Clinical Trials in Apitherapy – the State-of-the-Art

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Therapeutic clinical trials

- They are preferred in the clinical practise in order to promote a new intervention, application scheme or other combined interventions
- Clinical trials for new medicines should be mandatory preceded by:
- Formulation of a fundamental concept
- *In vitro* and *in vivo* studies (chemistry, pharmacology, toxicology on animal model)
- Human pharmacology researches (studies) through:
- Phase I – define the safety and tolerance of the products (a small number of patients)
- Phase II – specify the optimal efficacy of the treatment
- Phase III – specify the efficacy for extended, randomized studies
- Phase IV – specify (after marketing) the long term effects, new indications / undesired side effects

General concerns related to natural products

- Doses may be based on those most commonly used in available trials, or on historical practice.
- However, with natural products (bee product are included) it is often not clear what the optimal doses are to balance efficacy and safety.
- Preparation of products may vary from manufacturer to manufacturer, and from batch to batch within one manufacturer
- Because it is often not clear what the active component(s) of a product is, standardization may not be possible, and the clinical effects of different brands may not be comparable.

Clinical practice

- 1. Diagnosis (clear and precise)
- 2. Choosing the therapy (the most efficient / the less harmful)
  - *The results of the COMPARATIVE CLINICAL TRIALS*
  - *(*studied before, similar groups, the same group before /after the treatment, on self-selected groups
- Clinical trials (randomized or non-randomized)

Question:

- Are clinical trials needed for all the therapeutic approaches?
- What results are more useful: those with *placebo* groups / treated with other medicines?
- What are the consequences of applying the results of “false trials”?

Impact of clinical trials

The Impact of Studies

Other clinical trials have not been as successful for a variety of reasons:

- Medications did not work as in laboratory
- Loss to Follow-Up of too many patients
- Harmful substance
- Unethical & poorly conducted study (Ex: Tuskegee Study & recent Gene Replacement Study)

NY/VI AETC

Propolis

Honey Clinical Studies

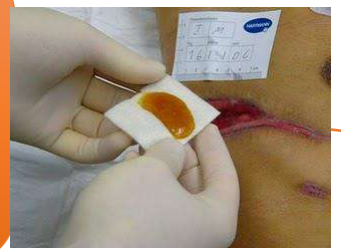
Dry eyes –
Contact lens
Manuka
honey

Chronic
rhinosinusitis

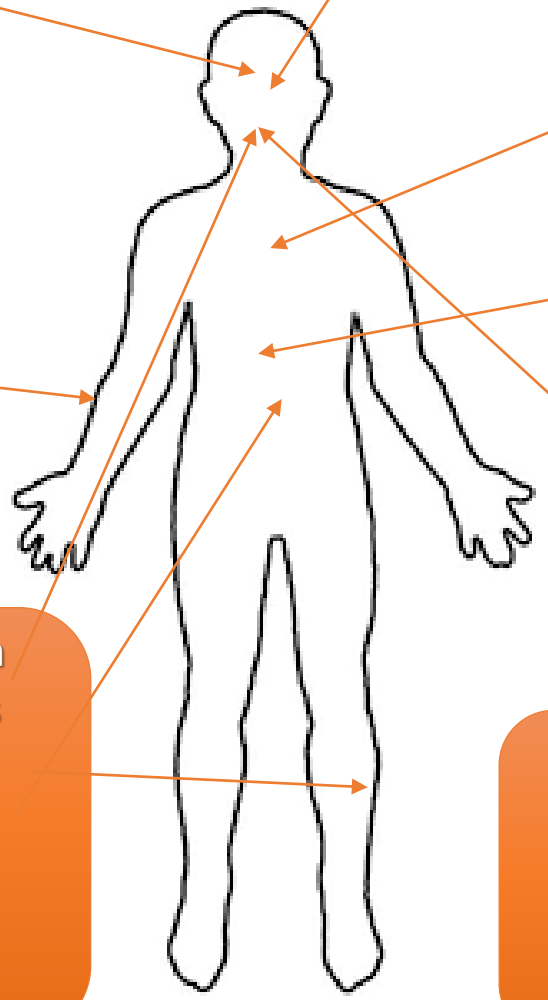
Cough, (children)
Lung cancer

Esophagitis
Gastroenteritis

Skin
Wounds,
Burns
Cosmetics



Herpes simplex labialis
Kanuka honey



Radiation-induced mucositis in head and neck cancer patients
Diabetes (diabetic foot ulcer)
Radiation induced diarrhea
Skin grafts
Stomatitis etc.

Inflammatory markers
Cervicitis
Vagitinis
Hormonal disorders

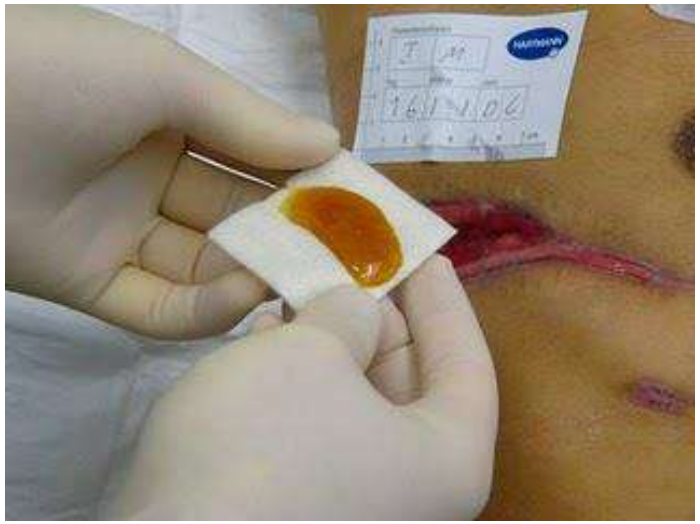
A randomized-controlled clinical trial of high fructose diets from either *Robinia* honey or free fructose and glucose in healthy normal weight males*,**

C. Despland et al. / Clinical Nutrition ESPEN 19 (2017) 16e22

- Isocalorically, replacing 25% total energy from starch with free **fructose and glucose** during 8 days slightly decreased postprandial plasma glucose and insulin concentrations, but **HAD NO SIGNIFICANT EFFECTS** on fasting and postprandial plasma triglyceride concentrations, oral glucose tolerance, or suppression of endogenous glucose production after ingestion of an oral 75 g glucose load.
- **The metabolic effects were not significantly different when free fructose and glucose were provided as natural honey or as a pure fructose: glucose mixture!!!**

- **Limitations of the study**
- ***First*, only metabolic responses to honey included in weight-maintenance diet were monitored but the hypothesis that the metabolic responses to overfeeding with honey or fructose: glucose mixture may differ, cannot be discarded.
- *Second*, the fructose : glucose ratio and oligosaccharides/polyphenols vary widely according to the type of honey; the conclusions therefore apply to *R. pseudoacacia* honey, but do not exclude that the metabolic effects of other types of honeys, such as buckwheat or honeydew honeys, may be different.
- *Third*, natural honey was compared with mixtures of hexoses matching its fructose : glucose content; by doing that, essentially the specific effects of the non-glucose, non-fructose component of honey (i.e., polyphenols, oligosaccharides) were assessed.

HONEY PRODUCTS FOR CLINICAL APPLICATIONS



Randomized Clinical Trial With Manuka Honey For Lung Cancer

Enrollment

ed (N=163

Allocation

Allocated to Supportive Care (n=53)	Allocated to Liquid Honey (n=54)	Allocated to Lozenge Honey (n=56)
Excluded (n=0)	Excluded (n=1) · Not meeting inclusion criteria (n= 1)	Excluded (n= 2) · Not meeting inclusion criteria (n=2)
Follow-Up		
Did not receive allocated intervention (n=2) · Patient refusal (n=2) Supportive care treatment delivery (n=53) · Per protocol/acceptable variation (n=46) · Unacceptable deviation (n=3) · Not evaluable (n=4)	Did not receive allocated intervention (n=1) · Patient refusal (n=1) Modality Reviews (n=53) · Per protocol/acceptable variation (n=38) · Unacceptable deviation (n=10) · Not evaluable (n=5)	Did not receive allocated intervention (n=1) · Patient refusal (n=1) Modality Reviews (n=54) · Per protocol/acceptable variation (n=40) · Unacceptable deviation n= (9) · Not evaluable (n=5)
Analysis		
Analyzed for primary endpoint (n=40) Excluded from analysis (n= 11) · Missing baseline form (n=3) · Patient Withdrew consent (n=6) · Other (n=2)	Analyzed for primary endpoint (n=41) Excluded from analysis (n=11) · Missing baseline form (n=2) · Patient Withdrew consent (n=2) · Refused due to illness (n=2) · Other (n=3) · Not Specified (n=2)	Analyzed for primary endpoint (n=38) Excluded from analysis (n=15) · Missing baseline form (n=1) · Patient Withdrew consent (n=7) · Refused due to illness (n=1) · Unable to contact patient (n=1) · Other (n=3) · Not Specified (n=2)

Recent clinical trials with honey

Randomized controlled trial of honey versus mupirocin to decolonize patients with nasal colonization of meticillin-resistant *Staphylococcus aureus* Poovelikunnel, T. T.; Gethin, G.; Solanki, D.; et al. *Journal Of Hospital Infection* Volume: 98 (2): 141-148 Published: **FEB 2018**



- ***Methods***: Patients colonized in the nose with MRSA and age >= 18 years were recruited. Participants received either one or two courses of MGH or mupirocin 2%, three times per day for five consecutive days. (MGH = medical grade honey)
- ***Findings***: The proportion of patients who were decolonized after one or two courses of treatment was not significantly different between MGH [18/42; 42.8%; 95% confidence interval (CI): 27.7-59.0] and mupirocin 2% (25/44; 56.8%; 95% CI: 41.0-71.7). Non-nasal MRSA colonization was significantly associated with persistent nasal colonization (odds ratio: 5.186; 95% CI: 1.736-5.489; P = 0.003). The rate of new acquisition of mupirocin resistance was 9.75%.
- **Conclusion**: Although not significant, **a decolonization rate of 42.8% for MGH was impressive**. Our findings suggest that this strategy, which has the potential to combat antimicrobial resistance, should be assessed in similar but **larger studies.**

Recent clinical trials with honey

Honey for acute cough in children Oduwole, Olabisi; Udoh, Ekong E.; Oyo-Ita, Angela; et al. *Cochrane Database Of Systematic Reviews* Issue: 4 Article Number: CD007094 Published: **2018**

Six randomized controlled trials involving 899 children plus three studies (331 children) in this update, were included.

Studies compared honey with dextromethorphan, diphenhydramine, salbutamol, bromelain, no treatment, and placebo. Five studies used 7-point Likert scales to measure symptomatic relief of cough; one used an unclear 5-point scale. In all studies, low score indicated better cough symptom relief.

Using a 7-point Likert scale, honey probably reduces cough frequency better than no treatment or placebo. Honey may have a similar effect as dextromethorphan in reducing cough frequency; Honey may be better than diphenhydramine in reducing cough frequency.

Adverse events included nervousness, insomnia, and hyperactivity, experienced by seven children (9.3%) treated with honey and two children (2.7%) treated with dextromethorphan (risk ratio (RR) 2.94, 95% Cl 0.74 to 11.71; I (2) = 0%; 2 studies; 149 children; low certainty evidence). Three children (7.5%) in the diphenhydramine group experienced somnolence (RR 0.14, 95% Cl 0.01 to 2.68; 1 study; 80 children; low-certainty evidence).

Recent clinical trials with honey

- Clinical Efficacy of a Topical Lactic Acid Bacterial Microbiome in Chronic Rhinosinusitis: A Randomized Controlled Trial Martensson, Anders; Abolhalaj, Milad; Lindstedt, Malin; et al. LARYNGOSCOPE INVESTIGATIVE OTOLARYNGOLOGY Volume: 2 Issue: 6 Pages: 410-416 Published: **DEC 2017**

Type of study: randomized, double-blinded, crossover, and sham-controlled design.

- *Methods*: Twenty patients received 2 weeks' treatment administered using a nasal spray-device. The subjects were monitored with regard to symptoms (SNOT-22 questionnaire, i.e., the primary efficacy variable), changes to their microbiome, and inflammatory products (IL-6, IL-8, TNF-, IL-8,a, and MPO) in nasal lavage fluids.
- *Results*: Neither symptom scores, microbiological explorations, nor levels of inflammatory products in nasal lavage fluids were affected by LAB (c.f. sham).
- *Conclusion*: Two weeks' nasal administration of a honeybee LAB microbiome to patients with CRS sNP is well tolerated but affects neither symptom severity nor th microbiological flora/local inflammatory activity.

PROPOLIS – CLINICAL TRIALS

Propolis Clinical Studies

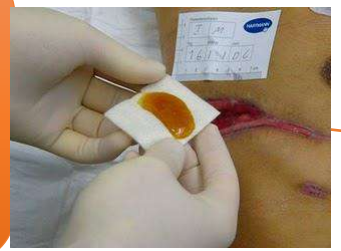
Ophtalmology

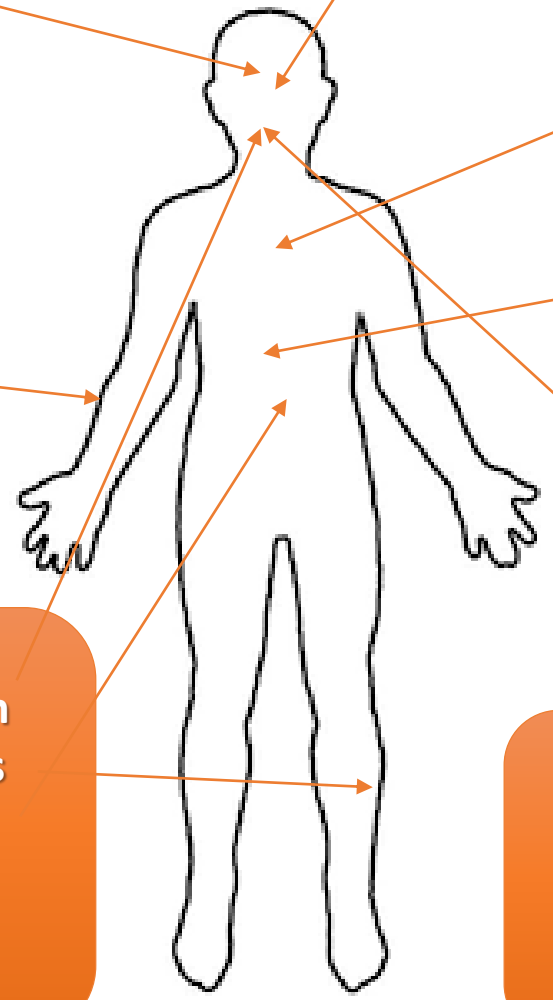
Otorhino laryngology

Cough, (children)
Lung cancer

Gastric ulcer with
Helicobacter pylori

Skin
Wounds,
Burns
Cosmetics





Herpes simplex

Radiation-induced mucositis in head and neck cancer patients
Diabetes (diabetic foot ulcer)
Stomatitis, parodontosis etc.

Inflammatory markers
Cervicitis
Vagitinis
Hormonal disorders

PROPOLIS – CLINICAL TRIALS

- most of them in dentistry / gingivitis, stomatitis, decay, hygiene etc.
- cancer / but most on cancer cell lines /Phase I of the research; animal studies (rats, mice), very few on humans;
- no standardized extract
- no specified dose / various doses
- several types of propolis (Brazilian green, red Cuban) or main compounds (artepillin, CAPE, quercetin etc.)

Propolis dentistry

- In Dentistry, propolis has been used for the treatment of aphthous ulcers, candidiasis, acute necrotizing ulcerative gingivitis (ANUG), gingivitis, periodontitis and pulpitis.
- Studies on propolis applications have increased because of its therapeutic and biological properties.

Current research involving propolis in dentistry spans many fields and highlights its antimicrobial and anti-inflammatory activities, particularly in cariology, oral surgery, pathology, periodontics, endodontics, prosthetic dentistry etc.

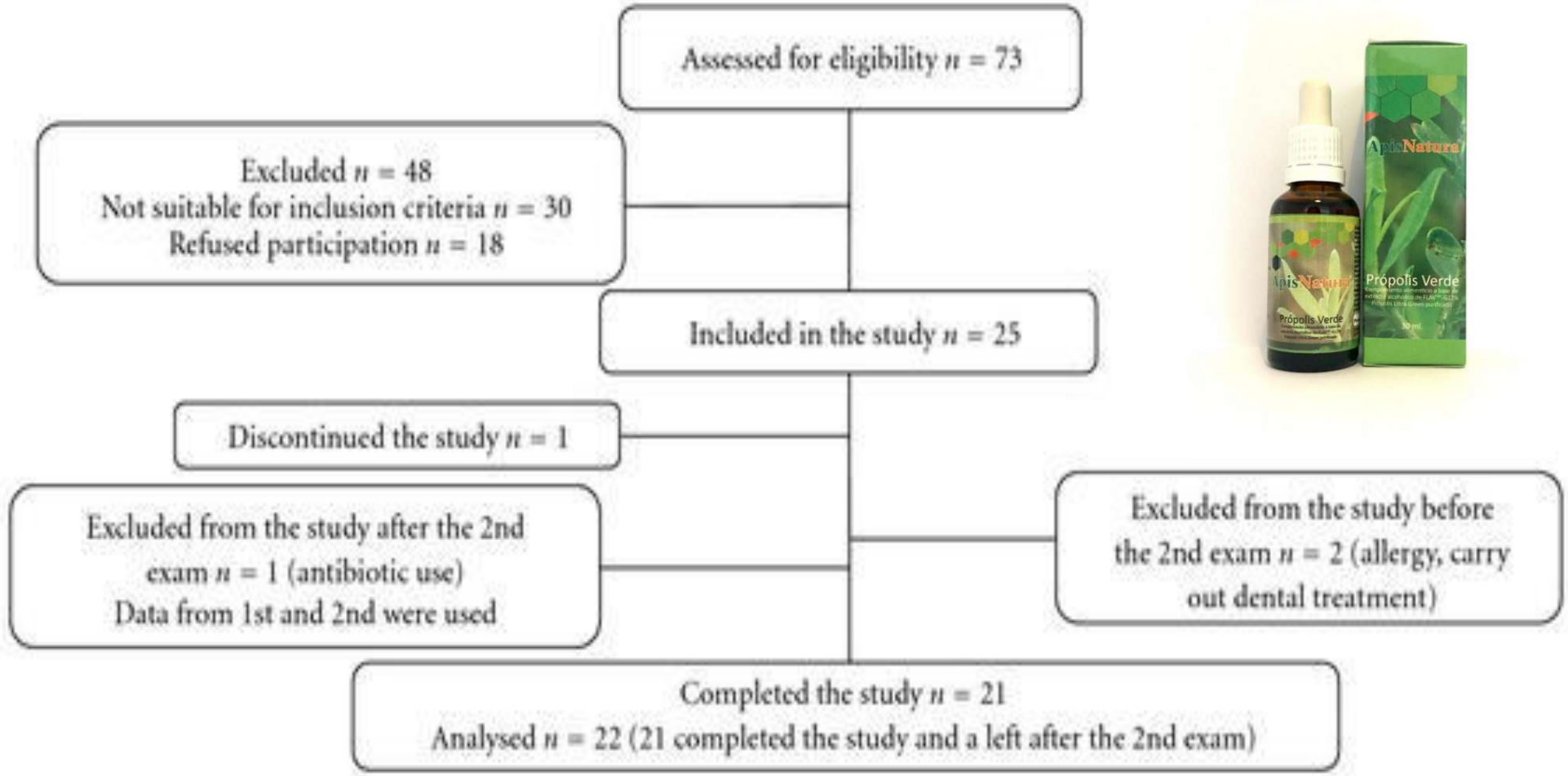
The application of propolis in dentistry is probably the most well scientifically documented and now practically applied in many countries, mostly the developing ones.

The different applications were reviewed in different publications.

Literature survey

- **Clinical Evidence of the Efficacy of a Mouthwash Containing Propolis for the Control of Plaque and Gingivitis: A Phase II Study** Evidence-Based Complementary and Alternative Medicine. 2011;2011 DOI 10.1155/2011/750249
- The protocol for the study was approved by the local ethical review committee—Committee of Bioethics in Research at the Federal University of Minas Gerais (COEP/UFMG-0600/09), and registered in Clinicaltrials.gov (NCT01142843).

Clinical Evidence of the Efficacy of a Mouthwash Containing Propolis for the Control of Plaque and Gingivitis: A Phase II Study Evidence-Based Complementary and Alternative Medicine. 2011;2011 DOI 10.1155/2011/750249



Limitations of the study

- The presence of unexpected reactions to the product and as a ***probable allergic reaction***, that did not deserve any concern, but resulted in the exclusion of an individual and decreased the sample.
- the difficulty to control the compliance to the study, how to get in touch with the patients every time they needed to return for evaluation.
- Despite the imposition of a control-use mouthwash (return the empty bottle), clinical trials have limitations with respect to the veracity of the suitability of the product by patients that are generally beyond the control of the researcher.
- The MGP 5% produced significant reductions in supragingival plaque and gingivitis as adjunct to the oral hygiene procedures, when compared to baseline scores index with 45 and 90 days.
- **These findings are probably justified by the antibacterial and anti-inflammatory effects of propolis**.

According to the results, the use of propolis may improve the prognosis of several chronic diseases and potentially contribute to decreasing the risk of cardiovascular diseases.

Propolis in oxidative stress and lipid metabolism: a randomized clinical trial

Mujika Veronika et al., 2017

Evidence-Based Complementary and Alternative Medicine
Volume 2017, Article ID 4272940, 11 pages
https://doi.org/10.1155/2017/4272940

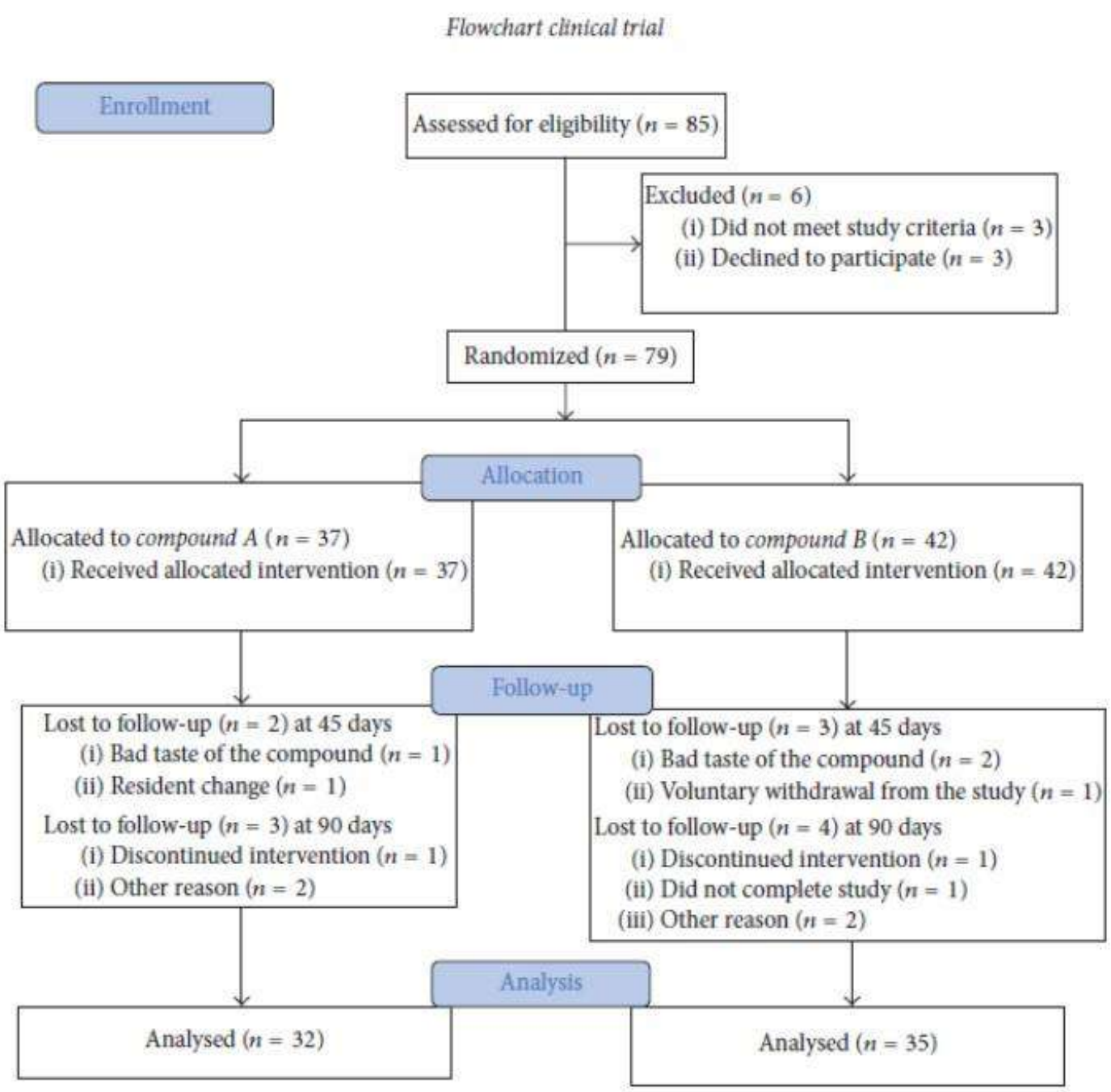


FIGURE 1: Eligibility, randomization, and patient follow-up. *Compound A* is placebo and *compound B* is propolis.

Propolis for Diabetic Foot Ulcers

Design: 108 patients with chronic diabetic foot ulcers

Group 1: standard wound care

Week 1: wound area reduced 16%

Week 3: wound area reduced 44%

Week 7: 5% of participants have fully healed ulcers

Group 2: standard wound care *plus* **topical propolis** once a week for 6 weeks

Week 1: wound area reduced **41%**

Week 3: wound area reduced **63%**

Week 7: 13% of participants have fully healed ulcers

Propolis for Asthma

Design:
48 people with chronic asthma; six month study

Dose:
Half receive propolis, half receive placebo

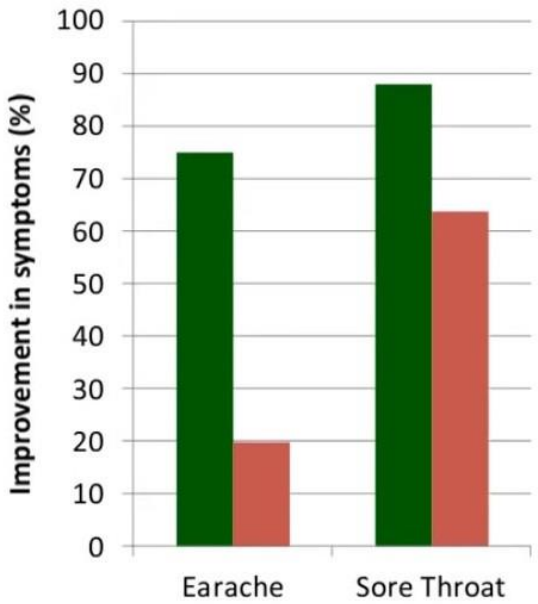
Measure:
Number of asthma attacks at night; lung function at study beginning and end

Result for Propolis:
Nighttime asthma attacks reduced 60%
Lung function increased 30%
Inflammatory compounds (prostaglandins and leukotrienes) reduced 30-40%

Result for Placebo:
No significant changes

https://www.fromnature2u.nl/Feiten-over-propolis

Propolis for Earache and Sore Throat



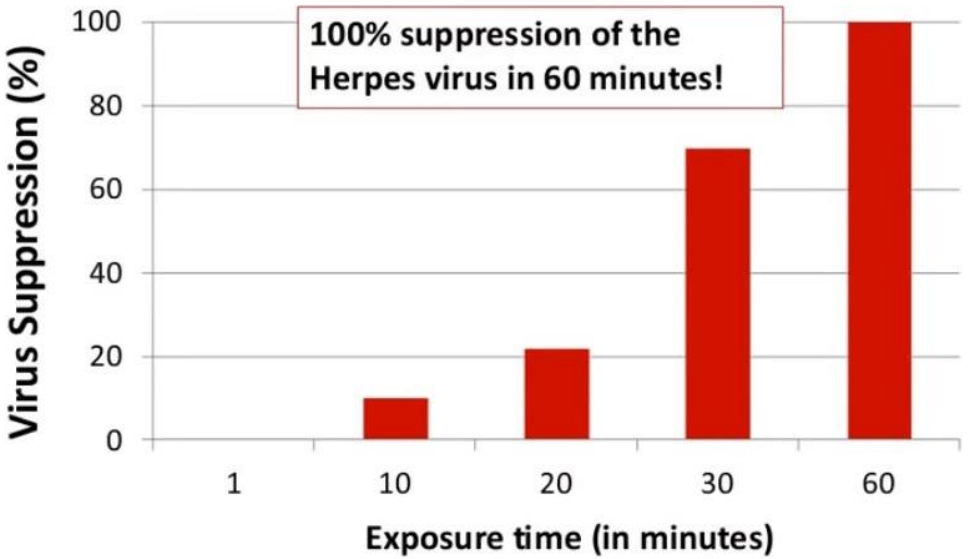
56 children (ages 2 and older) with earaches or sore throats (viral) were treated with propolis, or watched without any treatment, for 3 days.

Because these were **viral infections**, antibiotics were not prescribed.

After three days, the propolis group with earaches had a **75% improvement**, and the sore throat group an **88% symptom improvement.** The improvements for watch and wait children were only 20% and 64%.

Propolis for the Herpes virus

Herpes virus was incubated with a standardized propolis extract (GH 2002).



https://www.fromnature2u.nl/Feiten-over-propolis

External uses

- External Use of Propolis for Oral, Skin, and Genital Diseases: A **Systematic Review and Meta-Analysis**
  Soo-Hyun Sung, Gwang-Ho Choi, Nam-Woo Lee, Byung-Cheul Shin Evidence-Based Complementary and Alternative Medicine. 2017;2017 DOI 10.1155/2017/8025752

Royal jelly clinical trials

- The efficacy of topical **royal jelly** on healing of diabetic foot ulcers: a double-blind placebo-controlled **clinical trial**. Siavash M et al. Int Wound J. (2015)
- Effects of **royal jelly** supplementation on glycemic control and oxidative stress factors in type 2 diabetic female: a randomized **clinical trial**. Pourmoradian S et al. Chin J Integr Med. (2014)
- Effect of **royal jelly** ingestion for six months on healthy volunteers. Morita H et al. Nutr J. (2012)
- Electron Physician. 2016 Nov 25;8(11):3184-3192. doi: 10.19082/3184. eCollection 2016 Nov.
- **Comparison between vaginal royal jelly and vaginal estrogen effects on quality of life and vaginal atrophy in postmenopausal women: a clinical trial study.** Seyyedi F[1], Kopaei MR[2], Miraj S[3].

Royal jelly clinical trials

Electron Physician. 2016 Jun 25;8(6):2475-82. doi: 10.19082/2475. eCollection 2016 Jun. **Effect of Processed Honey and Royal Jelly on Cancer-Related Fatigue: A Double-Blind Randomized Clinical Trial.** Mofid B[1], Rezaeizadeh H[2], et al.,

PLoS One. 2017 Jan 6;12(1):e0169069. doi: 10.1371/journal.pone.0169069. eCollection 2017. **Clinical Evaluation of a Royal Jelly Supplementation for the Restoration of Dry Eye: A Prospective Randomized Double Blind Placebo Controlled Study and an Experimental Mouse Model.** Inoue S[1,2], Kawashima M[1], Hisamura R[1], Imada T[1], Izuta Y[1], Nakamura S[1], Ito M[3], Tsubota K[1].

Pharm Biol. 2017 Dec;55(1):497-502. **Hypocholesterolemic efficacy of royal jelly in healthy mild hypercholesterolemic adults.** Chiu HF[1], Chen BK[2], Lu YY[3], Han YC[2], Shen YC[4], Venkatakrishnan K[2], Golovinskaia O[5], Wang CK[2].

Royal jelly clinical trials

- Pharm Biol. 2017 Dec;55(1):497-502. **Hypocholesterolemic efficacy of royal jelly in healthy mild hypercholesterolemic adults.** Chiu HF[1], Chen BK[2], Lu YY[3], Han YC[2], Shen YC[4], Venkatakrishnan K[2], Golovinskaia O[5], Wang CK[2].
- Can J Diabetes. 2016 Aug;40(4):324-8. doi: 10.1016/j.jcjd.2016.01.003. Epub 2016 Mar 22. **Effect of Royal Jelly Intake on Serum Glucose, Apolipoprotein A-I (ApoA-I), Apolipoprotein B (ApoB) and ApoB/ApoA-I Ratios in Patients with Type 2 Diabetes: A Randomized, Double-Blind Clinical Trial Study.** Khoshpey B[1], Djazayeri S[1], Amiri F[1], Malek M[2], Hosseini AF[3], Hosseini S[4], Shidfar S[5], Shidfar F[6].
- Chin J Integr Med. 2014 May;20(5):347-52. doi: 10.1007/s11655-014-1804-8. Epub 2014 Mar 7. **Effects of royal jelly supplementation on glycemic control and oxidative stress factors in type 2 diabetic female: a randomized clinical trial.** Pourmoradian S[1], Mahdavi R, Mobasseri M, Faramarzi E, Mobasseri M.

Royal jelly clinical trials

- Int Wound J. 2015 Apr;12(2):137-42. doi: 10.1111/iwj.12063. Epub 2013 Apr 8. **The efficacy of topical royal jelly on healing of diabetic foot ulcers: a double-blind placebo-controlled clinical trial.** Siavash M[1], Shokri S, Haghighi S, Shahtalebi MA, Farajzadehgan Z.
- Nutr J. 2012 Sep 21;11:77. doi: 10.1186/1475-2891-11-77. **Effect of royal jelly ingestion for six months on healthy volunteers.** Morita H[1], Ikeda T, Kajita K, Fujioka K, Mori I, Okada H, Uno Y, Ishizuka T.

Bee Venom Clinical Trials

- BMJ Open. **2014** Nov 7;4(11):e006140. doi: 10.1136/bmjopen-2014-006140. Lee JA., et al., **Bee venom acupuncture for rheumatoid arthritis: a systematic review of randomised clinical trials.**

CONCLUSIONS:

- There is **low-quality evidence**, based on **one trial**, that BVA can significantly reduce pain, morning stiffness, tender joint counts, swollen joint counts and improve the quality of life of patients with RA compared with placebo (normal saline injection) control. However, ***the number of trials, their quality and the total sample size were too low to draw firm conclusions.***

- J Dermatolog Treat. **2015;**26(4):335-9. doi: 10.3109/09546634.2014.990411. Epub 2014 Dec 30. Eltaher S., et al., **Efficacy of the apitherapy in the treatment of recalcitrant localized plaque psoriasis and evaluation of tumor necrosis factor-alpha (TNF-α) serum level: A double-blind randomized clinical trial.**

RESULTS:

- A significant difference was found between the therapeutic responses of RLPP to the apitherapy and placebo groups ($p<0.001$). In the apitherapy group, complete response was achieved in 92% of patients. There was statistically significant decrease in TNF-α in the apitherapy group compared to the placebo group. No recurrence was observed in the apitherapy group.

CONCLUSION:

- Apitherapy is effective and a safe treatment for recalcitrant localized plaque psoriasis, when other topical or physical therapies have failed.

Bee Venom Clinical Trials

- J Ethnopharmacol. 2016 Dec 24;194:774-780. doi: 10.1016/j.jep.2016.11.012. Epub 2016 Nov 10. Ahn YJ., et al., **Safety of essential bee venom pharmacopuncture as assessed in a randomized controlled double-blind trial.**

CONCLUSIONS:

- eBV and BV displayed comparable anti-inflammatory effects, and eBV pharmacopuncture presented less local allergic reactions. (Bee venom was filtered for PLA2 and histamine and than injected)

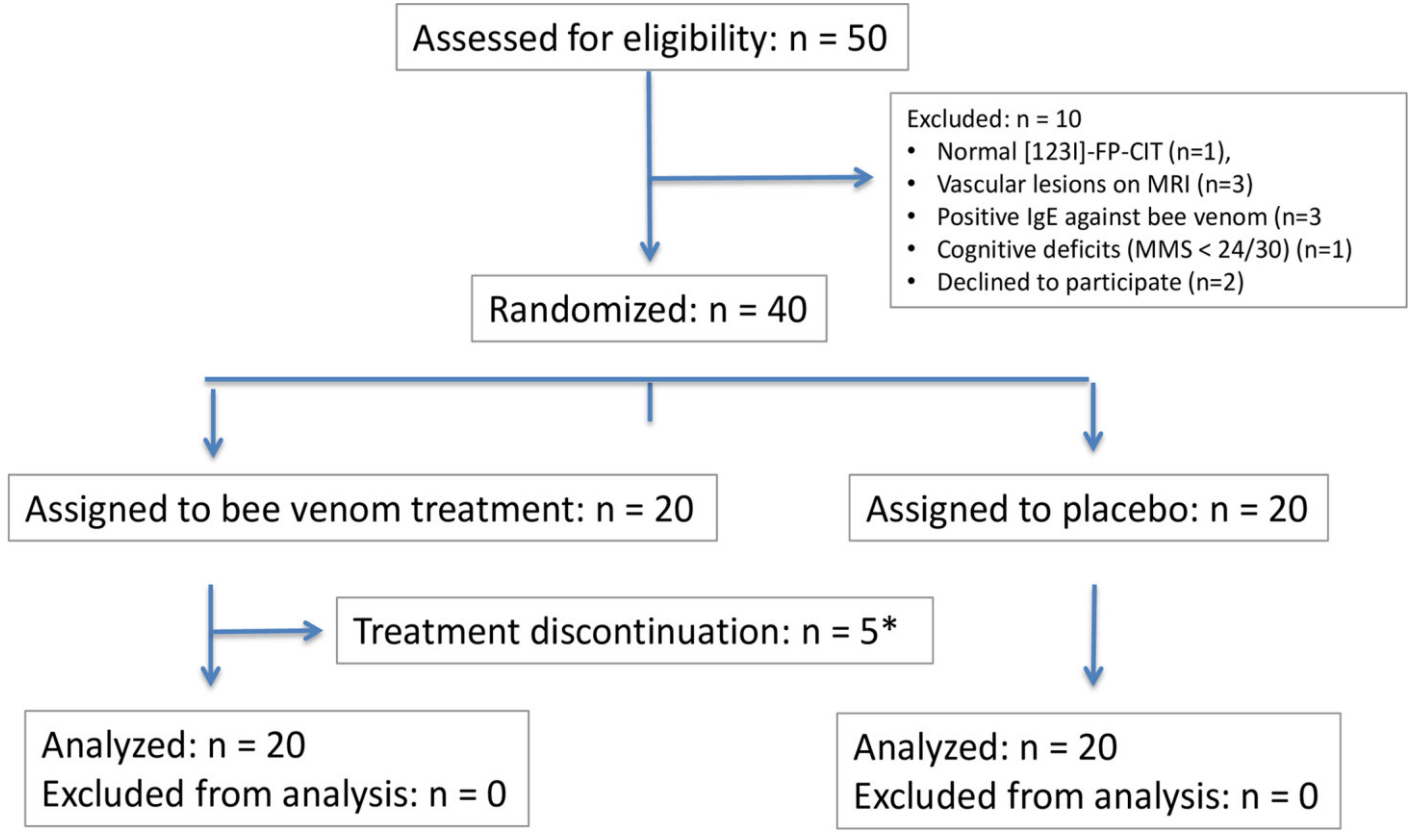
Toxins (Basel). 2017 Nov 7;9(11). pii: E361. doi: 10.3390/toxins9110361. Seo BK, et al., **Efficacy of Bee Venom Acupuncture for Chronic Low Back Pain: A Randomized, Double-Blinded, Sham-Controlled Trial.**

The results suggest that it can be used along with conventional pharmacological therapies for the treatment of CLBP.

Bee Venom for the Treatment of Parkinson Disease – A Randomized Controlled Clinical Trial

Hartmann A, Muellner J, Meier N, Hesekamp H, van Meerbeeck P, et al. (2016) Correction: PLOS ONE 11(9): e0162937. https://doi.org/10.1371/journal.pone.0162937

› **Trial Registration:** ClinicalTrials.gov NCT01341431



* According to the protocol, these patients were followed until the end of study

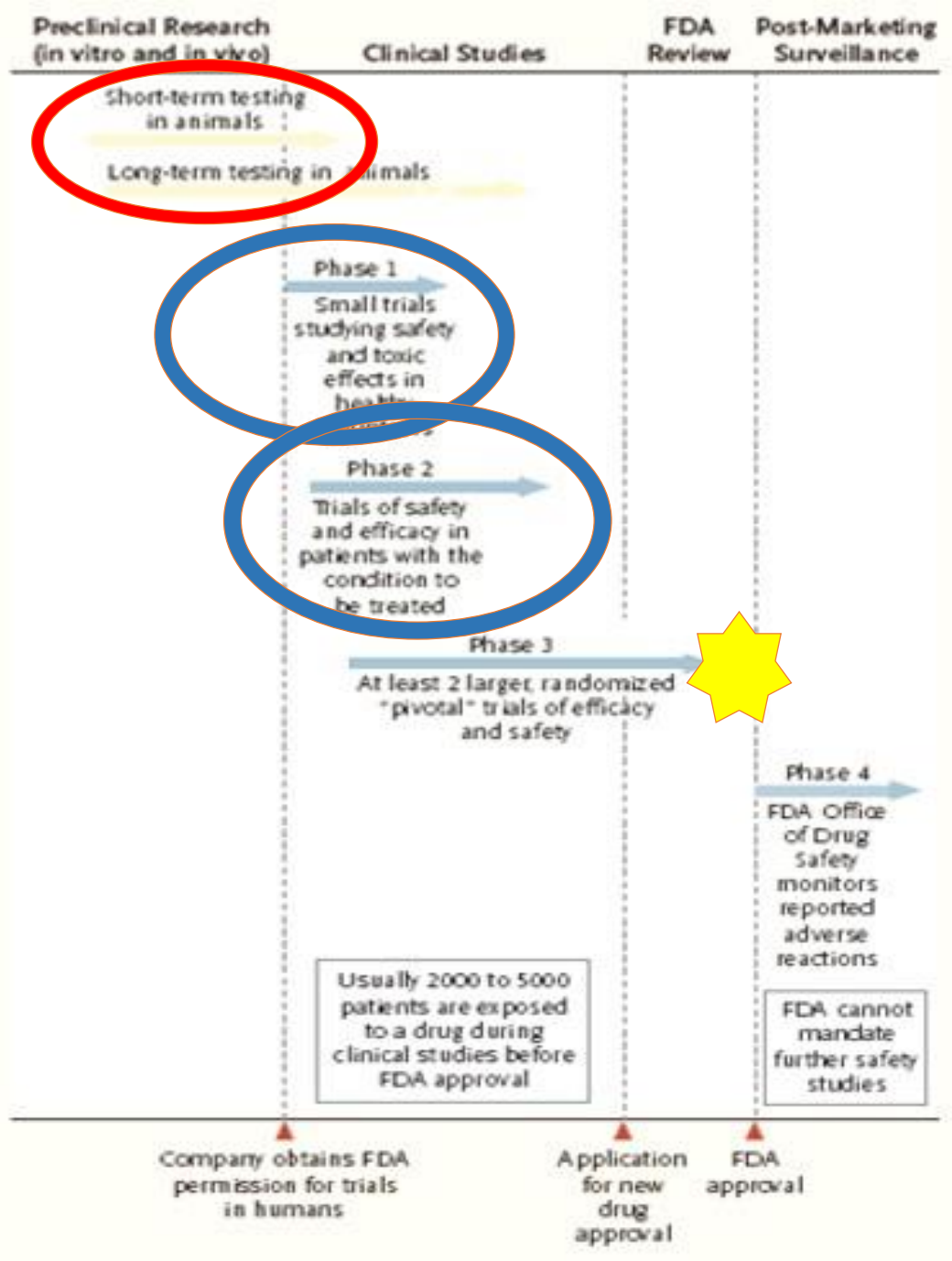
Bee venom clinical trials

› Parkinsonism Relat Disord. **2012** Sep;18(8):948-52. **Effectiveness of acupuncture and bee venom acupuncture in idiopathic Parkinson's disease.** Cho SY[1], Shim SR, Rhee HY, Park HJ, Jung WS, Moon SK, Park JM, Ko CN, Cho KH, Park SU.

› J Altern Complement Med. **2015** Oct;21(10):598-603.doi:10.1089/acm.2015.0078. Epub 2015 Jul 31. **A Prospective Open-Label Study of Combined Treatment for Idiopathic Parkinson's Disease Using Acupuncture and Bee Venom Acupuncture as an Adjunctive Treatment.** Doo KH[1,2], Lee JH[1,2], Cho SY[1,2], Jung WS[1], Moon SK[1], Park JM[1,2], Ko CN[1,2], Kim H[3], Park HJ[4], Park SU[1,2].

Where are we now?

- most studies are still *in vitro* on various microorganisms or cell lines (cancer research)
- short term preclinical studies on animals are prevailing
- some studies are in Phase 1 – healthy volunteers
- very few are checking the safety of the products in patients with a condition to be treated (very many Iranian trials on honey)
- the lots of patients are rather small;
- the trials are using various type of products (types of honey, types of propolis…)
- various doses of the products
- different protocols are used so the results cannot be compared



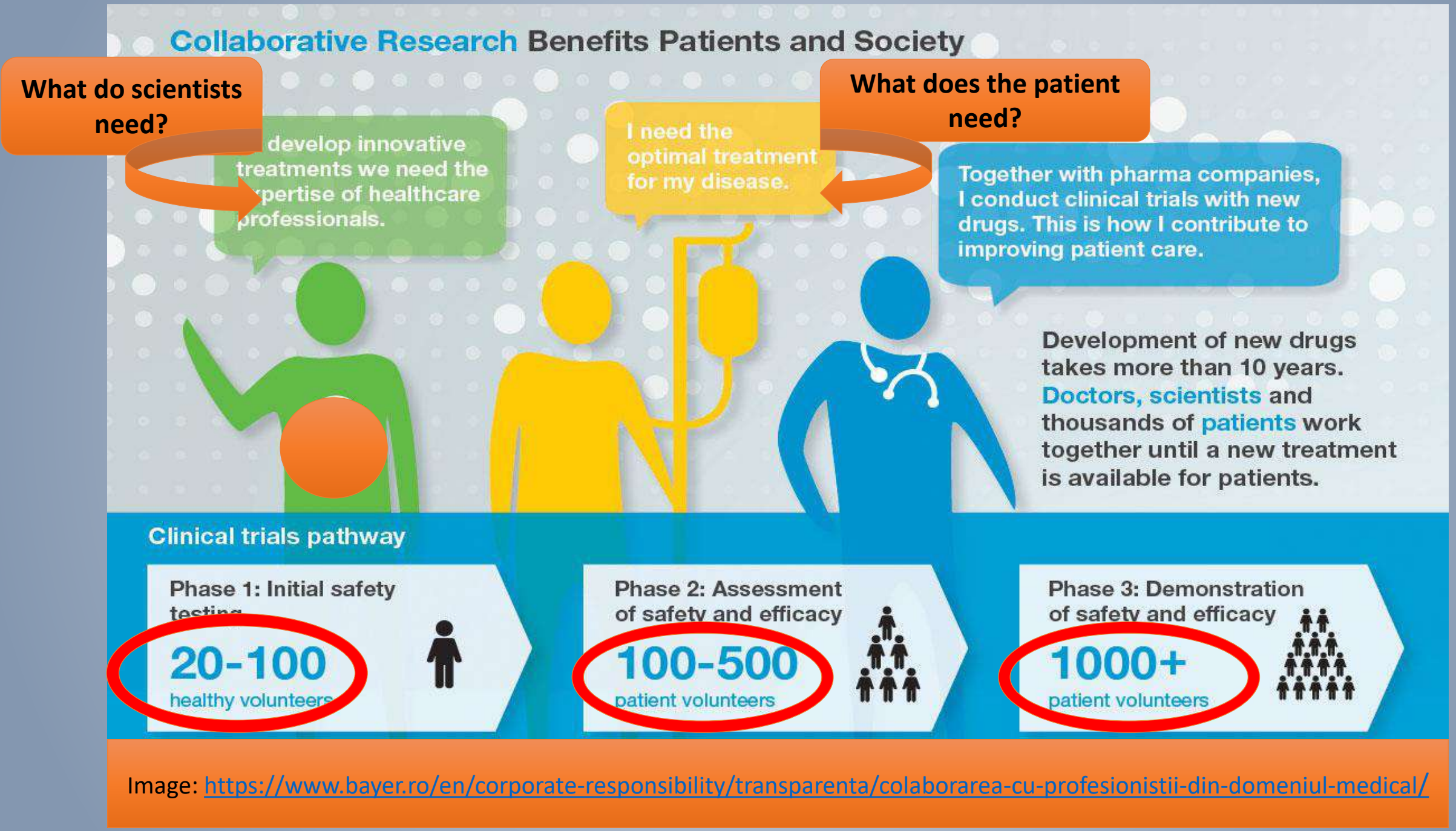


Image: https://www.bayer.ro/en/corporate-responsibility/transparenta/colaborarea-cu-profesionistii-din-domeniul-medical/

THANK YOU FOR YOUR ATTENTION!

MULTUMESC PENTRU ATENTIE!