meyer JM.

Abstract
Treatment for schizophrenia has evolved considerably since antipsychotic agents were introduced in the 1950s, with atypical antipsychotics supplanting the use of first-generation antipsychotics over the past decade. Despite the widespread belief that the atypical antipsychotics are superior to the conventional antipsychotics, clinicians lack compelling evidence about whether these new drugs really are safer or more effective than the older alternatives, or whether some atypical antipsychotics may be more effective than others. Both the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) and the Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study (CUTLASS 1) sought to determine if atypical antipsychotics were truly safer and more effective than typical antipsychotics, but the evidence provided did not support the superiority of the atypical antipsychotics as expected. However, differences between atypical antipsychotics and typical agents may accure over time, and the 2 trials may not have had a sufficient duration to determine this benefit. Long-term studies greater than 1 year may provide data to support the belief that atypical antipsychotics are more effective treatments for long-term safety and prevention of relapse in schizophrenia than older agents. While atypicals do have lower incidences of extrapyramidal symptoms and movement disorders than conventional antipsychotics, concerns about these adverse effects have been replaced by concerns about metabolic side effects. Given the widespread use of atypical antipsychotics, the psychiatric community has come to recognize that monitoring of metabolic side effects is the new standard of care for treating severely mentally ill patients.

PMID: 18284274
“There are multiple FDA-approved products indicated to treat the conditions proposed…”

The Harms of Antipsychotic Drugs: Evidence from Key Studies.

Moore TJ, Furberg CD.

Abstract

This safety assessment provides a detailed analysis of key studies and focuses on the six most-evidence include mechanisms of action, short-term treatment of psychosis, relapse prevention, comparisons between first- and second-generation agents, and flexible treatment algorithms. Common features were seen. All the agents obstruct normal signaling through widely dispersed psychotic relapse was the most frequent outcome in most key studies, ranging from 38 to 93%, to fail to demonstrate a substantial treatment benefit or difference from an active comparator. Motor system was confounded because of extensive neurological impairment from previous acute treatment, abrupt discontinuation effects, and high rates of concomitant medications to manage generation antipsychotic drugs have safety advantages over classical neuroleptic drugs and placebo. The extent of injury to and impairment of multiple body systems caused by antipsychotic drugs, clinical, and regulatory reappraisal of the appropriate use of these agents.
NIMH is supporting research on interventions that focus on a combination of approaches—symptom remission, family engagement, and functional recovery. The Recovery After Initial Schizophrenia Episode (RAISE) project combines low-dose medication with family psycho-education, supported education/employment, individual resilience training, and other interventions to focus on more than just the psychotic symptoms. Combining current treatments, as done in RAISE, looks like a promising approach.

We realize that for too many people, today’s treatments are not good enough. New, better treatments are essential if we are to improve outcomes for all— that is the promise of research. But in the meantime, we need to be thoughtful about the treatments we have. Clearly, some individuals need to be on medication continually to avoid relapse. At the same time, we need to ask whether in the long-term, some individuals with a history of psychosis may do better off medication. This is a tough call, where known risks need to be balanced against potential benefits. As the RAISE project has emphasized, shared decision-making between patients, families, and providers is essential for long-term management of psychotic disorders.

These new data on the long-term outcomes for people with “schizophrenia” remind us that 100 years after defining this disorder and 50 years after “breakthrough” medications, we still have much to learn.
PCCA’s Source of Pregnenolone

- FDA-registered & inspected cGMP facility
THANK YOU

Questions from the Committee?
Pharmacy Compounding Advisory Committee review: 7-Keto DHEA

Presenter: Tom Wynn, RPh
7-Keto-DHEA

- 7-Keto-DHEA is a metabolite of DHEA
- When administered to humans, 7-Keto-DHEA is metabolized to both 7-hydroxyepimers of DHEA

7-Keto-DHEA

DHEA (3β-hydroxyandrost-5-en-17-one) → 7α-hydroxyDHEA (irreversible) → 7β-hydroxyDHEA

7-Keto (7-oxodehydroepiandrosterone) via 11β-HSD type 1 (reversible)
7-Keto-DHEA

- In human therapy there are some undesired responses to administered DHEA because it elevates blood testosterone and dihydrotestosterone concentrations in women.

- The 7-oxo steroids should prove to be more useful therapeutic agents than DHEA, for they are more active, are not aromatized, and cannot be converted to testosterone.


7-Keto DHEA safety
7-Keto DHEA safety

“One Safety assessment is a Mammilian-microsome reverse mutation study using 3P-acetyl-7-oxo-DHEA and/or its metabolites in Salmonella-Escherichia coli. This evaluated the ability to induce reverse mutations at the histidine locus in the genome specific Samonella typhiurium tester strain and at the tryptophan locus in an Escherichia coli tester strain both in the presence and absence of exogenous metabolic activation system of mammalian microsomal enzyme (S9). In the presence and absence of S9 at doses of 0.1 to 5.0 mg per plate 3P-acetyl-7-oxo-DHEA did not cause an increase in the number of revertants per plant.”

• Conclusion: These results indicate that 3beta-acetyl-7-oxo-DHEA is safe and well tolerated in normal healthy men at doses up to 200 mg/d for 4 weeks.

Safety and pharmacokinetic study with escalating doses of 3-acetyl-7-oxo-dehydroepiandrosterone in healthy male volunteers.

Davidson M1, Marwah A, Sawchuk RJ, Maki K, Marwah P, Weeks C, Lardy H.

OBJECTIVES:
To evaluate the safety and pharmacokinetics of 3-acetyl-7-oxo-DHEA (3beta-acetoxyandrost-5-ene-7,17-dione) given orally.

DESIGN:
A randomized, double blind, placebo-controlled, escalating dose study.

PARTICIPANTS:
Twenty-two healthy men.

STUDY METHOD:
The participants received placebo (n = 6) or 3-acetyl-7-oxo-DHEA (n = 16) at 50 mg/d for 7 days followed by a 7-day washout; 100 mg/d for 7 days followed by a 7-day washout; and 200 mg/d for 28 days.

OUTCOME MEASURES:
Safety parameters, evaluated at each dose level, included measurement of total testosterone, free testosterone, dihydrotestosterone, estradiol, cortisol, thyroxin and insulin levels. Analyses for 7-oxo-DHEA-3beta-sulfate (DHEA-S), the only detectable metabolic product of the administered steroid, were conducted on plasma drawn from all subjects at 0.25, 0.5, 1, 2, 4, 6 and 12 hours after the final 100 mg dose of 3beta-acetyl-7-oxo-DHEA.

RESULTS:
There were no differences in the clinical laboratory values or in reported minor adverse experiences, between treatment and placebo groups. In general, blood hormone concentrations were unaffected by the treatment with 3beta-acetyl-7-oxo-DHEA and remained within the normal range. No changes in vital signs, blood chemistry or urinalysis occurred during treatment with 3beta-acetyl-7-oxo-DHEA compared to placebo. The administered steroid was not detected in the blood but was rapidly converted to 7-oxo-DHEA-S, the concentrations of which were proportional to dose. This steroid sulfate did not accumulate; plasma concentrations 12 hours after the 3beta-acetyl-7-oxo-DHEA dose at 7 and 28 days on the 200 mg/d dose were 15.8 and 16.3 microg/L respectively. The mean time to peak plasma level of 7-oxo-DHEA-S was 2.2 hours; the mean half life was 2.17 hours. The apparent clearance averaged 172 L/h, and the apparent mean volume of distribution was 540 L.

CONCLUSION:
These results indicate that 3 beta-acetyl-7-oxo-DHEA is safe and well tolerated in normal healthy men at doses up to 200 mg/d for 4 weeks.
An improved procedure for the synthesis of 3 beta-hydroxyandrost-5-ene-7,17-dione, a natural metabolite of dehydroepiandrosterone (DHEA) is described. The synthesis and magnetic resonance spectra of several other related steroids are presented. Feeding dehydroepiandrosterone to rats induces enhanced formation of several liver enzymes among which are mitochondrial sn-glycerol 3-phosphate dehydrogenase (GPDH) and cytosolic malic enzyme. The induction of these two enzymes, that complete a thermogenic system in rat liver, was used as an assay to search for derivatives of DHEA that might be more active than the parent steroid. Activity is retained in steroids that are reduced to the corresponding 17 beta-hydroxy derivative, or hydroxylated at 7 alpha or 7 beta, and is considerably enhanced when the 17-hydroxy or 17-carbonyl steroid is converted to the 7-oxo derivative. Several derivatives of DHEA did not induce the thermogenic enzymes whereas the corresponding 7-oxo compounds did. Both short and long chain acyl esters of DHEA and of 7-oxo-DHEA are active inducers of the liver enzymes when fed to rats. 7-Oxo-DHEA-3-sulfate is as active as 7-oxo-DHEA or its 3-acetyl ester, whereas DHEA-3-sulfate is much less active than DHEA. Among many steroids tested, those possessing a carbonyl group at position 3, a methyl group at 7, a hydroxyl group at positions 1, 2, 4, 11, or 19, or a saturated B ring, with or without a 4-5 double bond, were inactive.

"Within that single experiment, over the range 0.01 to 0.1% of the diet, 7-oxo-DHEA was 2.5 times as active as DHEA"
Ergosteroids IV: synthesis and biological activity of steroid glucuronosides, ethers, and alkylcarbonates.

Marwah P¹, Marwah A, Kneer N, Lardy H.

The 7-oxo derivative of dehydroepiandrosterone is more active than the parent steroid and is devoid of adverse side effects in rats, monkeys and humans. In anticipation of possible therapeutic use we have sought more active, longer lasting forms of 7-oxo- and 7beta-hydroxydehydroepiandrosterones. The 7-oxo- and 7-hydroxy steroids have been converted to glucuronides, ethers and carbonate esters. The syntheses of these compounds are described and their ability to induce the formation of liver thermogenic enzymes when fed to rats is reported. Some of the new derivatives were found to be somewhat more effective than the equimolar amounts of 7-oxo-DHEA with which they were compared in each experiment.
Commercial Distribution

- The FDA has accepted premarket notifications for 7-Keto DHEA from many dietary supplement distributors since as early as 1997, and allowed the distribution of varying doses of 7-Keto DHEA to the public.

- All manufactures of 7-Keto DHEA submitted safety profile information from the studies that are listed here today.

1. 75-Day Premarket Notification for New Dietary Ingredients -
   https://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0148-03-vol106.pdf &
   https://www.fda.gov/ohrms/dockets/dailys/03/Feb03/021303/95s-0316-rpt0148-01-vol106.pdf
7-Keto DHEA Efficacy
The effects of the ergosteroid 7-oxo-dehydroepiandrosterone on mitochondrial membrane potential: possible relationship to thermogenesis.

Bobyleva V, Bellei M, Kneer N, Lardy H.

Administered 3 beta-hydroxyandrost-5-ene-7,17-dione (7-oxo-DHEA) is more effective than 3 beta-hydroxyandrost-5-en-7-one (DHEA) as an inducer of liver mitochondrial sn-glycerol-3-phosphate dehydrogenase and cytosolic malic enzyme in rats. Like DHEA, the 7-oxo metabolite enhances liver catalase, fatty acylCoA oxidase, cytosolic sn-glycerol-3-phosphate dehydrogenase, mitochondrial substrate oxidation rate, and the reconstructed sn-glycerol 3-phosphate shuttle. The mitochondrial adenine nucleotide carrier is diminished by thyroidectomy and is restored to normal activity by administering 7-oxo-DHEA. The relationship between respiratory rate and proton motive force across the mitochondrial membrane was measured in the nonphosphorylating state. When treated with increasing concentrations of respiratory inhibitors liver mitochondria from rats treated with 7-oxo-DHEA or thyroid hormones show a more rapid decline of membrane potential than do normal liver mitochondria. Thus 7-oxo-DHEA induces an increased proton leak or slip as has been reported for the thyroid hormone by M.D. Brand [(1990) Biochem. Biophys. Acta 1018, 128-133]. This process may contribute to the enhanced thermogenesis caused by ergosteroids as well as by thyroid hormones. “Feeding 7-oxo-DHEA decreased body weight gain of rats”
Patients with Raynaud's phenomenon have abnormal digital vasoconstriction in response to cold. The pathogenesis remains unknown but may involve a local neurovascular defect leading to vasoconstriction. Diagnosis of primary Raynaud's phenomenon is based on typical symptomatology coupled with normal physical examination, normal laboratory studies and lack of observable pathology by nail fold capillaroscopy. Secondary Raynaud's phenomenon is known to occur associated with several connective tissue diseases, vascular injury due to repeated vibrational trauma, and other causes which produce demonstrable vascular and microcirculatory damage. Treatment of Raynaud's symptoms is conservative and aimed at prevention of attacks. Patients are advised to remain warm and, if possible, to live in warm climates. We suggest that an ergogenic (thermogenic) steroid, 7-oxo-DHEA (3-acetoxyandrost-5-ene-7,17-dione), which is available without prescription as the trademarked 7-keto DHEA, may be very helpful in prevention of primary Raynaud's attacks by increasing the basal metabolic rate and inhibiting vasospasm.
Conclusion

- 7-keto DHEA is well characterized using HPLC
- Its safety has been shown in both animal and human studies
- Its safety met no resistance from the FDA for Dietary Supplement status
- 7-keto DHEA has shown to be effective in weight loss and Raynaud’s
Fagron North America: Nominator

Pharmacy Compounding Advisory Committee review: Astragalus

Presenter: Tom Wynn, RPh
Astralagus Membranaceus

- Astralagus Membranaceus is a small bushy perennial plant
- Also referred to as Huang Qi
- The root is traditionally used for medicinal purposes
Characterization
Astragalus Root Dry Extract

**DEFINITION**
Astragalus Root Dry Extract is prepared from the dried root of *Astragalus membranaceus* var. *mongholicus* (Bunge) P.K. Hsiao or *Astragalus membranaceus* (Fisch.) Bunge (Fam. Fabaceae) subjected to aqueous or hydroalcoholic extraction. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of cycloartane saponins and isoflavonoids calculated on the anhydrous basis. It may contain suitable substances added as carriers.

**IDENTIFICATION**
- **A. HPTLC FOR ARTICLES OF BOTANICAL ORIGIN** (203)
  
  Standard solution A: 1 mg/mL of USP Astragaloside IV RS in methanol
  
  Standard solution B: 2 mg/mL of USP Daidzin RS and 1 mg/mL of USP Daidzein RS in methanol
  
  Standard solution C: 50 mg/mL of USP Astragalus Root Dry Extract RS in methanol. Sonicate for about 10 min, centrifuge, and use the supernatant.
  
  **Sample solution:** 50 mg/mL of Astragalus Root Dry Extract in methanol. Sonicate for about 10 min, centrifuge, and use the supernatant.

  **Chromatographic system**
  
  **Adsorbent:** Chromatographic silica gel with an average particle size of 5 μm (HPTLC plate)¹
  
  **Application volume:** 3 μL each of Standard solution A, Standard solution B, Standard solution C, and Sample solution as 8-mm bands
  
  **Relative humidity:** Condition the plate to a relative humidity of 33%.
  
  **Temperature:** Ambient, not to exceed 30°C
  
  **Developing solvent system:** Ethyl acetate, methanol, and water (100: 13.5: 10)

  [¹ Suitable commercially available plates are HPTLC Silica Gel 60 F254 from EMD Millipore (e.g., part no. 1.05642.0001).]

**B. HPLC**

**Analysis:** Proceed as directed in the test for Content of Isoflavonoids and Saponins.

**Acceptance criteria:** The Sample solution exhibits peaks at the retention times corresponding to those of calycosin 7-O-β-D-glucopyranoside, ononin, calycosin, formononetin, astragaloside IV, astragaloside I, and astragaloside II from Standard solution F.

**COMPOSITION**

**CONTENT OF ISOFLAVONOIDS AND SAPONINS**

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<th>Solution A (%)</th>
<th>Solution B (%)</th>
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</table>

**Table 1**

**Solution A:** Prepare a composite solution containing 0.4 mg/mL of USP Astragaloside IV RS, 0.1 mg/mL of USP Calycosin RS, 0.2 mg/mL of USP Calycosin 7-O-β-D-Glucopyranoside RS, 0.05 mg/mL of USP Formononetin RS, and 0.1 mg/mL of USP Ononin RS in methanol.

[NOTE—The extract of *Hedysarum polybotros*, a common adulterant, does not show orange bands corresponding to astragalosides I and II.]
Characterization

In order to ensure the superior quality and safety of the raw materials of the Chinese herbal medicine, a standard operating procedure for astragalus was established on the base of the GAP of the Chinese herbal medicine as well as practice investigation and experiments. **This standard operating procedure provides the technical requirements for astragalus's growing, field management, controlling of the diseases and pests, harvesting, processing, packing, storing, transporting and quality monitoring.**
PAPERS

New method for analysis of Chinese herbal complex prescription and its application

XIAO Hongbin¹, LIANG Xinmiao¹*, LU Peizhang¹ and CHAN Chikin²

1. Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116012, China;
2. Government Laboratory, Hong Kong
* Corresponding author

Abstract  Traditional Chinese medicine (TCM) is a great treasure of China, the analysis of which is an arduous task. The viewpoint that all chemical constituents of Chinese herbal complex prescription should be analyzed as a black box is elucidated for the first time. Intelligent multi-mode multi-column chromatographic system (IMMCC) with its hybrids is the basic method and HPLC Unified Method is the breakthrough for the black box analysis. Dang-Gui-Bu-Xue-Tang was selected as a typical TCM and a systematic separation method from non-aqueous mobile phase to pure water mobile phase was put forward in order to convert unknown sample to known sample. The $a, c$ values and UV spectra of 66 components of Astragalus, 78 components of Angelica and 71 components of Dang-Gui-Bu-Xue-Tang were obtained. Intelligent optimization and peak identification method and software for complex samples were developed and the optimum multi-step multi-binary gradient curve of mobile phase for Astragalus was ascertained. The maximum error and minimum error of predicted retention time for all components of Astragalus are 8.62% and 0.05% respectively. All components of Astragalus were compared with those of Angelica and it is found that many components of Astragalus are the same as those of Angelica, while the contents of these components are different. Many components of Dang-Gui-Bu-Xue-Tang are also the same as those of Astragalus and Angelica with different contents.
Fig. 1. Chart of HPLC unified method.
Safety

Safety evaluation of Astragalus extract mixture HT042 and its constituent herbs in Sprague-Dawley rats.
Song J¹, Lee D¹, Min B², Bae JS³, Chang GT⁴, Kim H⁵.

BACKGROUND:
Astragalus extract mixture HT042 is a combination of three standardized extracts from Astragalus membranaceus root, Eleutherococcus senticosus stem, and Phlomis umbrosa root, which has proven to stimulate children's height growth.

PURPOSE:
The aim of this study was to demonstrate the safety of HT042 and its three constituent herbs when administered orally.

METHODS:
Acute and sub-chronic oral toxicity studies were conducted using male and female Sprague-Dawley rats. In the acute toxicity study, HT042 and each of the herbs was administered at single doses of up to 5000 mg/kg. In the 13-week sub-chronic toxicity study, HT042 was administered at repeated doses of up to 4000 mg/kg/day.

RESULTS:
In the acute toxicity study of HT042 and each of the herbs, no deaths occurred and there was no indication of toxicity, on the basis of clinical signs, body weight, and necropsy findings. In the sub-chronic toxicity study of HT042, there were no deaths and no changes in clinical signs or the findings of ophthalmic examinations. Although there were some treatment-related changes in other findings, these alterations were not considered toxicologically significant because they remained within normal ranges or recovered during the recovery period.

CONCLUSION:
The oral approximate lethal doses of HT042 and each of the herbs were > 5000 mg/kg, and the no-observed-adverse-effect level of HT042 was 4000 mg/kg/day in male and female rats.
The study was designed to investigate efficacy and safety of Astragalus membranaceus (AM) in the treatment of patients with seasonal allergic rhinitis (SAR). AM is an active component in the herbal and mineral complex (HMC) registered in Croatia as a food supplement Lectranal. The study was designed as a 6-weeks, double-blind, placebo-controlled clinical trial and conducted in 48 adult patients with a moderate to severe SAR. The treatment efficacy was evaluated by the mean change in the symptom score (TSS), quality of life (QoL), specific serum IgE and IgG, nasal eosinophils, and physicians' and patients' global evaluation. Compared to placebo, HMC significantly decreased the intensity of rhinorrhea while for other primary efficacy variables the treatment groups did not differ. In contrast, investigators and patients equally judged the treatment with HMC as more efficacious. In addition, the analysis of changes from baseline inside the groups for TSS, QoL, and 4 main symptoms of SAR were strikingly in favor of the active treatment. In patients with SAR due to weed pollen allergy HMC significantly improved primary variables, reflective TSS and QoL. The study revealed a significant number of positive signals indicating the therapeutic effectiveness of the HMC in patients with SAR which should be further tested in larger, multicenter trials with more patients.

“Adverse events were not connected with the study drug”
Astragalus Membranaceus prevents airway hyperreactivity in mice related to Th2 response inhibition

Hua-HaoShenKaiWangWenLiYing-HuaYingGui-XinGaoXiao-BoLiHua-QiongHuang

Aim of the Study
Asthma is recognized as a common pulmonary disease throughout the world. To date, there has been a growing interest in herbal products in Traditional Chinese Medicine, which is considered to be effective to treat asthma. A Chinese herb Astragalus Membranaceus (AM) was found useful in treating allergic diseases. The purpose of this study is to determine whether this herbal injection could suppress allergic-induced AHR and mucus hypersecretion in allergic mice.

Materials and Methods
A mouse model of chronic asthma was used to investigate AM injection on the airway lesions as compared with glucocorticoids. The study was conducted on mice sensitized and challenged with ovalbumin and the whole body plethsmography was performed to assess AHR. The bronchoalveolar lavage (BAL), histopathology were examined.

Results
We found 28-day AM administration significantly decreased inflammatory infiltration and mucus secretion in the lung tissues of allergic mice. 28-day AM administration enhanced Ova-induced decreased IFN-γ, and the Ova-induced elevations of IL-5 and IL-13 in BALF were prevented by 28-day injection. We also showed 28-day AM injection markedly suppressed increased AHR in allergic mice.

Conclusions
Our results indicate Astragalus Membranaceus has a potential role in treating allergic asthma.
Astragalus and Allergic Rhinitis


Efficacy and safety of Astragalus membranaceus in the treatment of patients with seasonal allergic rhinitis.

Matkovic Z, Zivkovic V, Korica M, Plavec D, Pecanic S, Tudoric N.

The study was designed to investigate efficacy and safety of Astragalus membranaceus (AM) in the treatment of patients with seasonal allergic rhinitis (SAR). AM is an active component in the herbal and mineral complex (HMC) registered in Croatia as a food supplement Lectranal. The study was designed as a 6-weeks, double-blind, placebo-controlled clinical trial and conducted in 48 adult patients with a moderate to severe SAR. The treatment efficacy was evaluated by the mean change in the symptom score (TSS), quality of life (QoL), specific serum IgE and IgG, nasal eosinophils, and physicians' and patients' global evaluation. Compared to placebo, HMC significantly decreased the intensity of rhinorrhea while for other primary efficacy variables the treatment groups did not differ. In contrast, investigators and patients equally judged the treatment with HMC as more efficacious. In addition, the analysis of changes from baseline inside the groups for TSS, QoL, and 4 main symptoms of SAR were strikingly in favor of the active treatment. In patients with SAR due to weed pollen allergy HMC significantly improved primary variables, reflective TSS and QoL. The study revealed a significant number of positive signals indicating the therapeutic effectiveness of the HMC in patients with SAR which should be further tested in larger, multicentre trials with more patients.
Astragalus and Herpes Simplex

[Modulatory effect of Astragalus membranaceus on Th1/Th2 cytokine in patients with herpes simplex keratitis].
[Article in Chinese]
Mao SP¹, Cheng KL, Zhou YF.
OBJECTIVE:
To explore the influence of Astragalus membranaceus (AM) on serum cytokines, Th1, including interleukin-2 (IL-2) and gamma-interferon (gamma-IFN), and Th2, including interleukin-4 (IL-4) and interleukin-10 (IL-10), in patients with herpes simplex keratitis (HSK).
METHODS:
One hundred and six HSK patients were randomly divided into the AM treated group and the ribavirin treated group. Levels of serum IL-2, IL-4, IL-10 and gamma-IFN of all the patients and 62 healthy person, selected from donors for control group, were determined by sandwich enzyme-linked immunosorbent assay (ELISA) technique.
RESULTS:
Levels of serum IL-4 and IL-10 in HSK patients were significantly higher and those of IL-2 and gamma-IFN were significantly lower than those in the healthy control (all P < 0.01). These parameters were significantly improved in the patients of the AM group after treatment, but with no change in patients of the ribavirin group.
CONCLUSION:
AM can modulate the imbalance state of Th1/Th2 in HSK patients, improve their immune function disturbance, that shows important significance in treating HSK.
Astragalus and Wound Healing

Wound healing effect of an Astragalus membranaceus polysaccharide and its mechanism.

Zhao B¹, Zhang X¹, Han W², Cheng J³, Qin Y¹.
In the present study, a novel polysaccharide, APS2-1, was isolated and purified from Astragalus membranaceus using DEAE-cellulose and Sephadex G-100 chromatography. The effect of APS2-1 on the promotion of wound healing was evaluated and its preliminary mechanism was investigated. In vitro experiments showed that APS2-1 was able to promote human skin fibroblast (HSF) propagation and accelerate cell cycle progression. For further examination, a scalded mice model was used to verify the effect of APS2-1 and investigate its mechanism of action. The analysis of biochemical parameters, including cyclin D1, inhibitor of nuclear factor κBα (IκBα), transforming growth factor (TGF)-β1, basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) showed that APS2-1 inhibited the increase in cyclin D1 and IκBα, and promoted the expression of TGF-β1, bFGF and EGF, which was further confirmed by histopathological observation. These results suggested that APS2-1 possessed high potential in wound healing and its mechanism was associated with inhibiting inflammation, accelerating cell cycle and promoting the secretion of repair factors.
Astragalus and Wound Healing


The healing and anti-scar effects of astragaloside IV on the wound repair in vitro and in vivo. Chen X, Peng LH, Li N, Li QM, Li P, Fung KP, Leung PC, Gao JQ.

ETHNOPHARMACOLOGICAL RELEVANCE:
Astragaloside IV is the chief ingredient of Radix Astragali, which has been used in the Traditional Chinese Medicine as a major component of many polyherbal formulations for the repair and regeneration of injured organ and tissues. This study is to investigate the influence of astragaloside IV on both of the wound healing and scar formation.

MATERIALS AND METHODS:
For the in vitro evaluation, the influence of the astragaloside IV in the wound scratch test of keratinocytes and the secretion of transforming growth factor-β1, a key factor contributing to scar formation were determined. With the rat skin excision model, the in vivo regulation of astragaloside IV on wound closure, angiogenesis and collagen disposition were also evaluated.

RESULTS:
Astragaloside IV was shown to significantly promote the migration of keratinocytes in wound scratching assay. The superior effect of Astragaloside IV was observed at 100 μmol/L, in which the recover rates was increased with 2 and 3 folds after 48 h and 96 h respectively than that of blank control (P<0.01). Animal skin closure measurement showed that astragaloside IV could stimulate the wound healing, e.g. with 21% recover in contrast to the 8% of blank control at the 6th day. Biomechanic and Masson's trichrome stain analysis indicated that astragaloside IV may improve the strength of the repaired skin and promoted the angiogenesis and collagen synthesis. Meanwhile, the picrosirius-sirus red stain and Elisa test definitely showed the anti-scar effects of astragaloside IV by decreasing the levels of collagen I/III and TGF-β1 secretion by fibroblasts with a dose-dependent manner (25-100 μmol/L).

CONCLUSIONS:
Astragaloside IV was shown a promising natural product with both healing and anti-scar effects for wound treatment. These results give the evidence for the application of astragaloside IV in the treatment of injury.
Conclusion

• Astragalus is monographed in the USP dietary supplement section with specifications for standardization and HPLC analysis

• Astragalus has shown safety in both animal and human studies\textsuperscript{1,2}

• Astragalus has shown effectiveness in wound healing, herpes simplex, and allergic rhinitis\textsuperscript{3,4,5,6}


Fagron North America: Nominator

Pharmacy Compounding Advisory Committee review: Epigallocatechin Gallate (EGCg)

Presenter: Kim Kieffer, CPhT
Epigallocatechin Gallate (EGCg)

- Major polyphenolic compound found in green tea

- EGCGs antioxidant effects have been widely studied for the possible treatment of:
  - Cancer chemoprevention and therapeutic efficacies
  - Weight loss
  - Bone density
  - Alzheimer
  - Cardiovascular disease
  - Neuropathic pain
  - Fatty liver Disease
  - Dermatitis
  - Chronic kidney disease
  - Mood and cognition
  - Acne Vulgaris
  - Cholesterol lowering
  - General wellness
  - Inflammatory bowel disease
  - Wound care
  - Etc.
Typical Compounded Use

- Compounded into topical gels, creams and ointments for the treatment of wounds and scars
- Typical dosage ranges 0.1-1%
- Most often compounded in combination with topical steroids, anesthetics, skin lightening agents etc.
- Typical length of therapy is 1-3 months (anecdotally reported)
EGCG and Wound Healing

• Regulates the secretion of cytokines and the activation of skin cells\(^1\)

• Has been shown to have anti-inflammatory and anti-oxidant properties\(^2\)

• EGCG has been shown in studies to affect the role that TGF-beta1\(^2\)

• Enhances wound healing by accelerating re-epithelialization and angiogenesis - improves the cellular reorganization of granulation tissue\(^3\)

• EGCG has been shown in in vitro and in vivo studies to reduce fibrosis and the contraction often associated with scarring\(^4\)


Safety

“Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals.”

<table>
<thead>
<tr>
<th>Participants</th>
<th>Dosing</th>
<th>Length</th>
<th>Adverse Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 healthy men and women ≥18 years of age with Fitzpatrick skin type II or III</td>
<td>one of the five treatments: 800 mg EGCG once/day, 400 mg EGCG twice/day, 800 mg EGCG as Polyphenon E once/day, 400 mg EGCG as Polyphenon E twice/day, or a placebo once/day (8 subjects/group)</td>
<td>4 weeks</td>
<td>Excess gas Upset stomach Nausea Heartburn Stomachache Abdominal pain Dizziness Headache Muscle pain</td>
</tr>
</tbody>
</table>

### Safety

“Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals.”

<table>
<thead>
<tr>
<th></th>
<th>Placebo ($n = 8$)</th>
<th>800 mg EGCG once daily ($n = 8$)</th>
<th>800 mg EGCG as Polyphenon E once daily ($n = 8$)</th>
<th>400 mg EGCG twice daily ($n = 8$)</th>
<th>400 mg EGCG as Polyphenon E twice daily ($n = 8$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>3 (1)</td>
<td>2 (2)</td>
<td>0</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Stomach ache</td>
<td>0</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Upset stomach</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (1)</td>
<td>0</td>
</tr>
<tr>
<td>Heartburn</td>
<td>2 (1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>2 (1)</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Excess gas</td>
<td>1 (1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Nausea</td>
<td>1 (1)</td>
<td>5 (2)</td>
<td>3 (1)</td>
<td>0</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>0</td>
<td>1 (1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Muscle pain</td>
<td>0</td>
<td>1 (1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Safety

“Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals.”

• Adverse events were rated as mild events

• Common events included headache, stomach ache, abdominal pain, and nausea

• All adverse events noted were reported in subjects receiving green tea polyphenol treatment as well as placebo

• No significant changes in blood counts and blood chemistry were observed

• Conclusion: oral administration of EGCG or Polyphenon E at a daily dose of 800 mg (based on the EGCG content) for 4 weeks is safe and well tolerated in healthy human subjects.
## Safety

“Randomized, placebo-controlled trial evaluating the safety of one-year administration of green tea catechins”

<table>
<thead>
<tr>
<th>Participants</th>
<th>Dosing</th>
<th>Length</th>
<th>Adverse Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>49 men randomized to the treatment arm and 48 to the placebo group</td>
<td>Administered in a fed state, 200 mg twice a day</td>
<td>12 months</td>
<td>No liver or other toxicities were observed. A single report of grade III nausea was reported. No other dose limiting toxicities were observed.</td>
</tr>
</tbody>
</table>

- Daily intake of a standardized, catechin mixture containing 200 mg EGCG BID taken with food for 1 year accumulated in plasma and was well tolerated and did not produce treatment related adverse effects


### Evidence of safety

“Safety studies on epigallocatechin gallate (EGCG) preparations. Part 2: dermal, acute and short-term toxicity studies.”

<table>
<thead>
<tr>
<th>Assay</th>
<th>Analytical results (%)</th>
<th>Analytical results (%)</th>
<th>Analytical results (%)</th>
<th>Analytical results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EGCG</td>
<td>Other catechins</td>
<td>Water</td>
<td>Others^a</td>
</tr>
<tr>
<td>Rat acute dermal toxicity</td>
<td>93.0</td>
<td>1.77</td>
<td>4.1</td>
<td>1.11</td>
</tr>
<tr>
<td>Rabbit primary skin irritation</td>
<td>93.4</td>
<td>1.85</td>
<td>4.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Guinea pig dermal sensitization</td>
<td>80</td>
<td>5.28</td>
<td>4.3</td>
<td>10.43</td>
</tr>
<tr>
<td>Guinea pig maximization test</td>
<td>93.4</td>
<td>1.85</td>
<td>4.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Rabbit eye irritation study</td>
<td>93.0</td>
<td>1.77</td>
<td>4.1</td>
<td>1.11</td>
</tr>
<tr>
<td>Rat acute oral toxicity</td>
<td>93.4</td>
<td>1.85</td>
<td>4.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Rat 13-week study</td>
<td>77</td>
<td>3.7</td>
<td>5.6</td>
<td>13.7</td>
</tr>
<tr>
<td>Dog 13-week study (fasting)</td>
<td>80</td>
<td>5.28</td>
<td>4.3</td>
<td>10.43</td>
</tr>
<tr>
<td>Dog 14-day tolerance study</td>
<td>80</td>
<td>5.28</td>
<td>4.3</td>
<td>10.43</td>
</tr>
<tr>
<td>Dog graded dose study</td>
<td>91.2</td>
<td>3.67</td>
<td>3.9</td>
<td>1.24</td>
</tr>
<tr>
<td>Dog 13-week study (pre-fed)</td>
<td>91.8</td>
<td>2.06</td>
<td>4.7</td>
<td>1.42</td>
</tr>
</tbody>
</table>

^a Citric acid, caffeine, and other naturally occurring substances normally present in green tea extracts.
Safety

“Safety studies on epigallocatechin gallate (EGCG) preparations. Part 2: dermal, acute and short-term toxicity studies.”

- No systemic signs of toxicity were observed in any of the rats following the dermal application of 93% EGCG
- Minor dermal irritation was observed in rats and guinea pigs, but not rabbits
- Moderate dermal sensitizing in the guinea pig maximization test was observed
- Oral doses of 2000 mg EGCG preparation/kg was lethal to rats; whereas, a dose of 200 mg EGCG/kg induced no toxicity
- In the 13 weeks rat study no toxicity was observed at doses up to 500 mg/kg/day
Safety

“Safety studies on epigallocatechin gallate (EGCG) preparations. Part 2: dermal, acute and short-term toxicity studies.”

- No adverse effects were noted at 500 mg EGCG preparation/kg/day administered to pre-fed dogs in divided doses

- Morbidity did occur when administered to fasted dogs as a single bolus dose, however the authors assert that this model is unrealistic when applied to human condition

- From these studies a no-observed adverse effect level of 500 mg EGCG preparation/kg/day was established.

- From these results (using a safety/uncertainty factor of 100) a dose of 5 mg EGCG/kg/day would seem an acceptable daily intake for humans. For a 60 kg adult, this would be equivalent to 300 mg EGCG/day.

Safety

“Phase I study of topical epigallocatechin-3-gallate (EGCG) in patients with breast cancer receiving adjuvant radiotherapy”

- Topical EGCG was prepared in a spray and applied to grade 1 dermatitis as developed from radiation therapy to see if it would reverse or lesson irritation symptoms.

<table>
<thead>
<tr>
<th>Participants</th>
<th>Dosing</th>
<th>Length</th>
<th>Adverse Events</th>
</tr>
</thead>
</table>
| 24 (avg. age 44) woman with pathologically proven breast cancer with a planned course of radiotherapy to the chest wall after modified radical mastectomy | • EGCG concentration escalated from 40 - 660 μmol l⁻¹  
• Initiated once Grade 1 dermatitis occurred from radiation therapy  
• Applied 3 x daily | The median duration of the EGCG treatment was 4 weeks | • No Dose Limiting Toxicity was observed  
• No other obvious adverse effect were observed to be related to topical EGCG treatments |

- Conclusion: topical administration of EGCG was well tolerated and no dose limiting toxicity was observed


### Safety

**“Epigallocatechin-3-gallate ameliorates radiation-induced acute skin damage in breast cancer patients undergoing adjuvant radiotherapy”**

<table>
<thead>
<tr>
<th>Participants</th>
<th>Dosing</th>
<th>Length</th>
<th>Adverse Events</th>
</tr>
</thead>
</table>
| 49 woman with pathologically proven breast cancer with a planned course of radiotherapy to the chest wall after modified radical mastectomy | • EGCG concentration 660 μmol l⁻¹  
• Initiated once Grade 1 dermatitis occurred from radiation therapy  
• Applied 3 x daily | The median duration of the EGCG treatment was 4 weeks | • EGCG was well tolerated by all patients |
Evidence for Scar Reduction

“Ex vivo evaluation of antifibrotic compounds in skin scarring: EGCG and silencing of PAI-1 independently inhibit growth and induce keloid shrinkage.”

- Keloid Organ Control model tissue was maintained in either Dexamethasone (DEX) 50 $\mu$g/ml, as positive control, or EGCG 100 ug/mL dissolved in dimethyl sulfoxide, for up to 4 weeks.

- EGCG treatment stimulated cytotoxicity and significantly reduced metabolic activity from week 1 to week 4 compared with the vehicle-treated (dimethyl sulfoxide) control

- DEX induced higher cytotoxicity and lower metabolic activity in comparison

- EGCG and DEX both significantly reduced collagen I and collagen III transcription

- The EGCG group showed significant reductions in secreted collagen I & III compared with the DEX group that did not show significant changes

- The author concluded that overall, EGCG reduced intrakeloid collagen synthesis more efficiently than DEX

Evidence for Wound Healing

“Promotion of full-thickness wound healing using epigallocatechin-3-O-gallate/poly (lactic-co-glycolic acid) membrane as temporary wound dressing.”

- The study implies that EGCG regulates the secretion of cytokines and the activation of skin cells during wound healing.

- In this study, various concentrations of EGCG were added to the electrospun membranes composed of poly (lactic-co-glycolic acid) (PLGA), and its healing effects on full-thickness wounds created in nude mice were investigated.

- Cell infiltration of mice treated with electrospun membranes containing 1 wt % EGCG significantly increased after 2 weeks.

- Re-epithelialization at the wound site and formation of blood vessels also increased in the mice treated with 1% EGCG/PLGA membranes in comparison with the mice treated only with PLGA membranes.

- These results suggest that 1% EGCG/PLGA can enhance wound healing in full thickness wounds by accelerating cell infiltration, re-epithelialization, and angiogenesis.

Evidence for Wound Healing

“Enhanced wound healing by an epigallocatechin gallate-incorporated collagen sponge in diabetic mice.”

- Various concentrations (10, 100, and 1,000 ppm) of EGCG were incorporated into a collagen sponge (CS) in order to investigate its healing effects on full-thickness wounds created in type 2 diabetic mice.

- At 14 days the residual wound size of the mice treated with 10 ppm EGCG-incorporated collagen sponge (E-CS) decreased significantly faster than untreated mice.

- Significant increases in re-epithelialization, thickness of granulation tissue, and the density of the capillaries were observed in the wound sites exposed to 10 ppm E-CS in comparison with the others.

- These results suggest that a EGCG incorporated into CS at low concentrations can enhance wound healing in diabetic mice by accelerating re-epithelialization and angiogenesis as well as improving the cellular reorganization of granulation tissue by triggering the activity of myofibroblasts.

Conclusion

• Topical EGCG may improve wound healing and reduce scar formation

• Topical application studies in humans observe no dose limiting toxicity\textsuperscript{1,2}

• Ex vivo studies using keloid organ culture models conclude that EGCG reduces collagen synthesis\textsuperscript{3}

• Animal studies on wound closure suggest that topical EGCG enhances re-epithelialization, and angiogenesis\textsuperscript{4}


