APITSA Clinical Study

"Combination of supplements for treating Autistic Spectrum Disorder "

Protocol Cover Page

Protocol Title:	A pilot study to compare the efficacy of a ***combination of apitherapy supplements*** versus ***placebo*** in children with autistic spectrum disorder.
Protocol Number:	Bio-Forum Foundation 045 (B-F 045)
Protocol Date:	August 09, 2024
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Study Phase:	II
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Confidentiality Agreement

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LIST OF ABBREVIATIONS AND DEFINITIONS

The following abbreviations and specialist terms are used in this study protocol:

Abbreviation	**Explanation**
AE	Adverse Event
ASD	Autistic Spectrum Disorder
CNS	Central Nervous System
CRF	Case Report Form
GCP	Good Clinical Practice
Hgb	Haemoglobin
Hct	Haematocrit
IEC	Institutional Ethics Committee
IRB	Institutional Review Board
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
ml	milliliter
NSE	Neuron-specific enolase
RDW	Red cell distribution width
RBC	Red Blood Cells
SAE	Serious Adverse Event
SEM	Standard error of the mean
TSH	Thyroid-stimulating hormone
WBC	White blood cells

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PROTOCOL SYNOPSIS

TITLE	A pilot study to compare the efficacy of a ***combination of apitherapy supplements*** versus ***placebo*** in children with autistic spectrum disorder.		
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Study number:	B-F045		
Final Protocol:	August 2024	**Clean File:**	August 2024
Ethics Approval:	**August 2024**	**Statistical analysis:**	**October 2025**
Clinical Phase:	**II**	**Study Report:**	**November 2025**
OBJECTIVES:	To compare the efficacy of administering a ***combination of apitherapy supplements*** versus ***placebo*** in children with developmental disorders of the autistic spectrum (ASD).		
STUDY DESIGN:	A prospective, interventional, multi-centric, randomized, placebo-controlled, double-blind, crossover, pilot study conducted over a 6-month period which will compare treatment efficacy of a combination of apitherapy supplements with placebo. Participants will be screened at visit V1 and will be receiving the respective treatment: the active combination or placebo (A or B). At V2, at 12 weeks after V1; and at V3 - at 24 weeks after V1- the child will be evaluated by questionnaires and a psychotherapist or doctor trained in evaluating ASD. Parents will report adverse events and complete the ATEC and ASRS global questionnaires. Modifications of the scores on these questionnaires and the evaluations of the psychotherapist will be analyzed statistically as a quantitative measure of the child's improvement.		
SUBJECTS:	**Inclusion criteria** Males and females between 3 and 7 years of age: • parents informed of the nature of the study and will give written informed consent, • have body weights between 10-30 kg,		

	• have no clinically significant abnormal values on complete blood count with differential during screening, • make no changes on chronic medical treatments for 6 months • have no known allergies to substances contained in treatments **Exclusion criteria** • Any genetic or metabolic disease or condition which might compromise the hematopoietic, renal, endocrine, pulmonary, central nervous, cardiovascular, immunological, dermatological, gastrointestinal or any other body system. • History of allergic conditions – severe asthma, anaphylactic shock, allergy to components of supplements tested. • History of autoimmune disorders
TREATMENT TO BE EVALUATED	**Active Treatment:** A combination of 3 supplements containing apitherapy and plant extracts
DURATION	6 months per participant
ENDPOINTS:	**Primary: EFFICACY** 1. Behavioral improvement with validated scales for autistic spectrum disorders - ATEC and ASRS - in each 3-month interval; this will be assessed by a specifically trained medical provider **Secondary: SAFETY** 1. Adverse events, especially frequency and intensity of agitation episodes 2. Modification of laboratory tests: Hematology: RBC, MCHC, MCH, MCV, RDW, Hct, Hb, WBC Differential count – lymphocytes monocytes, neutrophils, eosinophils, basophils; Biochemistry: NSE laboratory, serum protein electrophoresis, ESR, ferritin, blood count, glycosylated hemoglobin **Secondary: Predictive biomarkers:** 1. Modifications of plasma levels of Neuron-specific enolase (NSE), and correlation with efficacy 2. Modifications of the plasma inflammatory and metabolic markers, and correlation with efficacy
SAMPLE SIZE:	A total of 40 patients meeting the inclusion criteria will receive sequentially the supplements and placebo and will be tested at 3 and 6 months afterwards;
STATISTICAL ANALYSIS:	Summary statistics will describe the safety variables. Significance testing will be done using analysis of variance for continuous and two-tailed Student's t- test for categorical variables. (α = 0,05). Pearson or Spearman correlation coefficients (for parametric and non-parametric variables) between psychometric scores and biomarkers will evaluate their predictive quality for treatment efficacy.

1. Introduction

1.1 Background

Developmental disorder of the autistic spectrum (ASD) is an extremely heterogenous pathology, with genetic and environmental determinants, with more than 800 genes (Hewitson, Havdahl, Rylaarsdam) found to be associated with ASD, and also a plethora of pollutants and environmental contaminants which act as metabolic or endocrine disruptors or produce inflammatory reactions upon contact or ingestion, sometimes in conjuction with genetic factors (Pugsley, Wiśniowiecka-Kowalnik).
The inflammatory changes have most often been highlighted as an overactive immune system (Gesundheit, Usui) in which various inflammatory markers are abnormally elevated – TNF-alpha, C reactive protein (CRP), various interleukins (IL-1beta, IL-6, IL-8, etc), NFkB, most of these being present in the alpha-2 globulin fraction of the serum protein electrophoresis (Vargas, Molloy, Hughes).

Besides the inflammatory modifications mentioned above, which were shown to be present in at least 70% of the ASD children tested, recently there were evidenced pathological values of serum neuronal specific enolase - NSE - in ASD children, which point to increased neuronal destruction associated with this pathology (Stancioiu 2023, 2024).

Presenting with such heterogenous causality, ASD warrants a complex treatment – not just with a single substance or extract – that addresses both the rebalancing of the immune system and the production of healthy neurons which can replace the lost ones and ensure proper brain function.

Natural products offer such a possibility, as plant extracts and apitherapy products are a combination of tens of substances, as opposed to pharmaceutical products, which offer the great benefit of standardization but are limited to 1-3 active substances in one pill, the drawback being the individual variations between preparations, including quality-wise.
A number of natural substances were found to have a beneficial effect on ASD pathology when studied in animal models (Erten, Khera, Singh), among those being bee pollen which was shown to improve behavior in an animal model of ASD (Alfawaz)

1.2 Study rationale

Another pervasive and constant aspect in ASD pathology, besides the presence of inflammation (various inflammation markers) and neuronal destruction (pathological values of NSE), is the fact that there is a much higher number of boys affected by ASD compared to the number of girls (approximately 4:1), and this ratio remains high even after eliminating the influence of the known X-linked pathologies (Santomauro, Foley).

Another observation worth considering is the simultaneous and seemingly relentless increase in ASD incidence in the last 2 decades, which cannot be explained by the increased access to healthcare and diagnostic of various populations (Chiarotti). Because it is very unlikely that a general and concomitant modification in population genetics has occurred in

the span of one generation on a global level, it is much more likely that this is due to environmental conditions which affect the prenatal and early postnatal life.

Putting these two things together – the recent increase in incidence and a male predominance in ASD-, on the top of the shortlist of suspects we can place the brain aromatase as a differentiating behavioral factor females vs males. Furthermore, bisphenol A, a common plastifiant and pollutant is known to inhibit the brain aromatase, and its effect is differentiated in females vs males. Even more interesting, a recent study showed that inhibition of cerebral aromatase in boys by bisphenol A can be reversed by the administration of 10-hydroxy-2-decanoic acid (10-OH DA), followed by the improvement of cognitive deficits resulting from aromatase inhibition (Simeonides, 2024),

In this context it is important to observe that there are natural sources for this substance and 10-hydroxy-2-decanoic acid (10HDA) is found in abundance in royal jelly, and by administering it to ASD children, and especially in children age 8 or over in whom the observed progress is much less ample and difficult to achieve, an effective treatment can be confirmed especially in this age group.

The combination proposed for this clinical trial is composed of apitherapy products and plant extracts that have the above effects; the main components of the supplement studied are the following:

Royal jelly - contains 29 essential amino acids, enzymes, lipids and numerous mineral salts (potassium, calcium, sodium, zinc, iron, copper, manganese, etc.) and vitamins (B1, B2, B3, B5, B6, B7, B8, B9.

Short-chain fatty acids are responsible for most of the biological processes of pure royal jelly. Pure royal jelly contains 10-hydroxy-2-decanoic acid (10HDA) called factor R and which is only found in pure royal jelly.

Scientific studies show that royal jelly can contribute to increased immunity, longevity and promotes cell regeneration.

Royal jelly is the only natural source of acetylcholine with an essential role in the transmission of nerve impulses and the proper functioning of the brain. It influences the emotional state, the ability to concentrate, memory and the speed with which we make decisions. Research has shown that pure royal jelly stimulates the production of new neurons and their connections from nerve stem cells, intervening in the brain's plasticity mechanism.

Pure royal jelly stimulates the development of new cells by acting directly on stem cells. It is also beneficial in inherited genetic conditions and has been used in dystrophic and underweight premature babies.

The administration of pure royal jelly leads to an emotional balancing reducing depression, agitation, fear. It leads to healthy, healthy sleep and feelings of optimism, joy and self-confidence. It can also be administered during pregnancy, being important for the health and development of the fetus.

Astragalus in traditional oriental medicine is a "tonic of the spleen, blood and IQ" that causes the growth of stem cells in the bone marrow and acts on the lymphatic tissue.

It stimulates the activity of macrophages, T lymphocytes and the body's resistance to pathogenic factors. Astragalus increases the level of antibodies, IgA, IgG and induces the production of interferon.

Ginseng improves nervous system functions, memory, behavior, and emotional state. In vitro and animal studies have shown protective effects of ginseng on the brain affected by free radicals. A study of 6422 adults, aged III, demonstrated that regular consumption of ginseng for at least 5 years is associated with cognitive function even in old age. Another study shows that taking 200 mg of American ginseng significantly improves memory 3 hours after administration. It has favorable effects in reducing stress, anxiety and depression due to its rich antioxidant content.

Burdock has been used since ancient times for various ailments. The 1-year-old burdock root "cleanses the blood". Modern science has confirmed that the fibers in burdock root have the ability to incorporate toxic compounds, including those absorbed orally, making them easier to eliminate. The analyses showed that it contains several types of powerful antioxidants that protect the body's cells from the action of free radicals.

Chamomile is a medicinal plant used since ancient times for conditions involving tissues and organs derived from ectoderm, rich in flavonoids with anti-inflammatory effects, adjuvant in the treatment of numerous ailments.

Milk thistle is known as milk thistle and silymarin, widely used as hepatoprotection, is extracted from it. It supports cognitive functions and is traditionally used in various neurological conditions. In the laboratory, it has been shown to prevent oxidative damage to brain cells.

Frankincense tree (Boswellia extract) improves bowel function by reducing inflammation. A 2007 study shows that frankincense in combination with other herbal medications reduces abdominal pain, bloating, depression, and anxiety in people with irritable bowel. Another study shows that Boswellia extract taken daily for 4 weeks improved symptoms in people with ulcerative colitis.

Sage is rich in vitamin K and essential minerals (Mg, Zn, Cu).

There are a few studies that show that sage through its antioxidant content reduces oxidative stress and protects cells from free radicals. Various studies have found that those who consumed sage extract performed better on tests of measuring memory, problem-solving, reasoning, and other cognitive skills. Sage improves memory and brain function in adults.

In order to evaluate the effect of these preparations in the case of children with ASD, a combination of phyto-apitherapy products was administered to a few children with very good results, these were recorded in Dr. Selaru's presentation and summarized below:

Case 1: An 11-year-old male patient.

Diagnosis: Autism spectrum disorders, with delayed cognitive and neuromotor development. The boy is completely dependent on his parents to eat, dress, go to school, etc. He sleeps with difficulty, only with his parents who have to hold him in their arms all night.

Treatment: The studied beekeeping complex (10 gr. / 0 gr. / 10 gr.), Another phyto-apitherapy complex (5 gr. / 0 gr. / 5 gr.), Royal jelly in honey 20% (5 gr. / 0 gr. / 5 gr.). At the same time, he also does ABA therapy.

Results at 1 month: He sleeps much better alone (parents can rest for the first time at night, after 11 years!). He can eat on his own and even cut his own bread. He can get dressed without help, including putting on his socks – the most difficult operation for him.

Results at 6 months: The autonomy previously gained is maintained. In addition, he asks to be allowed to make his own bed and even try to do his homework on his own, although he has great memory problems.

Results at 18 months (in the meantime he was also introduced natural brain tonic (1 / 1 / 1): He is completely autonomous for his age and, for the first time, he asked to go unaccompanied to his grandparents, packing his own bags. The ability to learn is much better, memory disorders being greatly improved. Parents are no longer oriented towards ensuring the cognitive minimum, but towards achieving school performance, especially in mathematics.

Case 2: 4-year-old patients, twins, male.

Diagnosis: autism spectrum disorders, with delayed cognitive and emotional development. Boys do not articulate words or even syllables, do not focus their gaze, do not communicate in any way with their parents. They are often very agitated and commit acts of self-harm.

Treatment studied: apitherapy complex (10 gr. / 0 gr. / 10 gr.), phyto-apitherapy complex (5 gr. / 0 gr. / 5 gr.), Royal jelly in honey 20% (5 gr. / 0 gr. / 5 gr.). At the same time, they do various forms of behavioral therapy.

Results at 6 months: the degree of neuro-motor agitation decreased, as did the self-aggressive flare-ups. They manage to articulate a few syllables and even words. Episodically, they are more attentive to their parents and more present in the surrounding world.

Results at 12 months: From the ninth month, there was what parents call a "burst of brain development." Children can now articulate whole words and even compose sentences, expressing their needs. They are much more collaborative and their curiosity has developed.

Results at 18 months: Cognitive and communication skills developed at an accelerated pace. At the age of 5 and a half, they are able to speak in two languages, Romanian and English, they come from a mixed couple. They are able to name all colors and count to a hundred. When they are very stressed, they still tend to hit themselves, but much less often. Instead, they began to be able to have social interactions and relate to strangers.

Case 3: 4-year-old female patient

Diagnosis: autism spectrum disorders, with delayed cognitive development. The little girl does not speak coherently, the vocabulary is very restricted, being unable to communicate what she feels or needs. She is often very agitated, especially at night, having sleep disorders.

Treatment: Studied beekeeping complex (5 gr. / 0 gr. / 5 gr.) + Royal jelly in honey 20% (5 gr. / 0 gr. / 5 gr.).

Results after 2 months: The child became more present in reality and more attentive. He has increased his vocabulary and speaks much more coherently, both in terms of diction and in the ordering of words in sentences. They also fall asleep more easily, as the states of nocturnal agitation are diminished.

2 STUDY OBJECTIVES, PLAN AND PROCEDURE

2.1 Investigational Products and Treatments

Taking into consideration the good results obtained in the above children with ASD, we have selected a combination of three supplements which are already on the market to be given to the ASD children enrolled in this study: Apicol 12 gamma; Royal jelly, and Telom R Cerebral.

We will also take into account the weight of the child, so that in children older than 6 years or with body weight greater than 30 kg will receive a higher dose of Royal Jelly (2 teaspoons/day vs 1 teaspoon for children younger than 6 years) and Telom R Cerebral (1 capsule/day vs 2 capsules/day) as indicated below, where details are also given on the ingredients in the three supplements (which are already available in stores, exemplified by the included links):

Apicol 12 gamma (bottle/jar, 200 ml) all participants will be administered 3 teaspoons/day, taken for 3 months (a total of 15 ml x 90 – approximately 6 jars per participant)

APICOL12GAMMA - "Mierea albastră" 200 ml - ApicolScience

Ingredients:

Polyfloral honey (organic): 90.39%; Raw royal jelly: 7%; 1:10 aqueous extracts of Burdock (Arctium lappa): 1% and Chamomile (Matricaria chamomilla): 1%; Spirulina (Arthrospira platensis), hydro saline extract, with min. 25% phycocyanin: 0.2%; Siberian ginseng (Eleutherococcus senticosus), root, aqueous extract with min. 0.8% eleutherosides, dry: 0.1%; Milk thistle (Silybum marianum), fruits, hydroalcoholic extract 40-50:1 (maltodextrin), dry, with 80% silymarin: 0.1(%); Korean ginseng (Panax ginseng), root, 4:1 hydroalcoholic extract (maltodextrin), dry, with 4% ginsenosides: 0.05(%); Astragalus (Astragalus membranaceus), root, hydroalcoholic extract 20/1 with min. 50% polysaccharides: 0.05(%); Frankincense tree (Boswellia serrata), concentrate of micellar emulsified extract in polysorbate 20, with min. 12% boswellic acid: 0.01 (%); Chamomile (Matricaria chamomilla),

essential oil 0.06(%); Serlai/Sweet Sage (Salvia sclarea), essential oil: 0.02(%); Sage (Salvia officinalis), essential oil 0.02(%).

Apicol 12 gamma (bottle/jar, 200 ml) – 1 teaspoon – 5 ml or approximately 5 g - will contain the following approximate amounts:

4.5 g honey; 0.3 g – royal jelly; 0.1 g Burdock + Chamomile + Spirulina; 0.1 g Siberian Ginseng, Milk Thistle, Korean Ginseng, Astragalus, Boswellia, Sage

Daily dose will be 3 x (3 teaspoons) of the above amounts (given per teaspoon)

Activated royal jelly (bottle, 200 ml)

LĂPTIȘOR DE MATCĂ ACTIVAT - util în anemii, depresie, oboseală - ApicolScience

For children age 6 or more - 1 teaspoon in the morning, 1 evening, (4 bottles for 3 months)
For children age 3-5 years – 1 teaspoon at lunchtime (3 bottles for 3 months)
Ingredients:
Polyfloral honey (organic): 161 g;
Royal jelly gel: 64.4 g;
Burdock leaves (Arctium lappa) 1:10 aqueous extract: 2.3 g;
Sweet Orange peel (Citrus sinensis) 1:10 aqueous extract: 2.18 g;
Grapefruit (Citrus paradisi) essential oils: 0.04 g;
Lemon (Citrus limon): 0.04 g;
Lime (Citrus aurantifolia): 0.04 g.

Telom-R Cerebral

Telom-R Cerebral, 120 capsule, Dvr Pharm : Farmacia Tei online

Children 6 years and older will receive 2 cp/day – 180 capsules for 3 months
Children 3-6 years old will receive 1 cp/day – 90 capsules for 3 months
Ingredients:

Astragalus (Astragalus membranaceus), root, hydroalcoholic extract 20:1, with min 50% polysaccharides, dry: 100 mg; and astragalus root, powder: 19.88 mg
Ginkgo biloba, leaves, 24/6 hydroalcoholic extract, with 24% flavonoids and 6% terpenoids: 80 mg; Chlorella (Chlorella vulgaris), powder: 60 mg;
Celery (Apium graveolens), fruit, powder: 60 mg;
Freeze-dried royal jelly: 40 mg;
Hawthorn (Crataegus oxyacantha), fruit, 4:1 hydroalcoholic extract: 40 mg;
Peppermint (Mentha piperita), essential oil: 0.04 mg;
Cloves (Eugenia caryophylus), essential oil: 0.04 mg;
Ravintsara (Cinnamomum camphora), essential oil: 0.04 mg.
hard gelatin capsule

Data from literature show that some of the components of this product may interact with anticoagulants, antiplatelet agents, thrombolytic agents, low molecular weight heparins, anticonvulsants, insulin, monoamine oxidase inhibitors, nonsteroidal anti-inflammatory drugs or thiazide diuretics when administered concomitantly.

Special attention needs to be given to patients with known allergy to celery and other plants of the Fam. Apiaceaea (Umbelliferae) – they should not receive this supplement.

2.2 Study design

Design: prospective, double-blind, placebo-controlled, crossover, multicenter.

All children diagnosed with ASD (about 40) will be tested before and after the combination of active substances (3 months) and placebo (3 months). The duration of participation in the study of each child will be approximately 6 months, and the total duration of the study at a clinical center (investigator) will be approximately 12 months, during which it is estimated that the 40 children can be enrolled and the study products will be administered individually for 6 months.

2.3 Study Population

For enrollment in the APITSA study we will include children who are already diagnosed with ASD and have received at least one treatment for this pathology; children who are currently receiving psychotherapy, age group 3-14 years. Consecutive children diagnosed with ASD and presenting as new patients at the clinical sites which enroll in this study (4 clinical sites, 2 in Bucharest, 1 in Constanta, 1 in Piatra Neamt), or existing children who meet the inclusion and exclusion criteria and will follow a stable therapy strategy for at least the duration of study participation (6 months for each individual child). Around 45 children will be enrolled, and assuming an attrition rate of 10%, we estimate that at least 40 children will be able to complete this study. Enrollment in the study will be positively stopped when 40 children have completed the study. Estimated enrolment of 40 patients at the 4 study sites: until April 30, 2025

2.3.1 Inclusion Criteria

Patients enrolled in the APITSA study will meet the following ***Inclusion*** criteria:

- age 3-14 years

- diagnosed with typical or atypical autism spectrum disorder (F84.0 or F84.1) and received at least one therapeutic intervention before enrolling

- the ability to follow an oral treatment, with TID/BID/QD administration

- the availability to perform 3 sets of blood tests at a clinical laboratory within 6 months (initial, at 3- and 6-months)

- the availability to perform 3 visits to the Investigator and 3 psychometric evaluations within 6 months

Exclusion criteria:

- patients enrolled in another interventional clinical trial currently or which ended less than 1 month prior to enrolling in this study

- known allergy to hive products or one of the substances studied

- inability to perform the study requirements (3 visits to the Investigator, 3 psychometric assessments, 3 sets of blood tests)

- diagnosis of diabetes

- history of seizures/epilepsy

- administration of anticoagulants, antiplatelets, thrombolytic agents, low molecular weight heparins, anticonvulsants, insulin, monoamine oxidase inhibitors, non-steroidal anti-inflammatory drugs or thiazide diuretics

- allergy to celery and other plants of the Fam. Apiaceaea (Umbelliferae)

- abnormally elevated baseline (at V1) values of ferritin, homocysteine, HgbA1c, ESR

2.3.3 Scheduled clinic visits and assessments

For each patient, three evaluations are made:

- initially (upon enrollment in the study), - V1

- after the first treatment (12 weeks after enrollment) – V2

- final (24 weeks after enrollment) – V3

At each of the three study visits, psychometric evaluation (ASRS and ATEC) and blood tests are performed, which include: NSE, serum protein electrophoresis, ESR, ferritin, blood count, glycosylated hemoglobin

Each patient will receive 2 treatments of 12 weeks (at Visit 1 – V1, respectively V2), so that each patient will take for 3 months both the active preparation (Combination A) and Combination B in random sequences (Combination A followed by Combination B, or vice versa), and the sequence is secreted both at the level of the Investigator and the patient.

2.3.4. Criteria for discontinuation

Patients can be discontinued on the study intervention in the following situations:

- severe allergy requiring emergency room treatment or administration of epinephrine as documented by a doctor or nurse

- new onset pathology requiring long-term treatment and which affects the immune or nervous systems (autoimmune disorder including type 2 diabetes, convulsions, asthma, others)

- at patient's request the participation in the study can be stopped without a reason, in which case the participant will reimburse the value of the blood tests and the medical evaluations.

2.4 Treatment administration and schedule

The treatment consists of administering for 6 months, daily, 3 types of preparations present in both Combination A and Combination B, the vials will be labeled in both cases with "A1/A2", A2/B2 and "A3/B3" respectively:

- Preparation 1 (A1 or B1). (blue) 1 teaspoon in the morning, lunch and evening

- Preparation 2 (A2 or B2) – 1 teaspoon in the morning and evening (children 7-14 years old); 1 teaspoon at lunch (2-6 years)

- Preparation 3 (A3 or B3) – 1 capsule in the morning and evening (children 7-14 years old); 1 capsule at lunch (2-6 years)

The approximately 40 patients are psychometrically evaluated 3 times: initially (at V1); after 12 weeks with Combination A, or with Combination B (at V2) and again after 12 weeks (at V3).

The investigator also sends the 3 sets of blood tests to the laboratory at V1, V2, and V3 for collection and receives the results of these tests.

Treatments (3 months active combination and 3 months placebo combination), doctor/therapist visits (350 lei x 3) and blood tests (200 lei x3) are free for the patient.

There will be different doses of supplement 2 and 3 for children 3-6 years old vs children 7-14 years; at each visit we give the following preparations that are enough for 3 months (90 days)

- 6 bottles of A1 or B1 (blue honey, 3 teaspoons/day)

- 4 bottles of yellow honey A2 or B2 for children between 7-14 years old (2 teaspoons/day), and 3 bottles of yellow honey for children 2-6 years old (2 teaspoons/day)

- 1 capsule/day from A3/B3 bottle for children 2-6 years old (total 90 capsules), respectively 2 capsules/day from A3/B3 bottle for children 7-14 years old (total 180 capsules)

2.4.1 Identity of Study Treatments

The identity of treatments (active combination and placebo combination) will be unknown to both patients/family and also the medical provider administering the treatment. At V1 and V2 the study investigator will note on the CRF the type of the combination dispensed (A or B). At the end of the study the manufacturer will disclose to the investigators and to

the sponsor the identity of treatments A and B (which is the one with the active substance); this way the double-blind characteristic of the study will be observed.

2.4.2 Storage and Accountability

Supplements and placebo kits will be stored in carton boxes at room temperature (between 18-25 Celsius) and humidity interval between 25-60% (normal conditions), protected from direct sunlight, rain and temperatures outside the interval mentioned.
The number and types of vials will be recorded upon reception by the sponsor from manufacturer and also at each time vials are distributed to the study investigators; the sponsor and the investigators are accountable for the vials received.
In the CRF the study investigator will note the type and vials dispensed at each study visit to the patients, and if there are special situations (discontinuation, returns, lost vials, etc.), the study investigator will note the occurrence and communicate to the sponsor and to the manufacturer, respectively.

2.4.3 Allowed medication

As a general rule, there should be no modifications of treatments for the child during the 6 months of the study. This means that acute treatments (ex. flu or colds) should be kept to a minimum of intervention and for the shortest possible time (ex: anti-inflammatories or antipyretics should be given for no more than 2 weeks), and treatment of other occurrences should be also minimal (ex. traveler's diarrhea) and also noted in the CRF on the respective study visit. If a new pathology is identified during the 6 months of the study (ex: asthma, diabetes) the patient should be discontinued and withdrawn from the study.

3. STUDY MEASUREMENTS AND ENDPOINTS

3.1 Primary Study Endpoints

Primary: EFFICACY

1. Behavioral improvement with validated scales for autistic spectrum disorders - ATEC and ASRS - in each 3-month interval; this will be assessed by a specifically trained medical provider acting as study investigator

3.2 Secondary Study Endpoint

Secondary: SAFETY

1 Adverse events, especially frequency and intensity of agitation episodes
2 Modification of laboratory tests: Hematology: RBC, MCHC, MCH, MCV, RDW, Hct, Hb, WBC Differential count –lymphocytes monocytes, neutrophils, eosinophils, basophils;

Biochemistry: NSE laboratory, serum protein electrophoresis, ESR, ferritin, blood count, glycosylated hemoglobin

Secondary: Predictive biomarkers:

3. Modifications of plasma levels of Neuron-specific enolase (NSE), and correlation with efficacy

Modifications of the plasma inflammatory and metabolic markers, and correlation with efficacy

3.3 Measurements at each visit

The specific evaluations at each visit are detailed in the table below

Enrollment/ Initial Visit - V1	12 weeks interval	Second Visit - V2	12 weeks interval	Final study visit – V3
Psychometric assessment (ASRS and ATEC)		Psychometric assessment (ASRS and ATEC)		Psychometric assessment (ASRS and ATEC)
NSE laboratory, serum protein electrophoresis, ESR, ferritin, blood count, glycosylated hemoglobin		NSE laboratory, serum protein electrophoresis, ESR, ferritin, blood count, glycosylated hemoglobin		NSE laboratory, serum protein electrophoresis, ESR, ferritin, blood count, glycosylated hemoglobin
A/B Combination Administration		A/B Combination Administration		

3.4 Specific detail on measurements

For ASRS administration, the answers to questions 1-36 are written down (for the respective age category of the child, 2-6 years or 6-14 years), scores from 0 to 4 (strictly in the last 4 weeks prior to the evaluation), then on the next page the corresponding figures are put and the subtotals are made at the bottom of the page, then the same questions 37-71.

Add up the scores at the bottom of the pages for the 2 pages and get the raw scores: SC, UB, SR, DSM IV-R, PS, AS, SER, AL, ST, BR, SS and AT

These are noted for each of the three study visits

For the ATEC test, the questions from the online questionnaire must be answered (it is also in Romanian) and the total score must be noted on the subscales

Understanding the ATEC score - Autism Research Institute

3.4.1 Laboratory Investigations

In addition to psychometric evaluations, the following blood tests will be performed at each of the 3 study visits:

NSE (neuron-specific enolase)
VSH
Ferritin
Serum protein electrophoresis
Complete Blood Count with differential
Hgb glycosylated (Hgb A1c)

When receiving the blood test results from the laboratory, the following guidelines need to be observed:
1. If ferritin is elevated to pathological values at V1 (>100 ng/ml), or or HgbA1c > 6.5 ESR >20, the patient is excluded or re-tested after specific treatment

2. If ferritin has normal values at V1 (between 20 and 99) then ferritin analysis is no longer performed at visits 2 and 3

3. If ferritin is abnormally low at V1, the ferritin analysis is repeated at Visit 2 and 3

3.4.2 Adverse Events

The parents will be instructed on how to look for and identify possible adverse events (AEs) during the study, and how to report them to study investigator or study coordinator.
As with any medication and treatment, there may be side-effects associated with the administered therapies, the most common being episodes of agitation
Some procedures used in this study may cause some discomforts (e.g. the withdrawal of blood may cause slight bruising or possible fainting, there may be discomfort during infusion or after administration of sedation, etc), but these will be no more than that experienced in a typical medical examination.
The observed adverse reactions are temporary and completely reversible upon discontinuation of administration of the respective product.

4 STATISTICAL METHODS

4.4 Determination of sample size

Based on previous experience with the combination in study, an effect size of approximately 20% was estimated as compared to no treatment, and using the two-tailed z test, confidence interval 95%, effect size 0,5 (medium) and a difference in scores average between the groups of 5 and the standard deviation of group A 10, SD for group B 5, then p value in this scenario is 0.02 with acceptable power.

4.5 Statistical analysis

Summary statistics will describe the safety variables. Significance testing will be done using analysis of variance for continuous and two-tailed Student's t- test for categorical variables. ($\alpha = 0,05$). Pearson or Spearman correlation coefficients (for parametric and non-parametric variables) between psychometric scores and biomarkers will evaluate their predictive quality for treatment efficacy.
For randomization the following table was used from graphpad

Subject #	Group Assigned
1	B
2	A
3	B
4	A
5	B
6	A
7	A
8	A
9	B
10	B
11	A
12	B
13	A
14	A
15	A
16	A
17	B
18	B
19	B
20	B
21	B
22	A
23	B
24	A
25	A
26	B
27	A
28	A
29	A
30	B
31	A
32	A
33	A
34	A
35	B
36	A
37	B

38	B
39	B
40	B
41	B
42	B
43	A
44	A
45	B
46	A
47	A
48	A
49	A
50	A

4.6 Changes to the protocol

Any changes to the protocol will be approved by the Ethics Committee of the Bio-Forum Foundation and will be marked in the updated version.

5 **ETHICS**

5.4 Ethics review

The APITSA clinical study has been reviewed and approved by the Ethical Committee of the Bio-Forum Foundation and found to be properly conducted, taking into consideration that the products tested have been sold in Romania for more than 5 years and there were no complaints

5.5 Ethical conduct of the study

The study will be performed in accordance with the ethical principles in the Declaration of Helsinki of the World Health Organization (see Appendix 1), and that are consistent with Good Clinical Practice and applicable regulatory requirements.

5.6 Subject information and consent

All participants in the APITSA clinical study will be properly informed – the children's parents – and will start evaluation and administration of the treatments only after carefully reviewing the Informed Consent Form and signing it – both parents are required to sign.

The investigator must store the original, signed Subject Informed Consent Form and a copy must be given to the subject. Samples of the English version of the Subject Information and Consent Forms are enclosed (Appendix 1).

If you are harmed by your participation in the study, you will be compensated according to the guidelines of the pharmaceutical industry relating to clinical trials (Association for British Pharmaceutical Industry ABPI guidelines). If you are harmed due to someone's negligence then you may have grounds for legal action. You are not waiving your legal rights by signing this form.

In case of study related questions please contact:

Doctor: Dr Felician Stancioiu or Site Investigator
Contact Numbers: 0727500402

5.7 Subject data protection

The Subject Information and Consent Form will explain that study data will be stored in a computer database, maintaining confidentiality. Subjects in this database will be identified by initials or enrolment code / subject number only.
All participant data is anonymized and will not be shared publicly or with other researchers, making it impossible to gather individual participant data without consent from family.
GDPR regulations will be observed throughout the study.

6 STUDY TIMETABLE AND TERMINATION

APITSA clinical study will begin in December, 2024 and is planned to end in September 2025. Termination of the study will occur after 40 patients will complete the study or if occurrences of serious adverse effects will be deemed to jeopardize participant safety.

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ANNEX A

DECLARATION OF HELSINKI

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000
Note of Clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002

INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

4. Medical progress is based on research, which ultimately must rest in part on experimentation involving human subjects.

5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.

6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best-proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.

8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. **BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH**

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

12. Appropriate caution must be exercised in the conduct of research, which may affect the environment, and the welfare of animals used for research must be respected.

13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.

16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.

17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.

18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

20. The subjects must be volunteers and informed participants in the research project.

21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. **ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE**

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.
29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists. See footnote

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best-proven prophylactic, diagnostic and therapeutic methods identified by the study.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgement it offers hope of saving life, re-

establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

FOOTNOTE:
NOTE OF CLARIFICATION ON PARAGRAPH 29 of the WMA DECLARATION OF HELSINKI

The WMA hereby reaffirms its position that extreme care must be taken in making use of a placebo-controlled trial and that in general this methodology should only be used in the absence of existing proven therapy. However, a placebo-controlled trial may be ethically acceptable, even if proven therapy is available, under the following circumstances:

- Where for compelling and scientifically sound methodological reasons its use is necessary to determine the efficacy or safety of a prophylactic, diagnostic or therapeutic method; or

- Where a prophylactic, diagnostic or therapeutic method is being investigated for a minor condition and the patients who receive placebo will not be subject to any additional risk of serious or irreversible harm.

All other provisions of the Declaration of Helsinki must be adhered to, especially the need for appropriate ethical and scientific review.

The Declaration of Helsinki (Document 17.C) is an official policy document of the World Medical Association, the global representative body for physicians. It was first adopted in 1964 (Helsinki, Finland) and revised in 1975 (Tokyo, Japan), 1983 (Venice, Italy), 1989 (Hong Kong), 1996 (Somerset-West, South Africa) and 2000 (Edinburgh, Scotland). Note of clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002.

ANNEX B

INFORMED CONSENT
(translation from Romanian)

Clinical Study "Apitherapy for Autistic Spectrum Disorder - APITSA"

Principal Investigator: Dr Felician Stancioiu

Date ___ / _____ / _____ Name_____

Date of birth _____ Diagnosis_Autistic Spectrum Disorder (ASD)

F84.0/F84.1

I (family name, first name) _____ declare that I agree that my child will participate in the clinical study "Apitherapy for Autistic Spectrum Disorder - APITSA"

I understand that during this study my child will have to consume for 3 months (12 weeks) either a supplement containing honey, royal jelly, astragalus and other plant extracts, or a placebo (a substance without direct physiological effects on the body).

These supplements can be purchased directly by consumers from specialized stores or online, so they have a good established safety profile; they will be provided for free during the study visits. The combination of supplements evaluated in this study was previously administered to a few children with autistic spectrum disorder (ASD) and was observed to have beneficial effects in children with ASD after several weeks of treatment. To date, there is no known treatment that will cure this pathology. This study will help to understand the benefit and to establish the limits of the effect on children with ASD and possibly shed new light onto this pathology.

Specifically, I understand that my child will have to take for 3 months, 2-3 times a day, 2-3 teaspoons and 1-2 capsules of the three supplements according to the body weight of the child. Each of the 3 supplements will have specific administrations schedule and for 3 months the child will be administered the supplement tested which contains the active ingredients, and for another 3 months, the placebo; the order in which the active combination will not be known by the patient nor the medical provider administering the treatments. It will also be necessary that three times during this study the child will be evaluated by a doctor or therapist, and to have blood tests done (initially at first visit, after 12 weeks – second visit- and after 24 weeks – third and last study visit).

I acknowledge that my child was previously evaluated by a clinician (medical doctor or psychotherapist) to assess the diagnosis of ASD, and it was established that the child has the diagnosis of ASD (F84.0 – developmental disturbance fron the autistic spectrum) or F84.1 (atypical ASD).

If I choose that my child will participate fully in the study, (ie to take the supplements and placebo for a total of 24 weeks and to do the 3 sets of blood tests at the three study visits during the 24 weeks of

participation). For these blood tests, as well as for the supplement administered and the 3 study visits, I will not have to pay.

If I choose that the child will not complete the study in the absence of objective reasons (illness, accident, etc.), I accept to pay the value of the tests and treatments performed as follows: each study visit - 300 lei, blood tests (hemoleucogram) - 250 lei. The respective amounts will be paid within 7 days from the date of withdrawal from the study to the study sponsor, The Bio-Forum Foundation, bank account at Banca Transilvania, SWIFT BTRLRO22, IBAN RO72BTRLRONCRT0056512501.

However, the decision of not continuing to participate in this study for whatever reason will not interfere with other medical care which the child is reasonably expected to receive in the future.

During the last five years in which these supplements were on the market, no adverse effects were reported when administering this type of supplement to patients without known allergies; however if there is an adverse reaction please report it as soon as possible to the study investigator or Dr Felician Stancioiu at 072750042; in the case of severe adverse reactions – difficulty breathing, swelling of tongue, throat, dizziness, first seek urgent evaluation and medical care.

By signing below, I acknowledge that I was informed about the following:

1. Variants and alternatives of treatment (medication, neurofeedback, psychotherapy, other supplements, oxygenotherapy, stem cells, etc.), and the natural evolution of ASD without treatment;
2. Possible benefits (improved behavior, cognitive status, verbalization, socialization, etc.), and side effects (allergic type reactions, including anaphylactic shock) as well as treatment needed in such situations (discontinuation of the supplement, and if allergic-type reaction - administration of anti-histamine medication, epinephrine or cortisone-based treatment)
3. The necessary or recommended analyzes (laboratory tests done from blood samples harvested from the antecubital vein via venipuncture), the treatment stages, the duration of my participation in this study and the fact that participation is free, but unmotivated withdrawal will incur costs on my part

I have understood the benefits and the risks of the administration of the treatment, I agree with the child's participation in the study in the above conditions, and I sign below:

Child's Name _____

Mother's Name

_____Signature_____

Father's Name _____Signature

_____

Date of signature ____ / _____ / _____

Study Investigator Name (Person obtaining the Informed Consent)

_____ Signature_____Date_____