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ORIGINAL ARTICLE

Avidekel Cannabis extracts and cannabidiol are as efficient as Copaxone in suppressing EAE in SJL/J mice

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Abstract
Multiple sclerosis (MS) is an autoimmune disease leading to the destruction of myelin with consequent axonal degeneration and severe physical debilitation. The disease can be treated with immunosuppressive drugs that alleviate the symptoms and retard disease aggravation. One such drug in clinical use is glatiramer acetate (Copaxone). The non-psychotropic immunosuppressive cannabinoid compound cannabidiol (CBD) has recently been shown to have beneficial effects on experimental autoimmune encephalomyelitis (EAE). The aim of our study was to compare the efficacy of CBD and standardized extracts from a CBD-rich, Δ^9-THC^{low} *Cannabis indica* subspecies (Avidekel) with that of Copaxone. Our data show that CBD and purified Avidekel extracts are as efficient as Copaxone to alleviate the symptoms of proteolipid protein (PLP)-induced EAE in SJL/J mice. No synergistic effect was observed by combining CBD or Avidekel extracts with Copaxone. Our data support the use of Avidekel extracts in the treatment of MS symptoms.

Keywords Avidekel extracts · Cannabidiol (CBD) · Cannabis · Experimental autoimmune encephalomyelitis (EAE) · Immunosuppression

Abbreviations

CBD	Cannabidiol
CNS	Central nervous system
EAE	Experimental autoimmune encephalomyelitis
MS	Multiple sclerosis
PLP	Proteolipid protein

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). The early phase of MS is characterized by relapses, while the later phase by progressive disability. Findings from animal models and immunological studies of patients with MS suggest that a peripheral immune response targeting various myelin components drives the disease process during the early phase, whereas immune reactions within the CNS dominate the progressive phase (Hemmer et al. 2015). Accordingly, treatment protocols have been developed based on immunosuppressive drugs, the aim of which is to alleviate the clinical symptoms and slow down disease progression (Reich et al. 2018). One outstanding drug in MS therapy is glatiramer acetate (Copaxone) that was accidently discovered by the research group of Prof. Ruth Arnon (Teitelbaum et al. 1971) when they tried to produce a synthetic antigen capable of inducing experimental autoimmune encephalomyelitis (EAE), an animal model of autoimmune inflammatory CNS disorders, including MS. Instead, they observed that Copaxone was protective in EAE models. Subsequent clinical evaluation resulted in FDA approval for the use of Copaxone in relapsing–remitting MS in 1996 (Arnon 1996).

Cannabidiol (CBD), the major non-psychotropic component of Cannabis, has long been known to have strong anti-inflammatory activities and has been shown in animal models to have beneficial effects on various autoimmune diseases such as rheumatoid arthritis, type 1 diabetes, and inflammatory bowel disease (Burstein 2015; Gallily et al. 2015; Malfait et al. 2000; Weiss et al. 2008). CBD has also been shown to alleviate the clinical symptoms of myelin oligodendrocyte glycoprotein (MOG_{35-55})-induced EAE in C57BL/6 mice (Rahimi et al. 2015). A major disadvantage of CBD is its bell-shaped dose–response curve resulting in a limited therapeutic dose range (Gallily et al. 2015;

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Malfait et al. 2000; Weiss et al. 2008). In contrast to purified CBD, standardized plant extracts of the *Cannabis indica* subspecies Avidekel (formerly known as Clone 202), which is highly enriched in CBD (18%) and barely contains the psychotropic Δ^9-tetrahydrocannabinol (THC) (1%), provide a correlative anti-inflammatory and anti-nociceptive dose–response when applied intraperitoneally or orally in an inflammatory mouse model (Gallily et al. 2015). The Avidekel extracts also contain trace amounts of other cannabinoids that might act in synergy with CBD (Gallily et al. 2015). Since Avidekel does not have psychotropic effects and also exhibit pain relieving activities (Gallily et al. 2015), it was worth studying the effects of Avidekel extracts on clinical symptoms of a mouse EAE animal model. Indeed, we found that Avidekel extracts had similar suppressive activity as purified CBD and Copaxone. No further suppression was observed when combining CBD or Avidekel extracts with Copaxone, suggesting for maximum suppressive effects using either drug alone.

Materials and methods

Materials

Purified CBD was purchased from THC Pharm. GmbH, Frankfurt, Germany. Flowers from the Avidekel *Cannabis indica* subspecies (formerly clone 202), which are rich in CBD (18%) while low in Δ^9-THC (1%) (Gallily et al. 2015), were supplied by Tikun Olam Company (A government-approved farm growing Medicinal Cannabis), Israel. CBD-enriched extract was prepared from the flowers of Avidekel grown under controlled temperature and light conditions. 100% ethanol (10 ml) was added to the chopped Avidekel dry flowers (100 mg) for 24 h with occasional shaking at room temperature. Following filtration, samples were taken for analysis as previously described (Gallily et al. 2015). Ethanol solutions of Avidekel extracts (10 mg/ml–20 mg/ml) were kept at − 20 °C in the dark. The extract was evaporated on Rotavapor (BÜCHI Labortechnik AG, Switzerland). For intraperitoneal injection, the dried Avidekel extract was emulsified in a vehicle composed of ethanol:Cremophor:saline at a 1:1:18 ratio. Purified CBD was emulsified in the same vehicle. Copaxone solution (20 mg/ml, Teva Pharmaceutical Industries Ltd, Israel) was diluted in PBS just before subcutaneous (s.c) administration.

Mice

Female SJL/J mice (Harlan Laboratories) were 6–7 weeks old at the beginning of the experiments. The mice were maintained at a constant temperature (20–21 °C) and a 12-h light/dark cycle in the SPF unit of the Hebrew University-Hadassah Medical School, Jerusalem, Israel. The animals were maintained on standard pellet diet and water ad libitum. The experimental protocols were approved by the Animal Care Ethical Committee of the Hebrew University-Hadassah Medical School, Jerusalem, Israel (Ethical Approval Number MD-16-14765-5).

PLP-induced EAE

Mice were immunized with proteolipid protein $PLP_{139-151}$ emulsified in Complete Freund's Adjuvant (CFA) together with pertussis toxin to induce relapsing–remitting EAE as described (McCarthy et al. 2012). In brief, 6–7-week-old female SJL/J were subcutaneously injected with an emulsion of 200 µg $PLP_{139-151}$ (GL Biochem., Shanghai, China) in 0.1 ml CFA (Sigma, Israel) on day 0, followed by intraperitoneal (i.p.) administration of 250 ng pertussis toxin (Sigma, Israel) in 0.2 ml PBS on day 0 and day 2. Upon signs of paralysis (usually after 9–11 days), the EAE mice were randomized into 4–6 groups depending on the experiment, with 6–8 mice per group. The mice (average weight of 20 ± 2 g at the beginning of the experiment) were then injected intraperitoneally with 0.1 ml vehicle (ethanol:Cremophor:saline at a ratio of 1:1:18) containing purified CBD (5 mg/kg) or Avidekel extract (50 mg/kg) 5 days a week for up to 60 days. Copaxone (50 mg/kg) was injected s.c. in 0.1 ml PBS. Control mice were injected i.p. with 0.1 ml vehicle only. In most of the experiments, PLP induced 3 phases of paralysis.

Neurological assessment

The mice were observed daily for the appearance of neurological paralytic symptoms and scored in a scale from 0 to 5 (McCarthy et al. 2012) according to the following signs: Grade 0: No neurological signs; Grade 0.5: Half paralyzed tail; Grade 1: Fully paralyzed tail; Grade 1.5: Fully paralyzed tail and weak or altered gait; Grade 2: Fully paralyzed tail and hind limb weakness; Grade 2.5: Unilateral hind limb paralysis; Grade 3: Complete hind limb paralysis; Grade 3.5: Complete hind limb paralysis and forelimb weakness; Grade 4: Full paralysis of all limbs (quadriplegia); Grade 5: Moribund state or death. Mice with clinical scores of 4–5 were euthanized.

Statistical Analysis

The results are presented as average ± standard error. Mice treated with CBD or Avidekel extracts were compared with control mice receiving the vehicle only or with mice receiving Copaxone. Mice treated with CBD and Copaxone or Avidekel extracts together with Copaxone were compared with mice treated with only one of the compounds. Raw *p* values were obtained from two-tail Mann–Whitney tests and

adjusted for multiple comparisons within each experiment using the Holm modification of the Bonferroni correction (Holm 1979). A p value equal to or below 0.05 was considered statistically significant. Six–eight animals were used in each treatment group for each experiment.

Results

Purified CBD and Avidekel extracts alleviate EAE symptoms

Experimental autoimmune encephalomyelitis (EAE) was induced in SJL/J mice by subcutaneous injection of $PLP_{139-151}$ emulsified in CFA followed by two intraperitoneal injections of pertussis toxin at days 0 and 2. The PLP-induced EAE model caused three distinct disease phases (I,

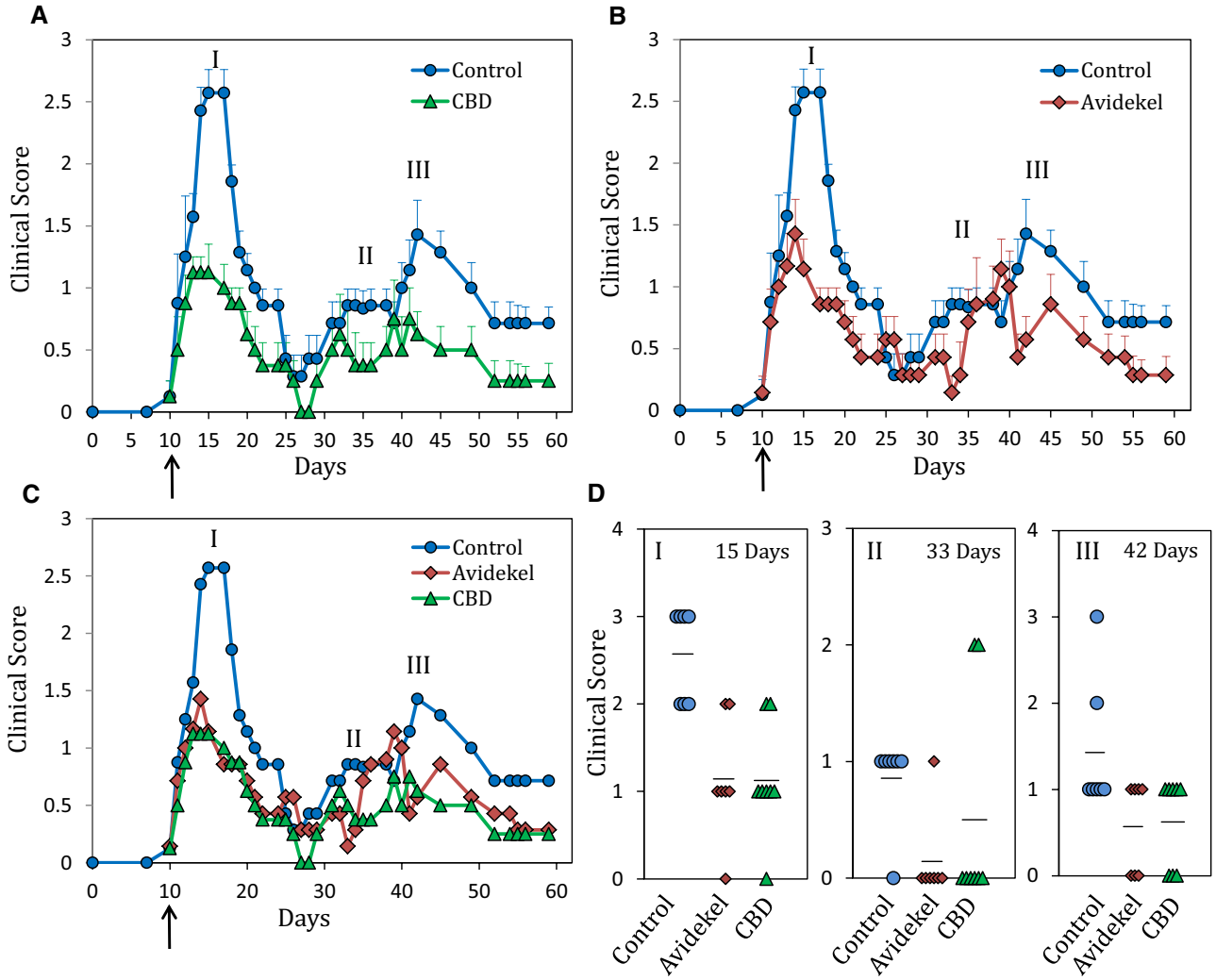

Fig. 1 Suppression of EAE symptoms by CBD and Avidekel extracts. EAE was induced by $PLP_{139-151}$ and at day 10 (indicated by an arrow), when the first neurological signs (Score 1) were observed, the mice were daily treated with CBD, Avidekel extracts or vehicle alone (Control) 5 days a week for 50 days. The clinical scores were monitored daily. Three relapse phases were observed as indicated (I, II, and III). Each group comprised 8 mice. **a**–**c** The graphs represent the average of data obtained from a representative experiment using 8 mice per treatment group. **a** Comparison of CBD with control mice. **b** Comparison of Avidekel with control mice. **c** The three treatment groups (Control, CBD or Avidekel) are presented together. Days 14–18 of phase I: $p<0.001$ for CBD vs control and Avidekel vs control. Days 31–35 of phase II: $p<0.005$ for CBD vs control and $p<0.01$ for Avidekel vs control. Days 41–49 of phase III: $p<0.001$ for CBD vs control and $p<0.03$ for Avidekel vs control. **d** The graphs present the clinical score of individual mice in each group at the peak of each relapse phase (I, II and III). The lines represent the average

II, III) (Fig. 1), which is in contrary to the MOG-induced EAE model where only one prolonged disease phase is observed (Rahimi et al. 2015). The first neurological symptoms (Score 1) were usually observed around day 10. From that day, the mice were daily injected intraperitoneally with purified cannabidiol (CBD; 5 mg/kg) or Avidekel Cannabis extracts (50 mg/kg), and the clinical scores were followed up daily. One of the 8 mice died in the control group in Phase I, while none died in the treated groups. Both CBD and Avidekel extracts efficiently inhibited the clinical symptoms appearing during all three relapse phases (Fig. 1). During days 14–18 of phase I, CBD suppressed the symptoms by $56.0 \pm 1.8\%$ ($p < 0.001$) and Avidekel extracts by $54.3 \pm 5.2\%$ ($p < 0.001$) at the average. During days 31–35 of phase II, CBD suppressed the symptoms by $39.1 \pm 8.1\%$ ($p < 0.005$) and Avidekel extracts by $48.9 \pm 11.9\%$ ($p < 0.01$) at the average. During days 41–49 of phase III, CBD suppressed the symptoms by $50.4 \pm 5.8\%$ ($p < 0.001$) and Avidekel extracts by $49.7 \pm 6.9\%$ ($p < 0.03$) at the average (Fig. 1). These data clearly show that Avidekel extracts are as efficient as CBD in suppressing EAE symptoms. Also, it is important to note the rapid onset of the therapeutic effects exerted by CBD and Avidekel extracts.

CBD and Avidekel extracts are at least as efficient as Copaxone in suppressing EAE symptoms

We next compared the efficacy of CBD and Avidekel extracts with that of Copaxone to suppress EAE symptoms. We observed that CBD at 5 mg/kg and Avidekel at 50 mg/kg were more efficient than the standard Copaxone dosage of 50 mg/kg during relapse phases I and II ($p < 0.05$), but showed similar suppression during relapse phase III (Figs. 2, 3, 4, 5). During days 11–13 of phase I, CBD suppressed the

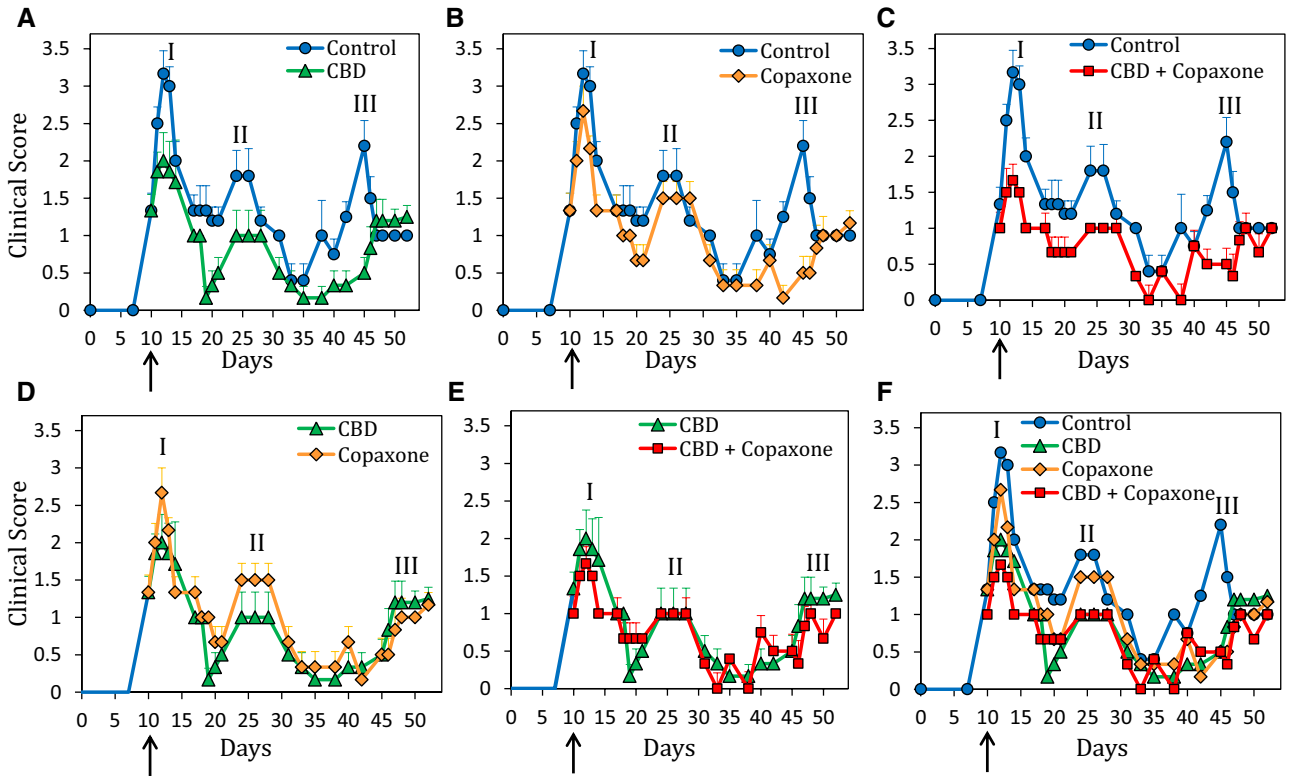

Fig. 2 CBD was at least as efficient as Copaxone to relieve EAE symptoms. EAE was induced by $PLP_{139-151}$ and at day 10 (indicated by an arrow), when the first neurological signs (Score 1) were observed, the mice were treated daily five times a week with CBD, Copaxone alone or in combination. Each treatment group comprised 6–8 mice. The graphs represent the average of data obtained from a representative experiment. **a** Comparison of CBD with Control mice. **b** Comparison of Copaxone with Control mice. **c** Comparison of CBD + Copaxone with control mice. **d** Comparison of Copaxone with CBD-treated mice. **e** Comparison of CBD + Copaxone with CBD-treated mice. **f** The four treatment groups (Control, CBD, Copaxone and CBD + Copaxone) are presented together. During days 11–13 of phase I: $p < 0.002$ for CBD vs control; $p < 0.006$ for Copaxone vs control; $p < 0.05$ for CBD vs Copaxone; $p < 0.05$ for CBD + Copaxone vs CBD; $p < 0.001$ for CBD + Copaxone vs Copaxone. During days 24–26 of phase II: $p < 0.01$ for CBD vs control; $p < 0.17$ for Copaxone vs control; $p < 0.05$ for CBD vs Copaxone; $p < 0.003$ for CBD + Copaxone vs Copaxone. During days 42–46 of phase III: $p < 0.0001$ for CBD, Copaxone and CBD + Copaxone vs control. In phase III there was no difference between the three treatment groups

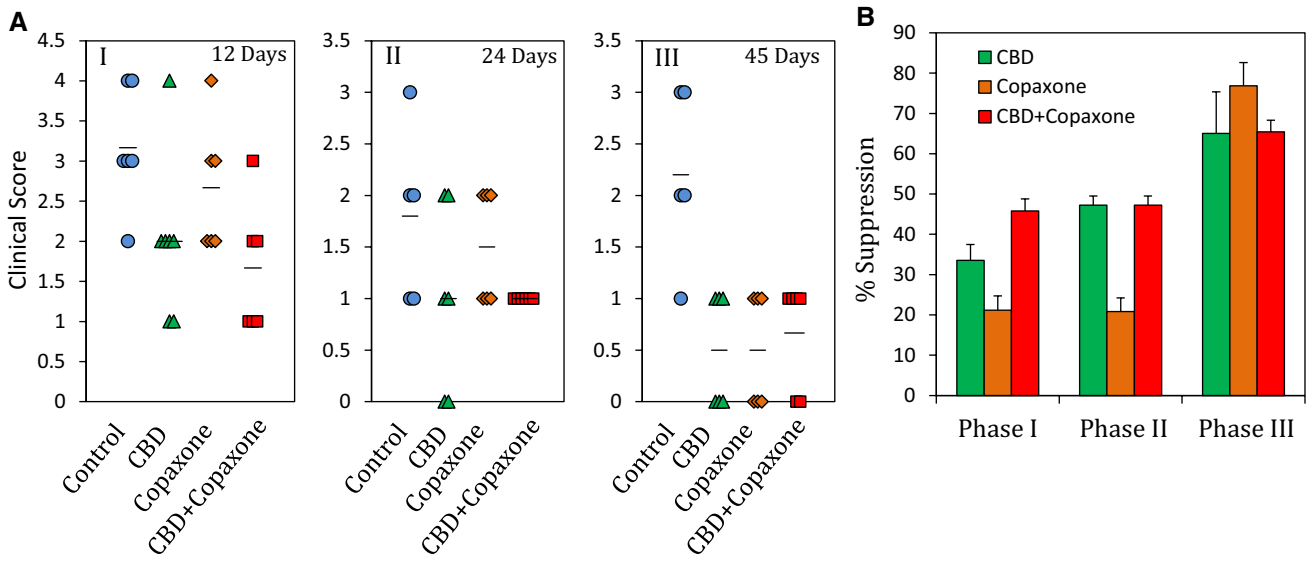

Fig. 3 **a** The graphs present the clinical scores of individual mice from the experiment presented in Fig. 2. The results from the peak of each relapse phase (I, II and III) are given. The lines represent the average. **b** The % of suppression achieved by each drug in the three relapse phases is given. The data are calculated from days 11–13 of phase I, days 24–26 of phase II and days 42–46 of phase III. In phase I, $p<0.05$ for CBD vs Copaxone and $p<0.05$ for CBD + Copaxone vs CBD, and in phase II, $p<0.03$ for CBD vs Copaxone. No differences were observed between the three treatment groups in phase III

symptoms by 33.5 ± 3.9% ($p<0.002$), Avidekel extracts by 40.3 ± 2.7% ($p<0.001$) while Copaxone only by 21.1 ± 3.5% ($p<0.006$) at the average. During days 24–26 of phase II, CBD suppressed the symptoms by 47.2 ± 2.2% ($p<0.01$), Avidekel extracts by 39.7 ± 2.6% ($p<0.03$), while Copaxone still only by 20.8 ± 3.4% ($p<0.05$) at the average. During days 42–46 of phase III, all three drugs showed strong suppression. During this phase, CBD suppressed the symptoms by 65.0 ± 10.3% ($p<0.0001$), Avidekel extracts by 80.0 ± 8.0% ($p<0.0001$), and Copaxone by 76.8 ± 5.7% ($p<0.0001$) at the average (Figs. 2, 3, 4, 5). Concurrent administration of CBD with Copaxone provided in general similar suppressive effects as CBD alone, with a slightly higher suppression during phase I ($p<0.05$) (Figs. 2, 3, 4, 5). Also, combined treatment of Avidekel extracts with Copaxone had in general similar suppressive effects as Avidekel alone, with a slightly higher suppression during phase II ($p<0.05$) (Figs. 4, 5). One of the 8 mice in the control group died in phase I, and three other control mice died in phase III. One of the 8 mice in the CBD-treated group died in phase I; all other mice survived. Altogether, our data show that CBD and Avidekel extracts are efficient in relieving EAE symptoms, and may, thus, be potential drugs in combined MS therapy.

Discussion

There are still no treatments that can cure MS patients. Since the main mechanism of injury appears to be inflammation, the drugs used for relapsing forms of MS usually target various parts of the immune system that aim to dampen the inflammation. Current drugs approved for relapsing forms of MS include interferon-β, Copaxone, mitoxantrone, natalizumab and fingolimod (Reich et al. 2018). Sativex, an oromucosal spray containing Δ^9-THC and CBD at a ratio of approximately 1:1, has been used to treat MS-related spasticity with improved quality of life (Giacoppo et al. 2017). The drawback of Δ^9-THC is its euphoric effects. CBD does not have psychotropic effects, but as a single agent, it usually gives a bell-shaped dose–response (Gallily et al. 2015), which makes it difficult to reach an optimal dose. Therefore, many attempts have been made to develop medical Cannabis subspecies with low Δ^9-THC content, while retaining the therapeutic benefits of Cannabis. One such species is Avidekel which contains high levels of CBD (18%), while very low levels of Δ^9-THC (1%) (Gallily et al. 2015). In contrast to

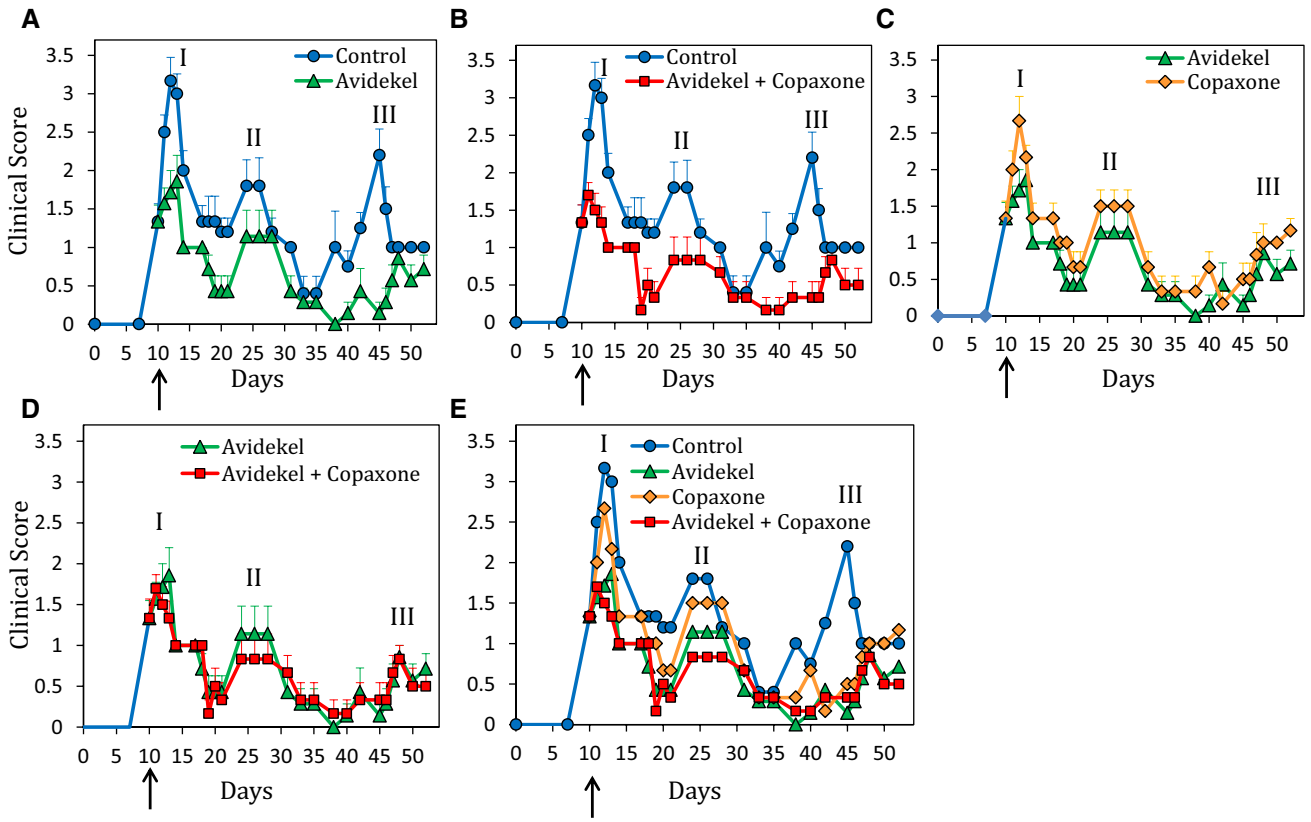

Fig. 4 Avidekel extract was at least as efficient as Copaxone to relieve EAE symptoms. EAE was induced by $PLP_{139–151}$ and at day 10 (indicated by an arrow), when the first neurological signs (Score 1) were observed, the mice were treated daily five times a week with Avidekel extracts, Copaxone alone or in combination. Each treatment group comprised 6–8 mice. The graphs represent the average of data obtained from a representative experiment. **a** Comparison of Avidekel with Control mice. **b** Comparison of Avidekel + Copaxone with control mice. **c** Comparison of Copaxone with Avidekel-treated mice. **d** Comparison of Avidekel + Copaxone with Avidekel-treated mice. **e** The four treatment groups (Control, Avidekel, Copaxone and Avidekel + Copaxone) are presented together. During days 11–13 of phase I: $p < 0.001$ for Avidekel vs control; $p < 0.006$ for Copaxone vs control; $p < 0.05$ for Avidekel vs Copaxone; $p < 0.001$ for Avidekel + Copaxone vs Copaxone. During days 24–26 of phase II: $p < 0.03$ for Avidekel vs control; $p < 0.17$ for Copaxone vs control; $p < 0.05$ for Avidekel vs Copaxone; $p < 0.002$ for Avidekel + Copaxone vs Copaxone and $p < 0.05$ for Avidekel + Copaxone vs Avidekel. During days 42–46 of phase III: $p < 0.0001$ for Avidekel, Copaxone, Avidekel + Copaxone vs control. In phase III there was no difference between the three treatment groups

purified CBD, Avidekel extracts provide a correlative dose response, with stronger effects upon increasing dosages. In addition to its anti-inflammatory properties, Avidekel also exerts anti-pain activity and causes relaxation. Both effects are beneficial for many severe disease conditions.

CBD is known for its strong anti-inflammatory effects (Burstein 2015; Gallily et al. 2015; Malfait et al. 2000; Weiss et al. 2008), and has recently been shown to have beneficial effects on EAE (Rahimi et al. 2015). Avidekel was shown to have strong anti-inflammatory as well as anti-nociceptive activities in an inflammatory mouse model (Gallily et al. 2015). Therefore, it was of high interest to study its ability to suppress EAE clinical symptoms. Both CBD and Avidekel extracts at the dosages given were more efficient than Copaxone during relapse phases I and II, while having similar strong suppressive effects during relapse phase III. This suggests for different therapeutic kinetics of these drugs. The immunosuppressive effect of Copaxone was achieved at a relative late time-point, while CBD and Avidekel extracts caused immediate relief. Upon prolonged treatment, the suppressive effects were more pronounced for all three drugs as seen by higher suppression in phase III in comparison to phases I and II. The combined treatment of CBD or Avidekel extracts with Copaxone in general did not increase the suppression above the one achieved with CBD or Avidekel alone, except for some periods were the suppression was slightly enhanced. Importantly, there were no antagonistic effects between CBD/Avidekel extracts and Copaxone, as was observed by Rahimi et al. for the combined treatment of CBD with palmitoylethanolamide (PEA)

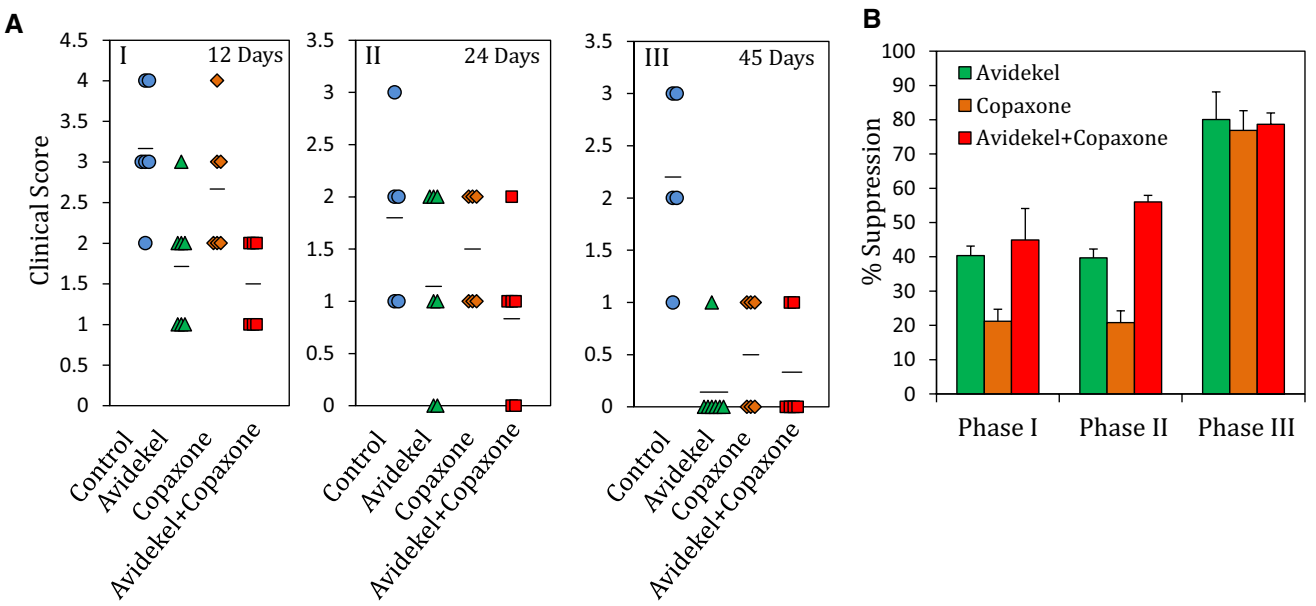

Fig. 5 **a** The graphs present the clinical scores of individual mice from the experiment presented in Fig. 4. The results from the peak of each relapse phase (I, II and III) are given. The lines represent the average. **b** The % of suppression achieved by each drug in the three relapse phases is given. The data are calculated from days 11–13 of phase I, days 24–26 of phase II and days 42–46 of phase III. In phase I, $p < 0.05$ for Avidekel vs Copaxone; and in phase II, $p < 0.05$ for Avidekel vs Copaxone and $p < 0.05$ for Avidekel + Copaxone vs Avidekel. No differences were observed between the three treatment groups in phase III

(Rahimi et al. 2015). Altogether, our study demonstrates strong immunosuppressive activities of CBD and Avidekel extracts that might be beneficial for MS patients. We, therefore, propose to combine Avidekel extracts with current treatment protocols.

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