Convolvulus pluricaulis extract can modulate synaptic plasticity in rat brain hippocampus

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The memory-boosting property of Indian traditional herb, *Convolvulus pluricaulis*, has been documented in literature; however, its effect on synaptic plasticity has not yet been reported. Two important forms of synaptic plasticity known to be involved in the processes of memory formation are long-term potentiation (LTP) and long-term depression (LTD). In the present study, the effect of *C. pluricaulis* plant extract on LTP and LTD were evaluated. The adult male Wistar rats were fed orally with 250, 500 and 1000 mg/kg of this extract for 4 weeks and the effect was determined on LTP and LTD in the Schaffer collaterals of the hippocampal cornu ammonis region CA1. We found that the 500 mg/kg dose of the extract could significantly enhance LTP compared to the vehicle treated ones. Moreover, the same dose could also reduce LTD while used in a separate set of animals. Also, a fresh group of animals treated with the effective dose (500 mg/kg) of plant extract were examined for memory retention in two behavioral platforms namely, contextual fear conditioning (CFC) and novel object recognition test (NORT). Increased fear response to the conditioned stimulus and enhanced recognition of objects were observed in CFC and NORT, respectively, both indicating strengthening of memory. Following up, ex-vivo electrophysiology experiments were performed with the active single molecule scopoletin, present in *C. pluricaulis* extract and similar patterns in synaptic plasticity changes were obtained. These findings suggest that prolonged treatment of *C. pluricaulis* extract, at a specific dose in healthy animals, can augment memory functions by modulating hippocampal plasticity.

NeuroReport 2020, **31**:597–604

Keywords: *Convolvulus pluricaulis*, contextual fear conditioning, hippocampus, memory, novel object recognition test, synaptic plasticity

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Received 15 February 2020 **Accepted** 29 February 2020

Introduction

Dementia or memory loss is a common problem among elderly individuals. Weakening of synaptic communication leads to gradual cognitive or memory impairment. The hippocampus is a major component of the limbic system present beneath the cerebral cortex of most vertebrates and in the medial temporal lobe in primates. Extensive studies on rodents have suggested that the hippocampus has been involved in performing critical functions like consolidating short-term to long-term memory and spatial memory formation, enabling navigation. It has been considered that long-lasting activity dependent changes in the efficiency of synaptic transmission in the mammalian brain are of central importance for the development of neural circuitry and storing information. The most reliable model for such changes has been long-term potentiation (LTP) and long-term depression (LTD) in the hippocampus [1]. The formation of memory involves experience dependent changes in the synaptic strength. These changes can exist as a form of persistent enhancements (LTP) or prolonged reduction (LTD) of synaptic transmission. Different areas of the hippocampus serve different functions viz. spatial memory, verbal memory and fear conditioning [2,3].

Discovery of drugs improving cognitive functions in healthy individuals is currently a topic of considerable scientific interest. In the Ayurveda system of traditional Indian medicine, various plant based medicinal preparations have been used to enhance cognitive function. *Convolvulus pluricaulis*, a perennial herb commonly known as 'Shankhpushpi', known to possess therapeutic benefits, has been reported in the Ayurveda literature for its memory enhancing potential, as well as in several preclinical and clinical studies [4–8]. One of the effects of the plant is thought to be on its cholinergic properties [4,9]. However, no effect demonstrating modulation of hippocampal synaptic plasticity including LTP or LTD, which are considered to play pivotal roles in learning and memory [10,11], has been reported. Notably, hippocampal LTP and LTD are regulated by *N*-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptors [1,12,13].

In the present study, the effect of chronic dosing of *C. pluricaulis* extract on hippocampal LTP and LTD in

DOI: 10.1097/WNR.0000000000001446

normal adult rats have been evaluated. We determined the effective dose, which modified hippocampal synaptic plasticity, and also assessed whether this dose could enhance memory and learning using two different models for studying animal behavior viz., novel object recognition test (NORT) and contextual fear conditioning (CFC) [14,15]. Finally, we tested through ex-vivo bath application of compound whether the active ingredient scopoletin can elicit similar positive modulations in synaptic plasticity [16].

Methods

Animals

Male Wistar rats, 4 weeks old and 100–120 g body weight were used in this study for dosing purposes. The animals were housed in groups of 3–4 in each ventilated cage at a temperature (around 25°C) and humidity (30–70%) controlled room with 12 h light and dark cycles. Food and water were provided *ad libitum*. All the study protocols were approved by the Institutional Animal Ethics Committee of TCG Lifesciences Pvt. Ltd., Kolkata, India.

Plant extract and chemicals

The dry extract of *C. pluricaulis* was obtained from Phyto Concentrates Pvt. Ltd., Ahmedabad, India. The chemical components of *C. pluricaulis* plant extract along with their molecular weights have already been reported in literature. LCMS finger printing of the extract was also performed more than once. Analysis of the LCMS spectra (-Q1) showed the presence of peaks which coincide with molecular weights of the extract components and the results are reproducible. The analysis of our plant extract showed peaks at 191, 175 and 227 representing molecular weights of scopoletin, ayapanin and myristic acid. These components are reported in the literature to be present in *C. pluricaulis* extract. All other chemicals were procured from Sigma-Aldrich (St. Louis, Missouri, USA).

Dosing

The *C. pluricaulis* extract was suspended in distilled water and dosed by oral gavage once daily for 4 weeks. Three different doses, viz. 250, 500 and 1000 mg/kg body weight, and in a dose volume 5 mL/kg, were used. The control animals (vehicle set) were treated with distilled water. For electrophysiological studies, six animals in each group and for behavioral experiments, ten animals in each group, were used.

Stock solutions of scopoletin were prepared in 0.5% dimethyl sulfoxide as required and bath-applied immediately [for 5 min duration, pre-high frequency stimuli (HFS)/low-frequency stimulation (LFS)].

Hippocampal slice preparation and electrophysiology study

The animals were sacrificed 24 h after the last dose by decapitation under deep anesthesia using halothane. Since the scopoletin solution was bath-applied, dosing was not required for the single molecule experiments. The brain was quickly removed and immersed in an ice-cold artificial cerebrospinal fluid (ACSF) solution (pH 7.4) containing: 118 mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_3$, 10 mM glucose, 1.2 mM NaH_2PO_4, 1.3 mM $MgCl_2$, 2.5 mM $CaCl_2$, for 1 min, under constant saturation with carbogen (95% O_2 and 5% CO_2). Then the brain was taken out, the hippocampi were carefully extracted and 400 µm thick transverse slices were prepared in the horizontal plane using tissue chopper (The Mickle Laboratory Engineering Co Ltd., Surrey, UK). The slices were collected in a holding chamber, where they were allowed to recover for 1.5 h at 30°C in ACSF saturated with carbogen. Following recovery, the slices were placed in the recording chamber, where they were continuously perfused with carbogenated ACSF at a flow rate of 2 mL/min at 30°C. Synaptic responses were evoked by stimulating the Schaffer collateral pathway (emanating from the CA3 region) using a bipolar platinum-iridium electrode (FHC, Maine, USA). The extracellular recording electrode, made of glass micropipette and filled with ACSF was placed in the 'stratum radiatum' of the CA1 region. The extracellular field excitatory post-synaptic potential (fEPSP) slope value was recorded using LTP software (WinLTP, Ver 1.11, UK) [17]. A test pulse (single current pulse of 100 µs duration at 0.033 Hz) was delivered to evoke a fEPSP. Once a response was evoked, the stimulus intensity was increased gradually to generate an input/output curve until a population spike was generated. The stimulus intensity was then decreased until it evoked 50–60% of the maximal response and this intensity was used for generating a stable baseline for the whole experiment. The evoked responses were amplified using a high-impedance differential AC amplifier (A-M Systems, Washington, USA) and digitized at 10 kHz A/D rate (National Instruments, Austin, Texas, USA). Once a stable baseline is obtained for 10–15 min, LTP or LTD is evoked using the following protocols.

LTP was induced by delivering HFS (a train of 100 pulses in 1 s that is, at 100 Hz; repeated three times at 10 s interval) at baseline stimulation intensity. Following HFS, recording was continued using test pulse for 45 min to determine the extent of LTP generated. The percentage LTP refers to the percentage increase in the fEPSP slope values during the last 5 min of the recording period compared to the 5-min period just preceding HFS.

LTD was induced by delivering LFS (a single train of 900 pulses in 15 min, i.e, at 1 Hz). Following LFS, recording was continued using test pulse for 45 min to determine the extent of LTD generated. The percentage LTD refers to the percentage reduction in the fEPSP slope values during the last 5 min of the recording period compared to the 5-min period just preceding LFS.

Separate groups of animals were used for LTP and LTD recordings to avoid any interference in the generation of synaptic plasticity.

Contextual fear conditioning

CFC is an associative learning task in which an animal learns to fear a harmless stimulus (conditioned stimulus) because of its association with an aversive condition (unconditioned stimulus). When the animal is re-exposed to only conditioned stimulus and no unconditioned stimulus, it demonstrates a fear response, such as, freezing, as it remembers or associates that environment with the aversive stimulus. The extent of freezing reflects the degree of fear memory. CFC was carried out in a fear conditioning chamber (Coulbourn Instruments, USA). CFC consisted of two sessions, training and testing. A day prior to training, the animals were acclimatized to the chamber for 5 min. During training each animal was placed inside the conditioning chamber for 5 s for context conditioning (conditioned stimulus). Following training, a foot-shock (unconditioned stimulus) of 1.5 mA for 2 s was delivered for conditioned stimulus-unconditioned stimulus pairing. Thirty seconds thereafter, the animal was placed back into its home cage and the conditioned stimulus chamber was cleaned with 70% alcohol. Twenty-four hours after the conditioning session, each animal was re-exposed to the conditioning chamber, for 5 min, during which no shock was delivered (only conditioned stimulus). During this period, the freezing time was recorded. Freezing behavior is defined as the absence of visible movement of the body except for respiratory-related movements.

Novel object recognition test

NORT is used to evaluate episodic memory in rodents based on the difference in exploration time for a novel object vs. a familiar one. In the present experiment, effect of the plant extract was evaluated in a time-induced memory deficit model of NORT, which involves gradual loss of memory with the passing of time. The experiment was carried out in a square-shaped animal exploration arena made of Plexiglass (50 × 50 × 40 cm). A 3-day protocol consisting of three sessions – acclimatization, familiarization and recognition, was done. On the first day, animals were allowed to freely explore the empty arena for 5 min in the absence of objects. On the second day during familiarization, two similar objects were placed in the box, and exploration of each object was recorded for 5 min. On the third day, the animals were exposed to one familiar and one novel object and allowed to explore for 5 min, and the exploration time for each object was again noted. The arena and the objects were thoroughly cleaned with 70% alcohol to remove any odor related cues. The memory readout is expressed in percentage of the object exploration time as a proportion of the total time spent in exploring the two objects. Due to the time gap between familiarization and recognition, untreated animals fail to discriminate between the two objects on the third day.

Separate groups of animals were used for CFC and NORT studies in order to avoid any interference in animal behavior.

Statistics

The data was analyzed using MS-Excel (Ver 2002) and GraphPad Prism (Ver 5). All data, presented as mean ± SEM, were compared by means of one-way analysis of variance with Dunnett's post-hoc test (for normally distributed data) or unpaired *t*-tests (two-group comparisons) with $P < 0.05$ considered statistically significant.

Results

Electrophysiological studies

Effect of Convolvulus pluricaulis extract on hippocampal long-term potentiation

Following 4 weeks of treatment with *C. pluricaulis* extract, hippocampal slices from sacrificed animals were prepared and LTP was generated by HFS (Fig. 1a). In all groups there was a robust and stable increase in fEPSP amplitudes following HFS, as demonstrated by the time-course profile and they remained stable about 45 min post-stimulus (Fig. 1a). The LTP values in the groups of animals treated with 250, 500 and 1000 mg/kg *C. pluricaulis* extract were found to be 53.2 ± 12.2%, 77.8 ± 16.2% and 60.9 ± 9.8%, respectively, while compared to the vehicle treated (37.5 ± 5.2%) animals. There was increase in LTP in all doses compared to the vehicle treated group; however, it was maximum at 500 mg/kg dose (Fig. 1c).

Effect of Convolvulus pluricaulis extract on hippocampal long-term depression

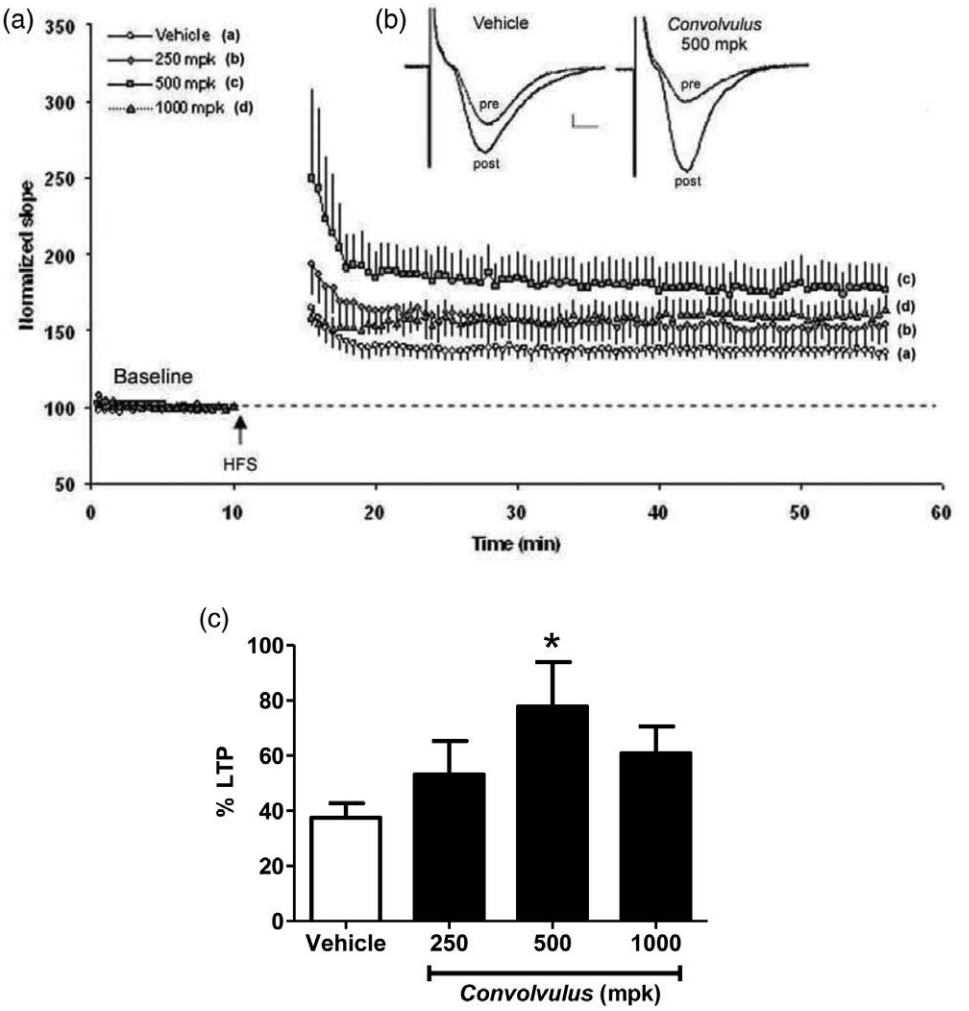
Following 4 weeks of dosing with *C. pluricaulis* extract, four groups (three extract treated and one vehicle treated) of animals were sacrificed, hippocampal slices were prepared and LTDs were generated by LFS (Fig. 2a). In all plant extract treated groups, LTD values decreased as compared to the vehicle treated group (37.4 ± 4.5%). The LTD values were reduced to 30.1 ± 3.8%, 22.7 ± 3.0% and 34.6 ± 5.2% with a dose of 250, 500 and 1000 mg/kg, respectively. However, the 500 mg/kg dose generated a significantly reduced LTD value with respect to the vehicle treated group (Fig. 2c).

Behavioral studies

Effect of Convolvulus pluricaulis extract on freezing behavior in contextual fear conditioning test

To determine the fear memory following 4 weeks of treatment with the vehicle and 500 mg/kg *C. pluricaulis* extract, animals were kept in the CFC chamber and the foot shock was applied. These two groups of animals were again kept in the CFC chamber next day, but no foot shock was given. It was found that the plant extract treated animals had significantly more fear memory compared to

Fig. 1



Effect of *Convolvulus pluricaulis* extract on hippocampal LTP at the CA1 area. (a) Time course profile of LTP at different doses of *Convolvulus* extract as shown by different symbols designated by (a), (b), (c), (d). (b) Representative waveforms. Scale bars: 0.2 mV, 10 ms. Pre, represents pre-HFS waveform at the end of baseline recording; post, represents post-HFS waveform towards the end of recording. (c) Percentage LTP expressed in mean ± SEM values. Percentage LTP refers to the percentage increase in the fEPSP slope values during the last 5 min of the recording period compared to the 5 min period just preceding HFS. Vehicle treated slices (n=12 from 6 animals, 2 slices per animal) and *Convolvulus* extract treated slices (n=8–11, from 6 animals, ~ 2 slices per animal). *P<0.05 with respect to Vehicle group, Dunnett's post-hoc test following a one-way ANOVA. ANOVA, analysis of variance; CA, cornu ammonis, HFS, high-frequency stimulation; LTP, long-term potentiation, mpk, milligrams per kilogram.

the vehicle treated group, as demonstrated by the extent of freezing behavior (Fig. 3). The vehicle treated group showed 24.2 ± 5.1% freezing whereas the 500 mg/kg of extract treated group showed 44.7 ± 8.6% freezing.

Effect of Convolvulus pluricaulis extract on object recognition memory in novel object recognition test

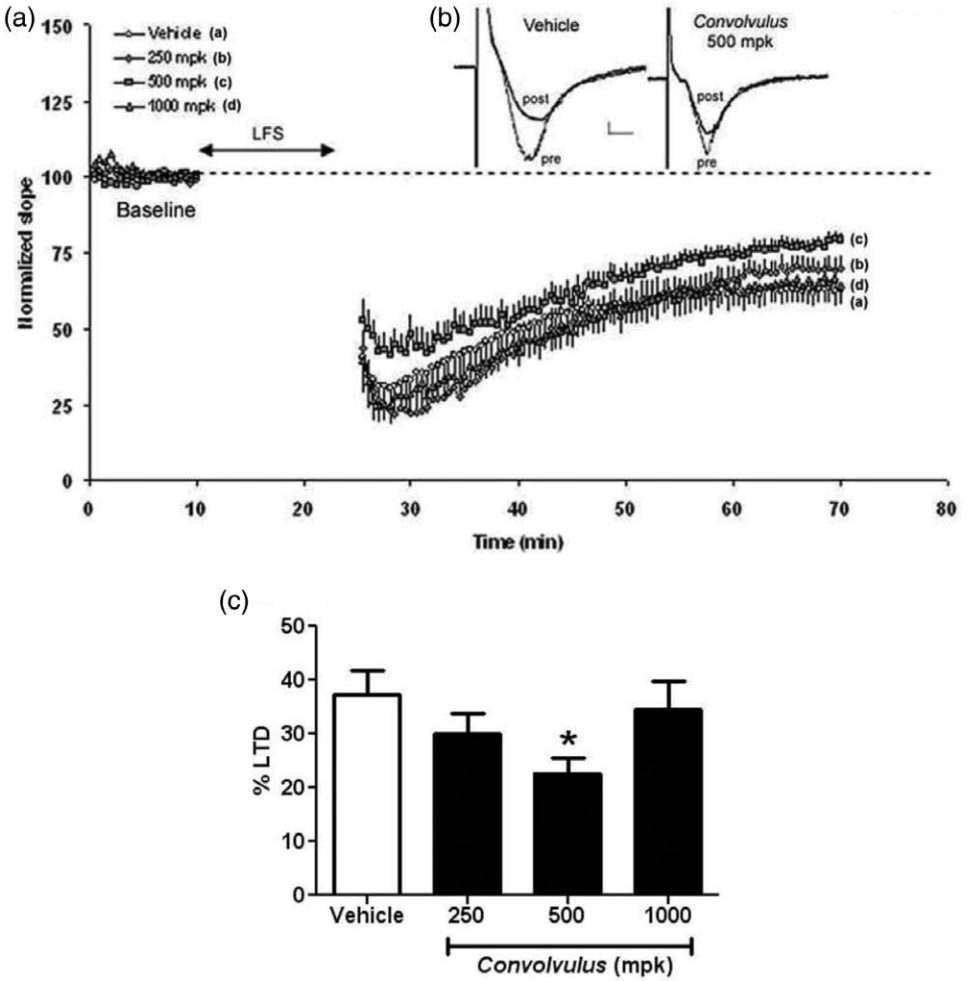
To determine the object recognition memory following 4 weeks of treatment, the vehicle and 500 mg/kg *C. pluricaulis* extract treated animals were exposed to the NORT arena where two similar objects were kept. Next day, one of the familiar objects was replaced with a novel one. On both the days, the extent to which the animals explored the objects was noted. It was found that on the first day both the vehicle and extract-treated animals explored the similar objects to an equal extent (Fig. 4a). On the next day, the extract-treated animals explored the novel object for significantly higher time (66.8 ± 5.8%), compared to the vehicle-treated group (43.1 ± 7.1%, Fig. 4b). The data suggest that the pretreatment of animals with the plant extract helps to retain object memory compared to the vehicle-treated control animals (Fig. 4b).

Ex-vivo single molecule electrophysiological studies

Effect of scopoletin on hippocampal long-term potentiation

Following electrophysiological and behavioral studies with the *C. pluricaulis* extract, the effect of the active

Fig. 2



Effect of *Convolvulus pluricaulis* extract on hippocampal LTD at the CA1 area. (a) Time-course profile of different doses of *Convolvulus* extract as shown by different symbols designated by (a)–(d) on LTD at the CA1 hippocampal area. (b) Representative waveforms, Scale bars: 0.2 mV, 10 ms. Pre represents pre-LFS waveform at the end of baseline recording and post represents post-LFS waveform towards the end of recording. (c) Percentage LTD expressed in mean ± SEM values. Percentage LTD refers to the percentage reduction in the fEPSP slope values during the last 5 min of the recording period compared to the 5-min period just preceding LFS. Vehicle treated slices (n=11 from 6 animals, ~2 slices per animal) and *Convolvulus* extract treated slices (n=11–12, from 6 animals, ~ 2 slices per animal). *P<0.05 with respect to vehicle group, Dunnett's post-hoc test following a one-way ANOVA. ANOVA, analysis of variance; CA, cornu ammonis; LTD, long-term depression, LFS, low-frequency stimulation, mpk, milligrams per kilogram.

molecule scopoletin was tested on hippocampal LTP. Scopoletin was bath applied (5 min pre-HFS) and LTP recording carried out as per the protocol mentioned above. In both the tested groups, there was a robust and stable increase in fEPSP amplitudes following HFS (Fig. 5). The LTP values in the groups of slices treated with 5 and 10 µM scopoletin were 74.5 ± 13.3% and 109.1 ± 21.8%, respectively, a marked increase compared to the vehicle treated animal group (37.5 ± 5.2%), and comparable with the effect of the 500-mg/kg extract dose (77.8 ± 16.2%).

Effect of scopoletin on hippocampal long-term depression

Following electrophysiological and behavioral studies with the *C. pluricaulis* extract, the effect of scopoletin was tested on hippocampal LTD. Scopoletin was bath applied (5 min pre-LFS) and LTD recording carried out as per the protocol mentioned above. In both the tested groups, there was a stable decrease in fEPSP amplitudes following LFS (Fig. 6). The LTD value in the groups of slices treated with 5 µM scopoletin was 16.6 ± 2.2%, a marked decrease compared to the vehicle treated animal group (37.4 ± 4.5%), and comparable with the 500 mg/kg extract dose (22.7 ± 3.0%). In case of 10 µM, the response returned to pre-LFS baseline values resulting in no LTD.

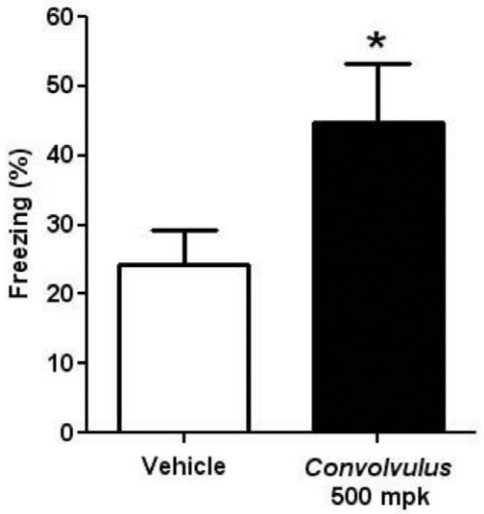
Discussion

C. pluricaulis is known as a traditional Indian medicine for enhancing memory. Although preclinical and a few

clinical studies in the past decades have validated the use of this plant as a nootropic agent [4–7], its mechanism of action and role on synaptic communication has not been studied. Interestingly, it has been shown that the extract of a similar nootropic medicinal plant *Bacopa monnieri* enhances LTP [18]. In the present study, we demonstrate for the first time that long-term treatment of healthy rats with *C. pluricaulis* extract leads to significant alteration in hippocampal synaptic plasticity viz. augmentation of LTP and diminution of LTD.

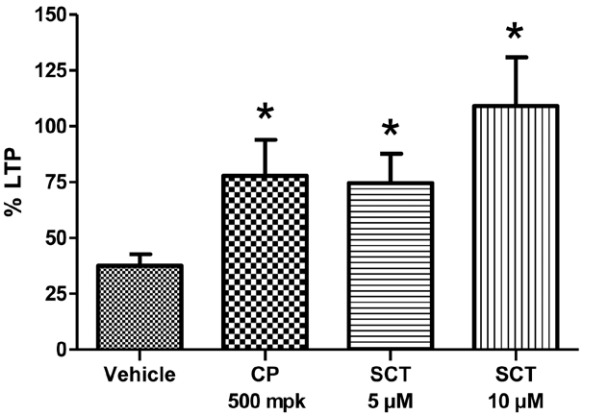
It has already been established that the synaptic plasticity processes, such as, LTP and LTD in the hippocampus, are important mechanisms that underlie the process of memory formation [19–21]. Traditionally LTP, by strengthening synaptic communication, is considered to play the key role in memory storage in the hippocampus. The role of LTD, as reported in literature, is equivocal. There are reports which suggest that it plays a role in forgetting or loss of memory due to factors like stress, there are other reports which suggest that it is required for memory formation since continuous potentiation of

Fig. 3



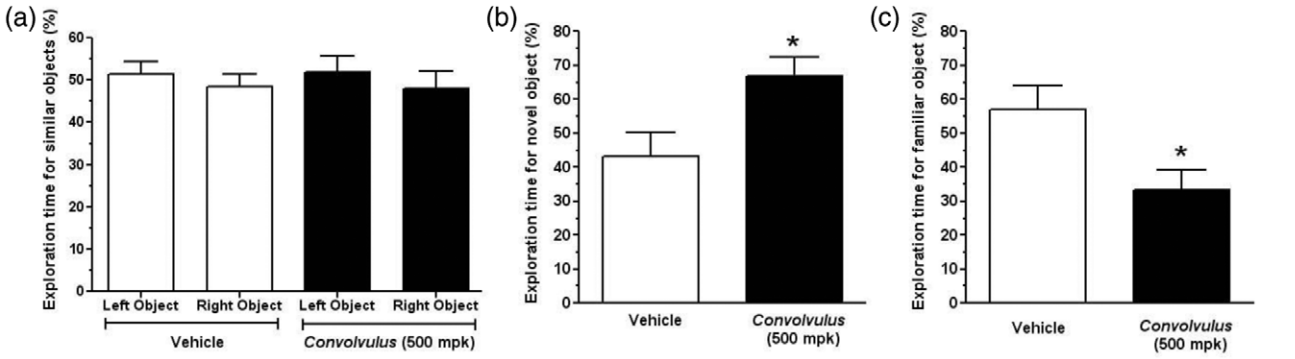
Effect of *Convolvulus* extract on freezing behavior in the CFC test. Bars represent the extent of freezing in the vehicle and extract-treated animal groups. Data represents mean ± SEM (n=10 animals). *P<0.05, unpaired t-test. CFC, contextual fear conditioning; mpk, milligrams per kilogram.

Fig. 5



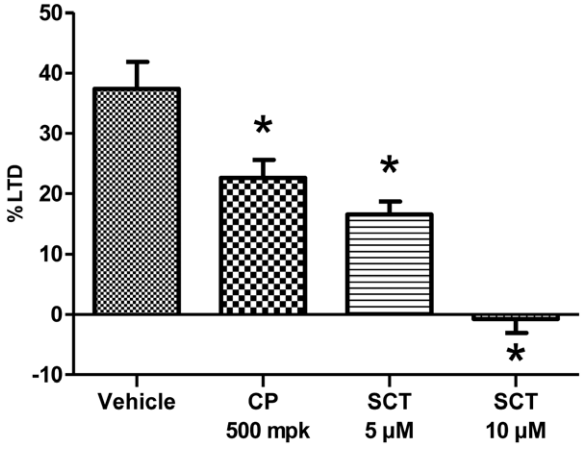
Effect of scopoletin on hippocampal LTP at the CA1 area. Bars represent the percentage LTP expressed in mean ± SEM values. Percentage LTP refers to the percentage increase in the fEPSP slope values during the last 5 min of the recording period compared to the 5 min period just preceding HFS. Scopoletin treated slices (n=8 from 4 animals, ~2 slices per animal). *P<0.05, unpaired t-test. CA, cornu ammonis; HFS, high-frequency stimulation; LTP, long-term potentiation, mpk, milligrams per kiligram.

Fig. 4



Effect of *Convolvulus* extract on exploration of the novel object in NORT. (a) Bars represent the extent of exploration of similar objects during the familiarization phase in the vehicle and extract treated animal groups. (b) Bars represent the extent of exploration of novel object during the recognition phase. (c) Bars represent the extent of exploration of familiar object during the recognition phase. Data represents Mean ± SEM (n=10 animals). *P<0.05, unpaired t-test. mpk, milligrams per kilogram; NORT, novel object recognition test.

Fig. 6



Effect of scopoletin on hippocampal LTD at the CA1 area. Bars represent the percentage LTD expressed in mean ± SEM values. Percentage LTD refers to the percentage reduction in the fEPSP slope values during the last 5 min of the recording period compared to the 5 min period just preceding LFS. Scopoletin treated slices (n=4–9 from 4 animals, ~2 slices per animal). *P<0.05, unpaired t-test. CA, cornu ammonis; LTD, long-term depression; LFS, low-frequency stimulation; mpk, milligrams per kilogram.

the synapse may lead to its inactivation through fatigue [21,22]. In the present study, we observed that the treatment with the plant extract for 4 weeks led to an increase in LTP and decrease of LTD, the percent decrease of LTD being less than half of the percent increase in LTP. Therefore, it is clear that LTP is the dominant phenomenon in our study.

Our results show that 4 weeks of treatment of *C. pluricaulis* extract, at 500 mg/kg dose increased LTP around two-fold compared to vehicle treatment. The U-shaped dose-response effect seen here has also been observed previously in scopoletin-treated behavioral experiments in mice [23]. This enhancement in synaptic transmission at the crucial CA3:CA1 synapse in the hippocampus can be considered to have played a seminal role in augmenting the cognitive capacities of the animals as seen in our behavioral tests for memory. It has been reported that *C. pluricaulis* treatment can enhance memory in both young and old experimental animals and also in human subjects [4,5]. A clinical trial utilizing the Weschler Memory Scale has also observed marked improvement in neuropsychological parameters demonstrating enhancement of memory in young individuals [7]. Thus, putting together our findings and available literature, we put forward that the nootropic effects of *C. pluricaulis* extract could be through its modulation of hippocampal synaptic plasticity.

Compounds scopoletin and ayapanin have been isolated from *C. pluricaulis* but only the former exhibited memory-enhancing activity [8]. A myristic acid analog also has been reported to have a role in learning in larval zebrafish [24]. Interestingly, an earlier study has demonstrated that scopoletin can amplify hippocampal LTP *in vitro* [16]. It also acts as an inhibitor of acetylcholinesterase [25]. This is important since loss of cholinergic transmission leads to cognitive deterioration and reducing the activity of the enzyme acetylcholinesterase will allow the neurotransmitter acetylcholine to continue to regulate synaptic signal transduction. Scopoletin also lowers the oxidative stress factor, reduces inflammation and inhibits monoamine oxidases, which increases the survival of neurons of the limbic system [26]. These are highly desirable in working towards a therapeutic for neurodegenerative Alzheimer's dementia [27]. In light of these findings, we decided to perform ex-vivo bath-application experiments (LTP and LTD) with scopoletin where the compound comes in direct contact with the hippocampal slices. The results obtained were promising and affected synaptic plasticity in a positive way. Future studies should focus on determining whether such components of the extract, either singly or in combination have synaptic modulatory potential and also the target(s) through which such pro-cognitive effects are elicited.

In order to ascertain whether the plant extract can really enhance cognitive abilities, we chose the dose which has shown best results in our electrophysiology studies, and applied in two different behavioral models of learning and memory, viz. CFC and NORT. While CFC is a paradigm for testing fear-induced memory formation [14], NORT evaluates spontaneous recognition memory of objects [15]. Though the role of hippocampus in NORT is debated, reports show that pathways underlying object recognition memory involve the medial dorsal thalamus, cortex (medial prefrontal, entorhinal, perirhinal) and hippocampus with the hippocampus playing a major role once the critical threshold of object exploration is reached [28]. Here, we must mention that since the route of administration of the extract being followed is oral and hence systemic; its effect may not be restricted to a particular region. So it is possible that other brain regions, in addition to the hippocampus, can contribute to the behavioral activity. Our results show that in both CFC and NORT tests, *C. pluricaulis* treated animals displayed enhanced cognition.

Conclusion

In conclusion, we showed by electrophysiology experiments using hippocampal slices that the *C. pluricaulis* extract modulates synaptic plasticity in the hippocampal CA1 region. The most effective dose also causes significant strengthening of memory across two different types of behavioral platforms. Taken together, our study validates the nutraceutical property of *C. pluricaulis* and implicates enhancement of synaptic communication at the hippocampal CA3:CA1 interface as an underlying mechanism. These directions of the study are

scientifically significant and provide the platform to discover new nootropic compounds.

Acknowledgements

The electrophysiological platforms and animal models used in this study were developed and validated at TCG Lifesciences Pvt. Ltd., Kolkata, India. R.D. is extremely grateful to seniors Dr. Rajib Barik, Dr. Chaitali Dhar, Mr. Sanjay Kumar, Dr. Rammohan Bera, Dr. Manish Banerjee, Dr. Subrata Chattopadhyay and Mr. Swapan Bhattacharya for their timely encouragement and assistance in advancing the study. The funding for this study has been provided by TCG Lifesciences Pvt. Ltd., Kolkata, India.

R.D.: performing the experiments, analysis of data and initial drafting of the manuscript.

T.S.: initial planning of the project and intellectual inputs while drafting of the manuscript. S.R.: drafting and critical review of the manuscript. S.C.: imparting knowledge on the techniques of electrophysiology and animal behavior, planning of electrophysiological studies, statistical analysis and review of manuscript. J.R.: planning and overall supervision of the study, intellectual input and critical review of the manuscript.

Conflicts of interest

There are no conflicts of interest.

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