ADHD-Relevant Motor and Cognitive Effects of a Fixed-Dose Combination of Caffeine, Gingko Biloba and β-Phenethylamine (PEA)

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Abstract
Background: The aim of the study was to evaluate Cogniben, a fixed-dose combination of caffeine, Ginkgo-biloba and β-Phenylethylamine (PEA) as a potential therapeutic option for attention deficit hyperactivity disorder.

Methods: Cogniben-treated mice were tested with the open-field test, object-recognition test, passive avoidance test and activity wheel test. In vitro, the effect of Cogniben and its constituents on release of NO from cultured murine macrophages was assessed. Finally, patients with ADHD were treated with Cogniben and the effect on ADHD Rating Scale-IV and clinical global impression was evaluated.

Results: Cogniben exhibited stimulant-like activity, as exhibited by both the open-field test and the activity wheel test. Treatment with Cogniben had positive effects of reducing anxiety and enhancing learning and memory in mice. In-vitro, the Cogniben combination reduced LPS-induced NO release from macrophages, to a similar extent to that of Dexamethazone. In the clinical evaluation, a 43% change from baseline was observed in the ADHD-RS-IV and a 34% reduction in severity from baseline was observed in the CGI-S following treatment with Cogniben.

Conclusion: Together, the beneficial effects of the Cogniben combination, suggest that it may be a valuable treatment for ADHD.

Keywords: Caffeine, Gingko Biloba, β-Phenethylamine (PEA), Attention-Deficit/Hyperactivity Disorder, Motor activity, Cognitive effects

Introduction

Attention deficit hyperactivity disorder (ADHD) has been estimated to affect as much as 3.4% of children and adolescents worldwide and the estimate in the USA is even higher, 11% as of 2011 [1, 2]. ADHD is a lifelong disorder with an estimated prevalence of 2.5%-4.7% in adults [3, 4]. Recent studies demonstrated that there is a constant rise in ADHD prevalence and a corresponding increase in drug prescription [5]. In the UK, the prescription rate increased by almost 800% between the years 2000-2015 and the treatment rate in males was 5 times higher than in females [6]. Moreover, much of the recent increase in ADHD diagnosis was in adults7 and hence the increase in medicated ADHD adult patients. The etiology of ADHD has not been fully elucidated; however, there is evidence in support of involvement of chronic inflammation in its pathophysiology. A potential role for the immune system in ADHD has long been suspected due to the increased prevalence of allergic diseases in ADHD patients. Allergic reactions could deregulate cholinergic and adrenergic activity in the central nervous system (CNS), leading to symptoms of ADHD. Moreover, children with atopic dermatitis displayed more ADHD-related symptoms, such as attention problems and impulsivity [7, 8].

As of 2014, a total of 17 ADHD medications were available in the USA, most of them stimulants such as methylphenidate and amphetamines [9]. Decreased appetite, growth decrease and impact on sleep (insomnia) are among the most common adverse effects associated with stimulant use [10]. Other adverse effects include weight loss, headaches and jitteriness. Recently, chronic use of methylphenidate has been shown to cause neuroinflammation in an animal model [11]. Hence, there is an unmet need for a highly effective herbal treatment for both pediatric and adult ADHD patients.

Cogniben is a novel herbal composition comprised of a synergistic fixed-dose combination of therapeutically effective doses of caffeine, gingko biloba and β-phenethylamine (PEA). **Caffeine** (1, 3, 7-trimethylxanthine; $C_8H_{10}N_4O_2$) is a natural compound found in the leaves, seeds, and fruit of many plants. Because of its high degree of lipid solubility, caffeine can easily cross the blood–brain barrier and reach the brain [12]. It is considered to be a mild stimulant of the CNS and as such, there is potential benefit for caffeine in the treatment of ADHD. A recent review article (2014) summarizing

the clinical and pre-clinical data about caffeine and ADHD, advises considering the merits of further investigating caffeine's therapeutic potential as a monotherapy or as an adjunctive agent in ADHD [13]. Caffeine and other xanthines affect the adenosine signaling system mainly via antagonism of A (2A) receptors. Through adenosine antagonism, caffeine has a significant indirect effect on various neurotransmitter systems including dopaminergic, noradrenergic, and glutaminergic signaling [13]. A (2A) receptors are now conceived as a normalizing device that promotes adequate adaptive responses in neuronal circuits, a role similar to that fulfilled by dopamine. Indeed, there is a vast body of research that supports the potential for adenosinergic antagonists as therapeutic options for the treatment of ADHD [14]. Through blocking adenosine receptors on microglia, caffeine may prevent adenosine from exerting its known pro-inflammatory effect. In a study conducted on rats, caffeine administration resulted in attenuation of the number of activated microglia within the hippocampus of animals with lipopolysaccharide (LPS)-induced and age-related inflammation [15]. While not as potent as methamphetamine or d-amphetamine, caffeine in children has been shown to have a therapeutic effect on ADHD, which is superior to placebo or to lack of any treatment [13]. In the US, caffeine has a Generally Recognized As Safe (GRAS) status. It is an FDA-approved product and a component of several over-the-counter and prescription products [16].

β-Phenylethylamine (PEA) is a natural endogenous member of the trace amines (TA), a group of amines that are found at much lower concentrations than the biogenic amines and neurotransmitters epinephrine, norepinephrine, serotonin, dopamine, and histamine. TAs exert their functions through binding of several molecular targets including the TA-associated receptor family (TAAR), monoamine transporters (mainly DAT), and dopamine/neuradrenaline extracellular modulator. It has been found that PEA is an endogenous agonist of the TA1 and TA2 receptors, with slightly more potency than other trace amines. Interactions with these receptors have been proposed as the mechanism through which PEA exerts its effects on adrenergic and dopaminergic neurotransmission. Secondary to the activation of TA1, PEA has been noted to both reduce the uptake of and increase the efflux of various neurotransmitters such as dopamine, serotonin, and noradrenaline in brain synaptosomes. In that respect, its mode of action echoes that of methylphenidate, which, through blockage of dopamine transporters, causes accumulation of dopamine in the synapse [17]. Injections of PEA in the periphery seem to be taken up in most brain areas evenly, and at rest, PEA seems to be dispersed across most brain regions although highest levels are found in areas with a higher catecholamine presence (nigrostriatal and mesolimbic regions such as the caudate–putamen, olfactory tubercles and nucleus accumbens).

PEA has been suggested as a biomarker for ADHD [18]. Several lines of evidence support a main role for PEA in the etiology of ADHD. Urine PEA levels were found to be significantly lower in individuals with ADHD than in controls [19]. However, no correlation was found between the severity of ADHD symptoms and urine-PEA levels. Kusaga and colleagues have shown that in children with ADHD treated with methylphenidate, PEA levels significantly increased after methylphenidate therapy in responders, whereas they did not increase in non-responders to methylphenidate treatment [20]. PEA was also significantly increased after d-amphetamine administration, and change in PEA excretion correlated significantly with d-amphetamine excretion [21].

The known PEA mechanism of action and the current understanding of ADHD etiology, together with the fact that PEA has a US GRAS status, suggest that elevating PEA levels might be considered as a therapeutic goal in any ADHD treatment approach [17, 22]. However, orally-ingested PEA undergoes extensive first-pass metabolism by gut monoamine oxidase B (MAO-B), preventing significant concentrations from reaching the brain. Hence, inhibition of MAO-B is essential to obtaining therapeutic benefits following PEA administration.

Ginkgo biloba was shown to influence neurotransmitter receptors of the CNS. In several studies it has been demonstrated that this effect is modulated via the involvement of cholinergic as well as the serotonergic systems. The serotonergic system is thought to be important for emotional states, learning and memory processes. Indeed, it has been found that Ginkgo extract can significantly modify 5-HT concentration and 5-HT turnover ratio in the prefrontal cortex and hippocampus, structures critically involved in spatial memory and behavioral flexibility [23]. It has also been shown that administration of Gingko extract enhanced levels of the stress-related neurotransmitter NA in the hippocampus, prefrontal cortex and striatum [24].

In addition to its direct effect on different neuronal systems, it was found that Ginkgo produces reversible inhibition of MAO-A/B [25]. Hence, in addition to its cognitive enhancement properties, Ginkgo supplementation, combined with PEA may markedly improve PEA bioavailability as a result of MAO-B inhibition. A recent study has found that Ginkgolide A, a component of the Ginkgo extract, has anti-inflammatory effects. Specifically, it was shown that Ginkgolide A suppressed the expression of pro-inflammatory mediators (cyclooxygenase-2 and nitric oxide) and pro-inflammatory cytokines (tumor necrosis factor-α, interleukins -6 and -1β) in LPS-treated mouse peritoneal macrophages, mouse macrophage RAW264.7 cells, and differentiated human monocytes in vitro. In addition, it attenuated the inflammatory response in LPS-treated BALB/c mice in vivo [26].

Ginkgo (80-120 mg/day) was found to be clinically effective in treatment of children and adolescents with ADHD receiving methylphenidate (20-30 mg/day) [27]. These study results were in agreement with previous studies demonstrating improvement in ADHD symptoms in pediatric patients treated with Ginkgo extract [28]. However, clinically, Ginkgo, when administered alone was found to be less effective than methylphelindate [29]. A recent Cochrane review has found Ginkgo Biloba to be safe with no excess side effects compared to placebo, however, as a single agent, was not found to be effective for the treatment of dementia or cognitive impairment [30].

In view of their potential additive or synergic mechanism we have decided to test whether in a fixed-ratio combination, these three compounds can extract a unique behavioral pattern, similar to the one obtained following methylphenidate administration. In addition, in this paper, we show the positive effects of Cogniben, the combination of the 3 components on various aspects relevant to ADHD.

Materials and Methods
Open Field Test in Mice
The well-known open field test (OFT) was used to assess anxiety and locomotor activity. The test box consisted of a 60 cm × 60 cm

square arena surrounded by a 50 cm high wall divided into two zones: center (11% of the entire area) and periphery [31]. The apparatus was placed on a table 80 cm above the floor. Mice were individually tested in the open field for 30 min at a time. At the beginning of each session, mice were placed at the center of the apparatus. The time in the central zone was measured during each test session. The test allows to explore the animals' spontaneous activity without external stimuli. Distance and velocity serve as a measure for locomotor activity while time spent in the center vs. perimeter and number of section crossings serve as a measure of anxiety.

Object Recognition Test (ORT)
The Object Recognition Test (ORT) was used to assess curiosity, short term memory and visual attention. The animals were brought to the testing room and left there undisturbed for at least 30 minutes for acclimation before the testing.

Habituation phase: The animals were allowed to explore the arena (described above) for 3 min without stimuli (without objects) 24 hours before the test. *Training phase (baseline)*: The mouse was placed in the arena, facing the center of the opposite wall and allowed to freely explore for 3 minutes two identical objects (A1 and A2) that were located in the corner, 15 cm from each adjacent wall. The time spent exploring each object was recorded using Ethovision software. The mouse was then moved to its home cage for a retention period of 60 minutes.

Test phase: The mouse was returned to the arena, where one of the familiar objects (A2) was replaced with a novel object (B). The animal was again allowed to explore the objects for 3 minutes and the cumulative time spent exploring the novel object (B) and the familiar object (A1) was recorded. Exploration of an object was defined as directing the nose to the object at a distance of 2 cm and/or touching it with the nose. The animal was then moved to its home cage and the arena was cleaned with alcohol after each animal.

Curiosity was measured as the time of exploring the new object (B), Short-term memory was expressed as the time of discriminating between the novel object (B) and the familiar object (A1), Visual attention was defined as the % of delta between the familiar and the novel objects.

A composite Differentiation Index (DI) of all variables derived from the ORT was defined, and calculated as:
DI= (new*-old**)/(new+old).
*new= Time with New Object (B); ** old= Time with Old Object A1

Passive Avoidance Test
The Passive avoidance test was used to measure memory deficits. It was performed as previously-described32–35 with a few modifications. The apparatus consists of two compartments of equal size (26×26 cm) separated by a sliding door (8×8 cm). The starting compartment is illuminated while the shock compartment is dark. A stainless-steel bar floor is used for delivery of scrambled constant current. The acclimation to the apparatus was performed on Day 0. Each experiment started with two acquisition trials, and a test trial performed 24 hours after the training trial.

Acclimation The mice were placed in the apparatus for two minutes and allowed to explore the compartments without activating electrical shock. *Acquisition 1* The mouse was placed in the illuminated

compartment for 60 seconds. The sliding door was raised and the latency to enter the dark compartment was recorded. Once all four paws were in the dark compartment, the door was closed and electrical current (3mA, 3 sec) was activated. 1 min following the foot shock, the mouse was returned to home cage. *Acquisition 2*: 30 minutes after Acquisition 1, the mice were returned to the PA apparatus, and an additional acquisition session was performed. The purpose of Acquisition 2 is assessment of short-term memory (30 minutes) as well as strengthening of the learning (an additional foot shock upon entering the dark compartment). *Testing* The retention test was performed 24 hours after the acquisition session. The mouse was placed in the illuminated compartment and allowed to step into the dark compartment (the electrical shock was not activated upon entry to the dark compartment). The step-through latencies were recorded over the 180-second testing period. After 180 seconds, the mouse was returned to its home cage.

Activity Wheel
Spontaneous activity was measured using the Activity Wheel Monitor (AWM) system. Mice were placed in the AWM cages at 4 P.M. and allowed to move freely in the cages until the next morning. The lights at the animal facility were automatically turned off at 7 P.M, i.e. 3 hours after the mice were placed in the AMW cages. The measuring was performed on Day 0 without administration of Test compounds, and on Days 1 and 4, 30 min after oral administration of Test compounds.

Evaluation of the Anti-Inflammatory Properties of the Combination of Caffeine, Ginkgo and PEA
105 macrophage murine cellular system (RAW 264.7 cells) were seeded in 96-well plates in (1% FBS, 1% Glutamine and 1% Pen/strep in DMEM). Cells were treated with Caffeine (6.67 µg/ml), Ginkgo (40 µg/ml) and PEA (20 µg/ml) for 22 hours together with LPS induction (5 ng/ml). LPS concentration was chosen based on previous studies that demonstrated no anti-inflammatory effect with the tested Items. In addition, positive control cells were treated with 20 µM dexamethasone. Nitric oxide (NO) release was tested after incubation at 37°C, 5% CO_2 using the Griess Reagent System.

Assessment of PEA, Neurotransmitters and their Metabolites in Urine of Human ADHD Patients
ADHD patients were treated for 1 week with Cogniben. Urine samples were taken at the end of Day 7 of administration and urine levels of PEA, catecholamine, Indolamine and their metabolites were measured (HPLC-ED).

A Pilot Study to Assess the Safety and Efficacy of Cogniben in Adults with Attention-Deficit Hyperactivity Disorder (ADHD)
A small pilot randomized, single-blind, dose titration, 7-week, outpatient study was performed to evaluate the efficacy and safety of Cogniben in adults (18 to 55 years) with ADHD (according to DSM-IV-TR criteria), following a washout period of previous medications. Patients with a history of psychotic symptoms, those with a psychiatric co-morbidity, mental or other significant neurological disorders, a positive human immunodeficiency virus (HIV) or Varicella zoster virus (VCV) status, significant gastrointestinal disease or a history of cancer within the 5 years preceding the screening visit were excluded.

10 subjects (females (n=6) median age 35.5 years, males (n=4), median age 41.5 years) with a mean the ADHD Rating Scale-IV

(ADHD-RS-IV) Total score of 39 and mean CGI-Severity (CGI-S) score of 5.3 were recruited. Each subject underwent a 2-week individual dose-titration period, followed by 5 weeks of maintenance with the selected dose (highest individually titrated dose).

The titration sequence was as follows:

Dose	Duration	Next dose	Increase following
½ caplet	2-days	1 caplet	Patient decision
1 caplet	5- days	2 caplets	Physician decision based on effects on ADHD and any reported adverse events
2 caplets	7-days		Maximal allowed dose in the study

The ADHD-RS-IV, that measures the symptoms of ADHD according to the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [36, 37]. Was used to evaluate treatment of ADHD. The primary efficacy endpoint was the mean change from baseline to different time points in ADHD-RS-IV total score. The CGI-S scale was used to assess the global ADHD illness severity state [38]. CGI-S was evaluated at each post-baseline visit using a 7-point scale with scores ranging from 1 (normal, not at all ill) to 7 (among the most extremely ill).

Results
Effect of Cogniben on Motor Activity in Mice
Open Field Test
In this test, Cogniben exhibited stimulant-like activity. Mice treated with Cogniben moved further from the box than mice treated with vehicle, achieving a distance similar to those treated with Methylphenidate (Figure 1A). The same was true of the time spent in the arena: Cogniben-treated mice spent more time in the arena than those treated with vehicle, and similar to those treated with Methylphenidate (Figure 1B).

Activity Wheel
The activity wheel measures spontaneous general activity in mice. In this test, mice treated with Cogniben were more active than those treated with vehicle over 2 hours after dosing during the "day phase" of their circadian rhythm (Figure 1C) as well as at different time intervals following administration during the "night phase" (Figure 1D) indicating general stimulatory effect.
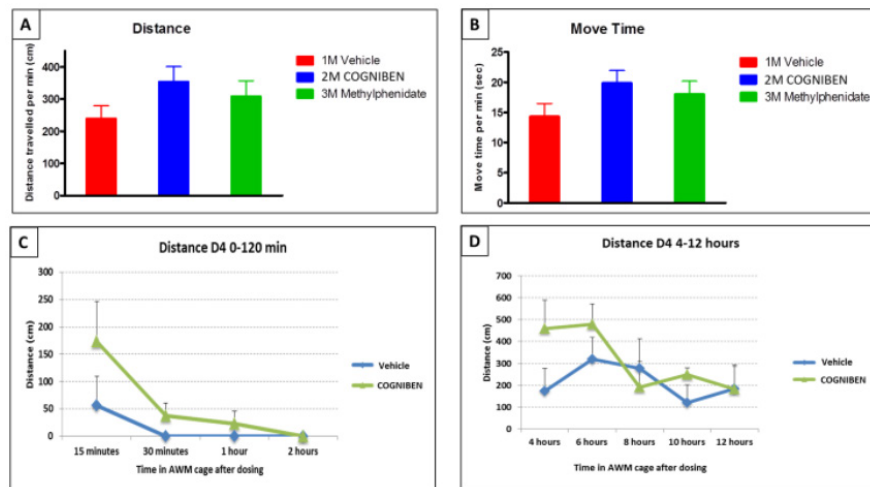

Figure 1: A and B Effect of COGNIBEN and Methylphelindate on mice motor activity. C and D Effect of COGNIBEN on spontaneous "home cage" motor activity

Cognitive Effects of Cogniben in Mice
Anxiolytic Activity
Mice treated with Cogniben ventured longer distances towards the center of the open arena than those treated with vehicle, indicating a lower level of anxiety (Figure 2A). When PEA was tested alone, and not as part of the Cogniben combination, this effect was shown to be dose-dependent. The distance that mice ventured towards the center of the open arena was larger, the higher the concentration of administered PEA. At doses ≥30 mg/kg, the distance was larger than in mice treated with vehicle (Figure 2B).
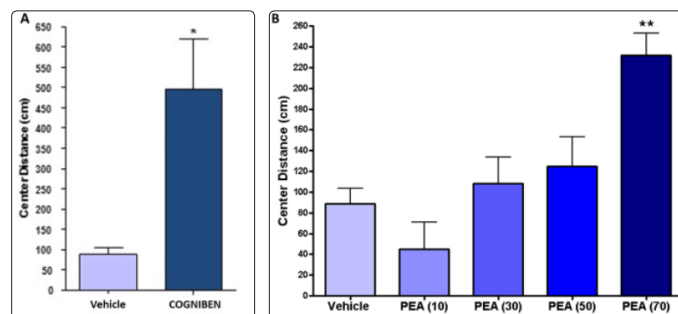

Figure 2: A Effect of COGNIBEN on the distance mice spent in the center of the open field arena. B Dose-dependent effect PEA on the distance mice spent in the center of the open field arena

Cognitive Enhancement

Mice treated with Cogniben exhibited increased curiosity, short term memory and visual attention compared to vehicle, as exhibited by the object recognition test (Figure 3A). Following treatment, the calculated composite differentiation index (DI) for all variables derived from the object recognition test (attention, learning and memory) was higher in all mice compared to baseline. Mice treated with Cogniben showed a much larger effect than those treated with vehicle and those treated with methylphenidate (Ritalin) (Figure 3B).

Mice treated with Cogniben exhibited a gradual lengthening of latency of entry to the dark compartment in the passive avoidance test, suggesting they learned and remembered that the dark compartment was associated with pain, as it was the location of the electrical shock. At the testing phase, Cogniben-treated mice exhibited a longer latency than control mice, and even than mice treated with Ritalin (methylphenidate) (Figure 3C).
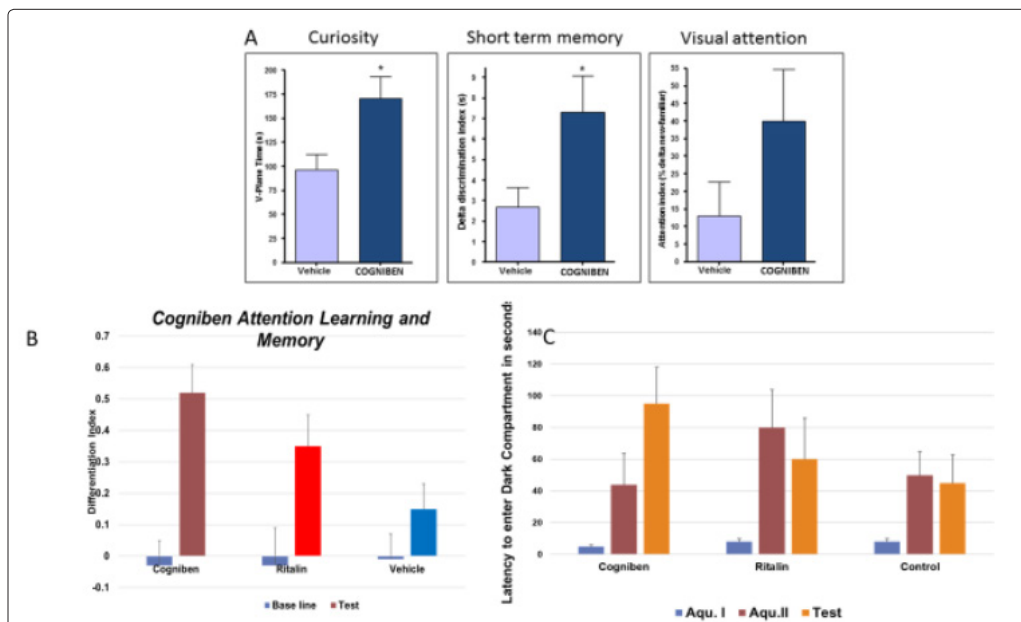

Figure 3: A and B: Effect of COGNIBEN on short term memory, curiosity and visual attention (object recognition test); C Effect of COGNIBEN on learning and memory (dark chamber test)

Anti-Inflammatory Properties of the Combination of Caffeine, Ginko and PEA

The best combination ratio that was achieved *In-vivo* and demonstrated elevation of motor and cognitive function was 1:6:3; Caffeinie: Ginko: PEA respectively. Therefore, this ratio was tested in this *Iv-vitro* inflammation model. LPS treatment caused an increase in nitric oxide (NO) release into growth medium to a final concentration of 33 μM. Each test item alone, did not attenuate LPS-induced NO release (105, 90 and 112% of NO concentration in the medium, following treatment with caffeine, Ginko and PEA, respectively, compared to level following LPS alone). In contrast, the combination of caffeine, Ginko and PEA led to a significant reduction in NO release following LPS induction (85% compared to LPS alone). This effect was comparable to that of Dexamethasone (63% of levels after LPS alone) (Figure 4).
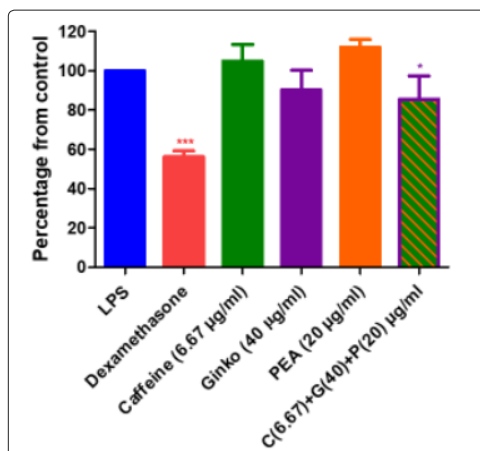

Figure 4: Anti- inflammatory effect of caffeine, Ginko and PEA combination on RAW 264.7 cells. Treatment: 6.67 μg/ml Caffeine, 40μg/ml Ginko or 20 μg/ml and combination of all three compound. Results presented as percentage from LPS treatment. (**) $p < 0.001$ compared to LPS treatment, using one way ANOVA

Improved Bioavailability of PEA Accompanied by Indicators for Changes in Relevant Neurotransmitters and Their Metabolites in Urine of ADHD Patients

When PEA was co-administered with caffeine and Ginko to ADHD patients, a significant elevation in urine PEA was demonstrated (Figure 5A). The observed elevation in PEA level was accompanied by an increase in Catecholamine and Serotonin urine levels (Figure 5B).
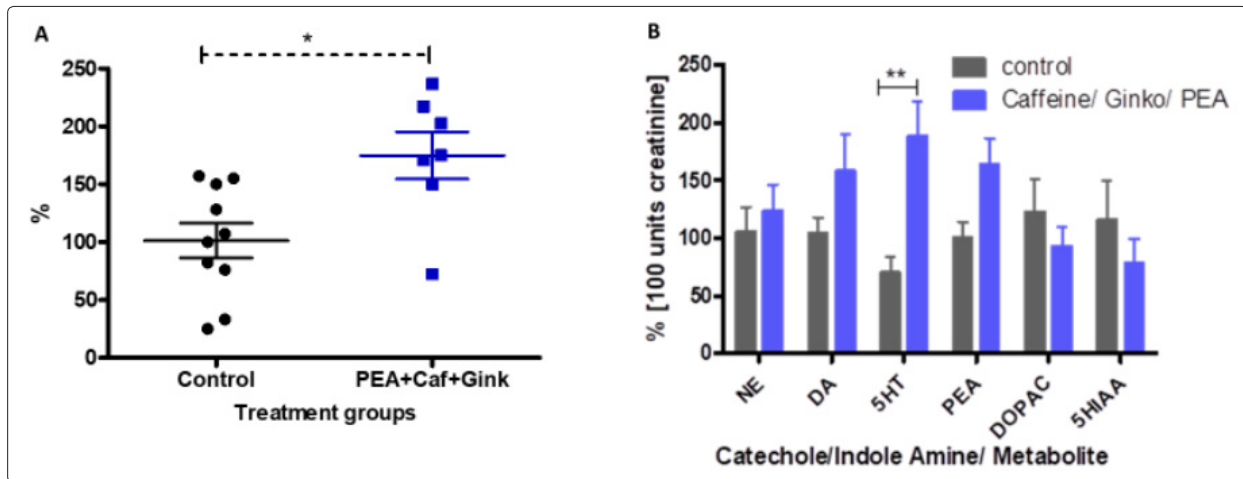

Figure 5: A: Effect of 1 week treatment with PEA, Ginko and caffeine on PEA urine levels, B: Effect of Ginko, Caffeine and PEA administration on catecholamine, Indolamine and their metabolite Urine level (% fro base line values)

Pilot Study to Assess the Safety and Efficacy of Cogniben in Adults with Attention-Deficit Hyperactivity Disorder (ADHD)
The primary efficacy endpoint was the mean change from baseline to different time points in ADHD-RS-IV total score. By Day 49, a 43% change from baseline was observed in the ADHD-RS-IV following treatment with Cogniben. The same result was observed for the ADHD-RS-IV Inattentive and Hyperactive- impulsive symptoms score (data not shown).

Table 1: ADHD-RS-IV total score and Change from Baseline

	Screening	Rand/ Bassline	Day 7	Day 14	Day 28	Day 49
Mean	38	39	34	27	24	22
Difference From baseline			6	12	15	17
% change			14%	32%	38%	43%

The CGI-S scale was used to assess the global ADHD illness severity state. By Day 49, a 34% reduction in severity from baseline was observed in the CGI-S scale (Table 2).

Table 2: CGI-Severity (CGI-S) scale and Change from Baseline

	Screening	Rand/ Bassline	Day 7	Day 14	Day 28	Day 49
Mean	5.2	5.3	4.4	3.7	3.6	3.5
Difference From baseline			0.9	1.6	1.7	1.8
% change			17%	30%	32%	34%

Treatment was found to be safe and well-tolerated, 9 subjects (90%) were able to titrate the dose to the maximal allowed dose level in the protocol, using the titration sequence defined in the study protocol. One subject (subject 10) did not tolerate the escalation to 2 caplets and experienced vasomotor "hot flash" activity, temporary anxiety, and short-term hyper-focus during regular daily tasks that lead to

a de-escalation back to one caplet treatment. The low body mass index of this subject (18 Kg/m^2) might have contributed to this effect. Subject 101 reported onset of depression mainly in the evenings and reported having suicidal thoughts. The subject was not considering to be actually suicidal but was scheduled for psychiatric evaluation in 4 days. According to the evaluation by the study primary investigator (PI), no hospitalization or immediate medical attention was needed. This AE was classified by the PI as non- serious, mild intensity and probably related to the study drug. No pharmaceutical was warranted and the treatment with the study medication was stopped on day 15. Follow-up safety call and subsequent office visit indicated a stabilization in mood with study product discontinuation and start of antidepressant. Participant showed significant improvement in focus, attention and mood while on study product, but experienced rebound depression (similar to the effects that he experienced with the use of Lisdexamfetamine) as product washed out of system in evening.

Discussion
In this study, the combination of caffeine, Ginko-Biloba and PEA was shown to have enhancing motor and cognitive effects on mice. Administration of PEA, concomitantly with caffeine and Ginko-Biloba increased the bioavailability of PEA. Interestingly, the observed elevation in PEA level was also accompanied by an increase in Catecholamine and Serotonin urine level, suggesting alteration in the catecholamines-Indolamines balance as a result of sub-chronic consumption of PEA, Caffeine and Ginko. The combination was also shown to have anti-inflammatory effects. Finally, a pilot assessment in humans showed a beneficial effect on ADHD symptoms.

PEA is considered to be an "endogenous amphetamine" as it releases dopamine and blocks its reuptake through the inhibition of the dopamine transporter (DAT). When PEA is administered alone, this effect is not observed because PEA is metabolized by MAO-B in the gut. Only a small proportion of ingested PEA gets into the

blood stream and an even lower amount crosses the blood-brain barrier. However, when co-administered with Ginkgo, MAO is actively inhibited and as a result, PEA bioavailability increases. Furthermore, although at plasma concentrations (approximately 1-10 μM) achieved by normal human consumption, the MAO inhibitory potencies of caffeine are unlikely to be of pharmacological relevance in humans, when co-administered with Ginkgo, this additional MAO inhibitory effect becomes highly relevant. It should be noted that in addition to interfering in adenosine receptors, caffeine interacts with acetylcholinesterase, monoamine oxidase, phosphodiesterase, ryanodine receptors and other receptors/ transporters together providing a stimulant-like activity. Both Ginkgo and caffeine have been previously shown to exert anti-inflammatory effects, that are also relevant to a few potential mechanisms of ADHD [15, 26, 39].

ADHD has been noted to be comorbid with a variety of psychiatric disorders. These include oppositional defiant and conduct disorders, as well as affective, anxiety, and learning disorders. Indeed, a significant proportion of adults with mood disorders have comorbid ADHD, and a significant proportion of adults with ADHD have comorbid mood disorders [40].

The observed anxiolytic effect in mice following Cogniben administration might be related to the expected MAO inhibition as a result of Ginkgo administration. Furthermore, these results are supported by previous studies attributing the anxiolytic effect of Ginkgo to its constitute Ginkgolide-A. Furthermore, this effect can also be attributed to the antagonism of the adenosine receptor as a result of caffeine administration. Indeed, in previous publications this effect could be observed pre-clinically [41]. Although caffeine at high doses is considered as anxiogenic, at lower doses it showed psychostimulatory action which might be related to the observed Cogniben effect (the clinical equivalent dose level of caffeine in the Cogniben mixture tested pre-clinically is equivalent to one cup of coffee or less). It is known that caffeine belongs to a group of compounds within the group of "cognitive enhancers" considered to be effective in treatment of anxiety disorders [12].

Interestingly, we have also demonstrated that PEA increases anxiolytic activity in a dose-dependent manner; hence, one should expect that the observed increase in PEA level following Cogniben administration will have an anxiolytic effect.

Cogniben, similar to classical stimulants, appears to improve several cognitive functions including learning, memory and attention. These observed effects may be related to the acceleration of ACh release, suggested decrease in 5-HT turnover (as revealed by the observed biochemical clinical profile of serotonin and its metabolite 5HIAA in the clinical sample) and potentially the inhibition of Adenosine A2 receptor as a result of caffeine administration.

The "cognitive-enhancement" properties of the Gingko component, together with the stimulant properties of the PEA and caffeine, can potentially induce a new spectrum of activities that might be linked to plasticity changes and, therefore, provide a broad spectrum of activities affecting multiple neuronal network expending ADHD therapeutic options. Of note, Cogniben has been shown to have anti-inflammatory effects, above and beyond those of its individual components. This effect is highly relevant, first due to the potentially etiological role of inflammation in ADHD, and second as a safety benefit, since methylphenidate has the potential to induce

neuroinflammation. Our findings support a potential for Cogniben as an effective treatment for ADHD. This notion is further supported by the encouraging results of the pilot clinical evaluation, showing improvement of ADHD symptoms. Together, the beneficial effects of the Cogniben combination, suggest that it may be a valuable treatment for ADHD, not only for the relief of symptoms, but also as a potentially disease-modifying therapy.

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