

Enhanced dendritic arborization of hippocampal CA3 neurons by *Bacopa monniera* extract treatment in adult rats

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Abstract

Objective: *Bacopa monniera* (BM), a traditional Ayurvedic medicine has been used in treatment for a number of disorders, particularly those involving anxiety, intellect and poor memory. The current study examined the effects of standardized extract of *Bacopa monniera* on the dendritic morphology in adult rats of hippocampal CA3 neurons, one of the regions concerned with learning and memory. **Materials and Methods:** Adult Wistar (2.5-month-old) rats were designated into 2-, 4- and 6-week treatment groups. Rats in each of these groups were divided into 20 mg/kg, 40 mg/kg and 80 mg/kg dose groups (n=8 for each dose). These rats along with age-matched control rats were then subjected to spatial learning (T-maze) and passive avoidance tests. Subsequent to the T-maze and passive avoidance tests, these rats were killed by decapitation, brains were removed and hippocampal neurons were impregnated with silver nitrate (Golgi staining). Hippocampal CA3 neurons were traced using camera lucida. Dendritic branching points (a measure of dendritic arborization) and dendritic intersections (a measure of dendritic length) were quantified. These data were compared with control rats. **Results and Conclusions:** The results showed improvement in spatial learning performance and enhanced memory retention in rats treated with BM extract. There was a significant increase in the dendritic intersections and dendritic branching points along the length of both apical and basal dendrites in rats treated with BM extract for four and six weeks. However, the rats treated with BM extract for two weeks did not show any significant change in hippocampal CA3 neuronal dendritic arborization. We conclude that constituents present in BM extract have neuronal dendritic growth stimulating properties.

Keywords: *Bacopa monniera*, spatial learning, passive avoidance, hippocampal CA3 neurons, dendritic arborization, memory.

Introduction

Herbs, a principal form of medicine in developing countries, are becoming popular throughout the developing and developed world. In western societies, there is increasing interest in herbal medicines, which are often perceived as a more 'natural' and 'soft' treatment compared to synthetic drugs [1]. Before the development of modern medicine, people relied on a large arsenal of natural remedies for the treatment of central nervous system (CNS) related maladies. The medicinal plants and/or their constituents which have been widely used for their reputed effectiveness in functions of CNS are as follows: *Acorus calamus*, *Bacopa monniera*, *Celastrus paniculatus*, *Centella asiatica*, *Clitoria ternatea*, *Convolvulus pluricaulis*, *Ginkgo biloba*, *Nardostachys jatamansi*, *Scutellaria baicalensis*, *Withania somnifera*.

Bacopa monniera (Linn.), syn. *Herpestis monniera* (Linn.), a member of the *Scrophulariaceae* family, is a small, creeping herb with numerous branches, small oblong leaves, and light purple flowers. The genus *Bacopa* includes over 100 species of aquatic herbs. It commonly grows in wet, marshy areas throughout India, Nepal, Sri Lanka, China, Taiwan, and Vietnam;

and is also found in Florida and other southern states of the USA. The herb can be found at elevations from sea level to altitudes of 4400 feet [2–6]. Flowers and fruits appear in summer and the entire plant is used medicinally [7].

This medicinal plant is popularly known as Brahmi. The name Brahmi is derived from the word "Brahma", the mythical "creator" in the Hindu pantheon. Because the brain is the centre for creative activity, any compound that improves brain health is called Brahmi. The plant has been used in Indian folklore as a nerve tonic [8].

In the ancient Indian system of medicine, viz., "Ayurveda", *Bacopa monniera* (BM) has been classified under "Medhya rasayana", i.e., medicinal plants rejuvenating intellect and memory. The ancient classical Ayurvedic treatises, viz., "Charak samhita", "Susruta samhita", and "Astanga hridaya", have prescribed BM for the promotion of memory, intelligence, and general performance. Therefore, this plant has been investigated in several laboratories in India for its neuropharmacological effect [9–11]. Its traditional memory-enhancing claim has been established experimentally in several animal experimental models of learning [12–15]. There

is no evidence which shows the effect of this plant extract on the brain regions involved in learning and memory, namely the hippocampus [16–18], amygdala and limbic cortex. The cornu ammonis (CA) region, particularly the CA3 sub region of the hippocampus, is the key structure of the brain involved in learning and memory [19–22]. The current study examined the effects of standardized extract of BM on the dendritic morphology of hippocampal CA3 neurons in adult rats.

Materials and Methods

Animals and experimental groups

Wistar albino rats of random sex, approximately 2.5-month-old and weighing about 150–200 g were obtained from “the central animal house”, Manipal University, Manipal, India. The total number of animals used for the study was 120 (72 experimental rats and 48 control rats). The experimental protocol was approved by the institutional animal ethical committee for experimental clearance IAEC/KMC/02/2005–2006. The rats were fed “Amrut rat and mice pellet” manufactured by Pranav Agro Industries Ltd., E/5–6, M.I.D.C., Kupwad Block, SANGLI – 416436 (Maharashtra), India. Four rats were housed in each polypropylene cage and maintained in a 12:12 hour cycle of dark and light.

Three time intervals (two, four or six weeks) were used and each time group was divided into dose groups ($n=8$ for each dose). Each dose group was fed 20 mg/kg, 40 mg/kg and 80 mg/kg of standardized extract of BM daily for two, four or six weeks. Age-matched normal control group (NC) and a gum acacia vehicle control group (GAC) ($n=8$ in both groups) were also maintained for each time period.

Extraction and administration of BM

Standardized plant extract of BM was supplied by the herbal manufacturer, M/s. Natural Remedies Private Limited, Bangalore, India. The shelf life of this extract is 2 years.

The first step was extraction of the botanically identified plant material with alcohol. The alcoholic extract was then re-extracted with water and the water-soluble matter was retained. The final re-extract was concentrated and dried to make a powder. Phytochemical analysis revealed that the final extract contained approximately 10% w/w (10% of the total mass of the extract) of the active ingredients (Bacosides A and B) by high-performance liquid chromatography (HPLC) and high-performance thin layer chromatography (HPTLC).

The BM extract was administered orally along with 5% gum acacia, using an oral feeding tube and syringe.

Behavioral tests

Following treatment, all groups (NC, GAC, and BM) were subjected to behavioral tests during the night (starting at 7 PM). The behavioral tests consisted of a spatial learning (T-maze) test and a passive avoidance test and were done as detailed earlier [23].

Rapid Golgi staining procedure

Subsequent to the behavioral tests, these rats were anesthetized with ether, sacrificed by cervical dislocation and the brains were removed. Each cerebral hemisphere was cut coronally to two equal pieces, cortex was removed from the posterior part and hippocampi were dissected (but were not compared) and fixed in rapid Golgi fixative. Tissue was processed for rapid Golgi staining as detailed previously [24]. Briefly, tissues were fixed for 5 days in Golgi fixative, and impregnated with 1.5% aqueous silver nitrate solution for 48 hours. Sledge microtome sections of 120 μ m thicknesses were cut (as many serial sections as possible in the tissue), dehydrated, cleared and mounted with Distrin plasticizer xylene mounting media.

Camera lucida tracing

8–10 hippocampal CA3 neurons of each rat were traced using camera lucida from the slide, and their dendritic branching points and dendritic intersections were quantified. Both right and left side hippocampal CA3 neurons were used. Neurons with minimal overlap of dendrites, heavily impregnated with silver nitrate and without truncate dendrites, were selected for tracing.

Quantification of dendritic branching points and dendritic intersections

The concentric circle method of Sholl DA (1956) was used for dendritic quantification [25]. Five concentric circles on a transparent sheet with a radial distance of 20 μ m between them were used for dendritic quantification (dendritic branching points and intersections). The sheet was placed on a neuron tracing such that the center of the cell body of the neuron coincided with the center of the concentric circles. The number of branching points between the two concentric circles, i.e. within each successive 20 μ m concentric zone (ring), was counted. The dendritic intersection is the point where a dendrite intersects the given concentric circle (Figure 1).

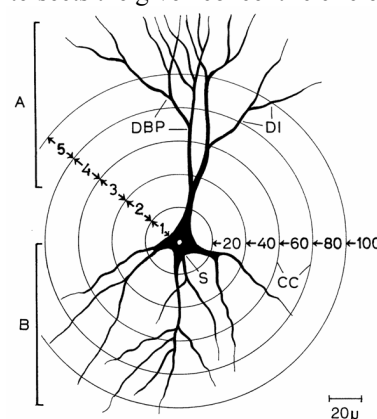


Figure 1 – Diagram showing a hippocampal CA3 neuron and the scheme of dendritic quantification. A – Apical dendrites, B – Basal dendrites, DBP – Dendritic branching points, DI – Dendritic intersections, S – Soma, CC – Concentric circles, and 1 to 5 – Concentric zones.

The dendritic intersections at each concentric circle were counted. Both branching points and intersections were counted up to a radial distance of 100 μ m from the

center of the soma. Mean number of dendritic branching points in each concentric zone and number of dendritic intersections at each concentric circle were calculated. This method of scoring was applied for both apical and basal dendritic quantification. The apical dendrite of pyramidal neuron in CA3 region is a single pole extending from the apex of soma. It divides into 2–3 main branches from which the secondary and tertiary branches arise. The basal dendrites of pyramidal neurons arise from several places along the base of the soma and these repeatedly branch producing a dense tuft (Figure 1).

Statistical analysis

Data was analyzed using analysis of variance (ANOVA) followed by Bonferroni's test (*post hoc*) using GraphPad Prism, version 2.01.

Results

The rats treated with all doses of BM showed improvement in spatial learning performance and enhanced memory retention compared to normal control rats [23]. Briefly, when treated for a longer duration (four and six weeks), rats showed significant improvement in their learning behavior in all (20, 40, and 80 mg/kg) dose groups. During spatial learning T-maze tests, they showed an increased number of alternations and decreased percentage bias during spontaneous alternation test and increased percentage of correct responses during rewarded alternation test. In the passive avoidance tests, there was no significant change in behavior during exploration. However, during the retention test, rats treated for four and six weeks at all three doses (20, 40, and 80 mg/kg) spent less time in the smaller compartment, suggesting improved memory retention [23].

Hippocampal CA3 neuronal dendritic quantification

Hippocampal CA3 neuronal dendritic analyses in BM extract (40 and 80 mg/kg) treated rats (four and six weeks) showed a significant increase in dendritic length and branching both in the apical and basal dendrites (Figures 2–11).

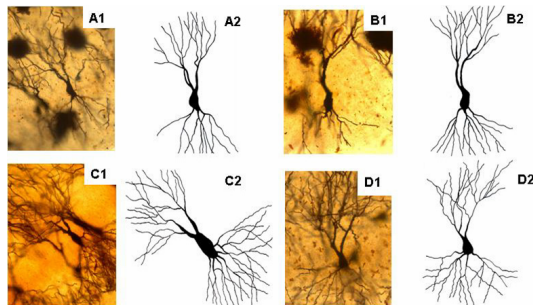


Figure 2 – Photomicrographs (A1, B1, C1, D1) and camera lucida tracings (A2, B2, C2, D2) of Golgi-stained hippocampal CA3 neurons from control rats (A1, A2) and rats treated with BM for four weeks at doses of 20 mg/kg (B1, B2), 40 mg/kg (C1, C2), and 80 mg/kg (D1, D2). A significant increase in dendritic arborization in BM 40 and 80 mg/kg groups is demonstrated.

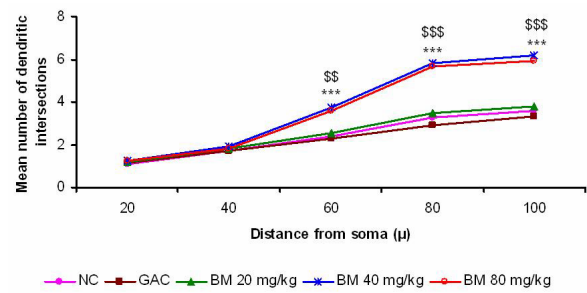


Figure 3 – Apical dendritic intersections of hippocampal CA3 neurons in rats treated with BM for four weeks, control and gum acacia rats. NC vs. BM 40 mg/kg: *** $p < 0.001$; NC vs. BM 80 mg/kg: $^{ss}p < 0.01$, $^{sss}p < 0.001$.

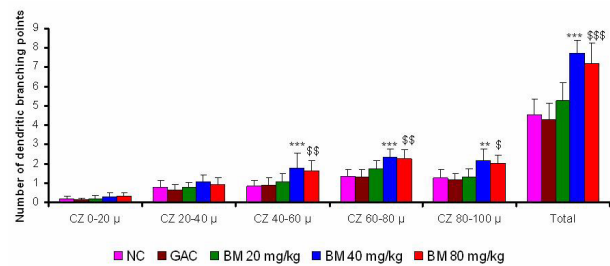


Figure 4 – Apical dendritic branching points of hippocampal CA3 neurons in rats treated with BM for four weeks, control and gum acacia rats at different concentric zones (CZ) and total number of branching points. Each value represents the mean + standard deviation of 8–10 neurons from each rat. NC vs. BM 40 mg/kg: ** $p < 0.01$, *** $p < 0.001$; NC vs. BM 80 mg/kg: $^s p < 0.05$, $^{ss}p < 0.01$, $^{sss}p < 0.001$.

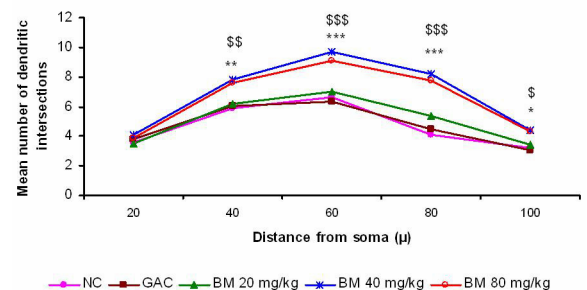


Figure 5 – Basal dendritic intersections of hippocampal CA3 neurons in rats treated with BM for four weeks, control and gum acacia rats. NC vs. BM 40 mg/kg: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; NC vs. BM 80 mg/kg: $^s p < 0.05$, $^{ss}p < 0.01$, $^{sss}p < 0.001$.

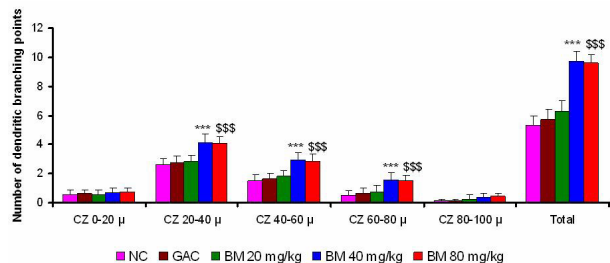


Figure 6 – Basal dendritic branching points of hippocampal CA3 neurons in rats treated with BM for four weeks, control and gum acacia rats at different concentric zones (CZ) and total number of branching points. Each value represents the mean + standard deviation of 8–10 neurons from each rat. NC vs. BM 40 mg/kg: *** $p < 0.001$; NC vs. BM 80 mg/kg: $^{sss}p < 0.001$.

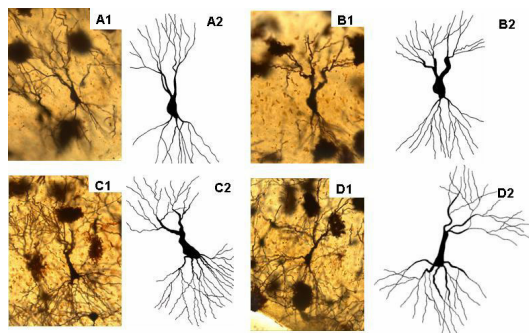


Figure 7 – Photomicrographs (A1, B1, C1, D1) and camera lucida tracings (A2, B2, C2, D2) of Golgi-stained hippocampal CA3 neurons from control rats (A1, A2) and rats treated with BM for six weeks at doses of 20 mg/kg (B1, B2), 40 mg/kg (C1, C2), and 80 mg/kg (D1, D2). A significant increase in dendritic arborization in BM 20, 40 and 80 mg/kg groups is demonstrated.

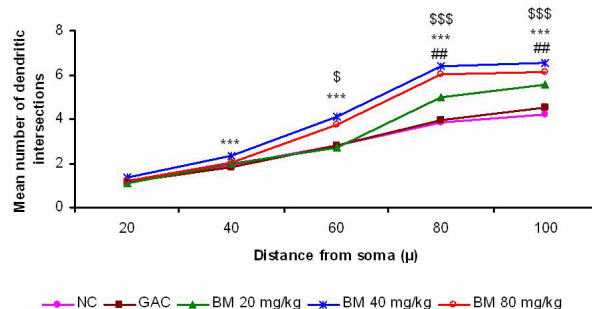


Figure 8 – Apical dendritic intersections of hippocampal CA3 neurons in rats treated with BM for six weeks, control and gum acacia rats. NC vs. BM 20 mg/kg: $^{\#}p<0.01$; NC vs. BM 40 mg/kg: $^{***}p<0.001$; NC vs. BM 80 mg/kg: $^{sss}p<0.05$, $^{ss}p<0.001$.

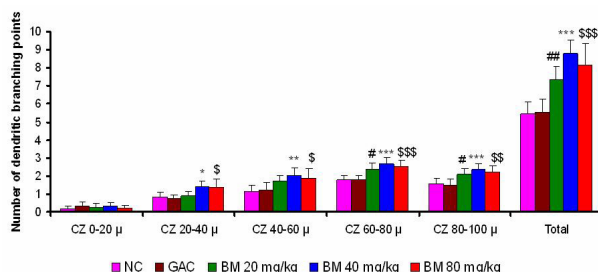


Figure 9 – Apical dendritic branching points of hippocampal CA3 neurons in rats treated with BM for six weeks, control and gum acacia rats at different concentric zones (CZ) and total number of branching points. Each value represents the mean + standard deviation of 8–10 neurons from each rat. NC vs. BM 20 mg/kg: $^{\#}p<0.05$, $^{##}p<0.01$; NC vs. BM 40 mg/kg: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$; NC vs. BM 80 mg/kg: $^{s}p<0.05$, $^{ss}p<0.01$, $^{sss}p<0.001$.

Treatment with all doses (20, 40 and 80 mg/kg) for two weeks did not alter the dendritic arborization. There was no difference in dendritic length and branching pattern between control and gum acacia treated rats, suggesting that daily handling of the rats (handling stress and vehicle) itself did not alter dendritic pattern. Since there was no significant difference in the dendritic length and branching between the control and vehicle groups, only comparisons between the control and experimental groups are detailed.

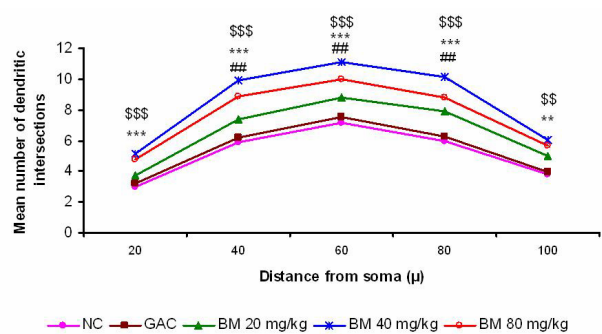


Figure 10 – Basal dendritic intersections of hippocampal CA3 neurons in rats treated with BM for six weeks, control and gum acacia rats. NC vs. BM 20 mg/kg: $^{\#}p<0.01$; NC vs. BM 40 mg/kg: $^{**}p<0.01$, $^{***}p<0.001$; NC vs. BM 80 mg/kg: $^{ss}p<0.01$, $^{sss}p<0.001$.

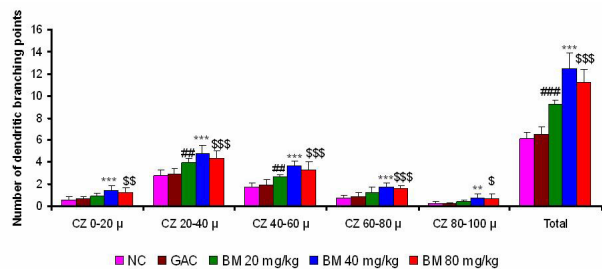


Figure 11 – Basal dendritic branching points of hippocampal CA3 neurons in rats treated with BM for six weeks, control and gum acacia rats at different concentric zones (CZ) and total number of branching points. Each value represents the mean + standard deviation of 8–10 neurons from each rat. NC vs. BM 20 mg/kg: $^{\#}p<0.01$, $^{##}p<0.001$; NC vs. BM 40 mg/kg: $^{**}p<0.01$, $^{***}p<0.001$; NC vs. BM 80 mg/kg: $^{s}p<0.05$, $^{ss}p<0.01$, $^{sss}p<0.001$.

Four weeks treatment

Apical dendritic intersections (Figure 3)

No significant change in dendritic intersections/concentric circle was noted in BM 20 mg/kg group when compared to normal control group. Both BM 40 and 80 mg/kg groups showed a significant increase in dendritic intersections at 60, 80 and 100 μ m concentric circles (60 μ m concentric circle: 2.41 ± 0.39 in normal control group vs. 3.76 ± 0.51 in 40 mg/kg group, $p<0.001$ and 3.59 ± 0.88 in 80 mg/kg group, $p<0.01$, 80 μ m concentric circle: 3.28 ± 0.60 in normal control group vs. 5.84 ± 0.69 in 40 mg/kg group, $p<0.001$ and 5.66 ± 0.73 in 80 mg/kg group, $p<0.001$, 100 μ m concentric circle: 3.59 ± 0.72 in normal control group vs. 6.20 ± 0.76 in 40 mg/kg group, $p<0.001$ and 5.93 ± 0.68 in 80 mg/kg group, $p<0.001$).

Apical dendritic branching points – At different concentric zones (Figure 4)

No significant change was observed in the dendritic branching points in any of the concentric zones in BM 20 mg/kg group. However both BM 40 and 80 mg/kg groups showed significant increase in dendritic branching points in 40–60, 60–80 and 80–100 μ m concentric zones (40–60 μ m concentric zone: 0.87 ± 0.27 in normal control group vs. 1.81 ± 0.74 in 40 mg/kg group, $p<0.001$ and

1.65±0.51 in 80 mg/kg group, $p<0.01$, 60–80 μm concentric zone: 1.38±0.33 in normal control group vs. 2.36±0.41 in 40 mg/kg group, $p<0.001$ and 2.27±0.45 in 80 mg/kg group, $p<0.01$, 80–100 μm concentric zone: 1.26±0.45 in normal control group vs. 2.17±0.62 in 40 mg/kg group, $p<0.01$ and 2.03±0.40 in 80 mg/kg group, $p<0.05$).

Total number of dendritic branching points

There was no significant change in the total number of branching points in BM 20 mg/kg group when compared to normal control group. However, in both BM 40 and 80 mg/kg groups there was a significant increase in the total number of branching points (4.52±0.85 in normal control group vs. 7.71±0.69 in 40 mg/kg group, $p<0.001$ and 7.21±1.05 in 80 mg/kg group, $p<0.001$).

Basal dendritic intersections (Figure 5)

There were no significant changes in the dendritic intersections at any of the concentric circles in BM 20 mg/kg group when compared with the normal control group. Both BM 40 and 80 mg/kg groups showed significant increase in dendritic intersections at 40, 60, 80 and 100 concentric circles (40 μm concentric circle: 5.86±0.81 in normal control group vs. 7.81±1.16 in 40 mg/kg group, $p<0.01$ and 7.58±1.24 in 80 mg/kg group, $p<0.01$, 60 μm concentric circle: 6.63±1.08 in normal control group vs. 9.67±1.32 in 40 mg/kg group, $p<0.001$ and 9.12±1.19 in 80 mg/kg group, $p<0.001$, 80 μm concentric circle: 4.13±0.72 in normal control group vs. 8.18±0.76 in 40 mg/kg group, $p<0.001$ and 7.74±0.83 in 80 mg/kg group, $p<0.001$, 100 μm concentric circle: 3.21±0.61 in normal control group vs. 4.43±0.62 in 40 mg/kg group, $p<0.05$ and 4.32±0.74 in 80 mg/kg group, $p<0.05$).

Basal dendritic branching points – At different concentric zones (Figure 6)

No significant changes were observed in the dendritic branching points at any of the concentric zones in BM 20 mg/kg group when compared to normal control group. However, both BM 40 and 80 mg/kg groups showed a significant increase in dendritic branching points in concentric zone 20–40 μm (2.63±0.39 in normal control group vs. 4.17±0.52 in 40 mg/kg group, $p<0.001$ and 4.11±0.42 in 80 mg/kg group, $p<0.001$), concentric zone 40–60 μm (1.53±0.42 in normal control group vs. 2.97±0.46 in 40 mg/kg group, $p<0.001$ and 2.83±0.53 in 80 mg/kg group, $p<0.001$), concentric zone 60–80 μm (0.53±0.27 in normal control group vs. 1.57±0.50 in 40 mg/kg group, $p<0.001$ and 1.48±0.39 in 80 mg/kg group, $p<0.001$).

Total number of branching points

There was no significant change in the total number of branching points in BM 20 mg/kg group when compared to normal control group. However, the total number of branching points was found significantly increased in BM 40 and 80 mg/kg groups (5.37±0.57 in normal control group vs. 9.72±0.73 in 40 mg/kg group, $p<0.001$ and 9.63±0.55 in 80 mg/kg group, $p<0.001$).

Six weeks treatment

Apical dendritic intersections (Figure 8)

BM 20, 40 and 80 mg/kg groups showed significant increase in dendritic intersections at 80 and 100 μm concentric circles (80 μm concentric circle: 3.83±0.46 in normal control vs. 5.01±0.76 in 20 mg/kg group, $p<0.01$, 6.38±0.67 in 40 mg/kg group, $p<0.001$ and 6.05±0.65 in 80 mg/kg group, $p<0.001$, 100 μm concentric circle: 4.20±0.47 in normal control vs. 5.55±0.79 in 20 mg/kg group, $p<0.01$, 6.55±0.64 in 40 mg/kg group, $p<0.001$ and 6.11±0.68 in 80 mg/kg group, $p<0.001$). BM 40 and 80 mg/kg groups also showed significant increased number of dendritic intersections in 60 μm concentric circle (2.80±0.35 in normal control vs. 4.12±0.84 in 40 mg/kg group, $p<0.001$ and 3.73±0.51 in 80 mg/kg group, $p<0.05$). In addition, BM 40 mg/kg group alone produced significant increased number of dendritic intersections in 40 μm concentric circle (1.90±0.16 in normal control vs. 2.32±0.24 in 40 mg/kg group, $p<0.001$).

Apical dendritic branching points – At different concentric zones (Figure 9)

All the groups treated with BM showed significant increase in the dendritic branching points in 60–80 μm and 80–100 μm concentric zones (60–80 μm concentric zone: 1.76±0.27 in normal control vs. 2.35±0.36 in 20 mg/kg group, $p<0.05$, 2.67±0.35 in 40 mg/kg group, $p<0.001$ and 2.51±0.39 in 80 mg/kg group, $p<0.001$, 80–100 μm concentric zone: 1.56±0.31 in normal control vs. 2.08±0.31 in 20 mg/kg group, $p<0.05$, 2.35±0.34 in 40 mg/kg group, $p<0.001$ and 2.18±0.37 in 80 mg/kg group, $p<0.01$). In addition, BM 40 and 80 mg/kg groups also showed significantly increased number of dendritic branching points in 20–40 μm , 40–60 μm concentric zones (20–40 μm concentric zone: 0.82±0.28 in normal control vs. 1.40±0.35 in 40 mg/kg group, $p<0.05$ and 1.37±0.45 in 80 mg/kg group, $p<0.05$, 40–60 μm concentric zone: 1.15±0.30 in normal control vs. 2.03±0.43 in 40 mg/kg group, $p<0.01$ and 1.87±0.56 in 80 mg/kg group, $p<0.05$).

Total number of dendritic branching points

Total number of branching points was found significantly increased in all the three groups treated with BM (20, 40 and 80 mg/kg) when compared to normal control group (5.46±0.67 in normal control vs. 7.32±0.74 in 20 mg/kg group, $p<0.01$, 8.77±0.75 in 40 mg/kg group, $p<0.001$ and 8.16±1.22 in 80 mg/kg group, $p<0.001$).

Basal dendritic intersections (Figure 10)

BM 20, 40 and 80 mg/kg groups showed significant increase in dendritic intersections at 40, 60 and 80 μm concentric circles (40 μm concentric circle: 5.91±0.56 in normal control vs. 7.40±1.03 in 20 mg/kg group, $p<0.01$, 9.90±0.88 in 40 mg/kg group, $p<0.001$ and 8.88±0.63 in 80 mg/kg group, $p<0.001$, 60 μm concentric circle: 7.15±0.87 in normal control vs. 8.82±0.49 in 20 mg/kg group, $p<0.01$, 11.08±0.82 in 40 mg/kg group, $p<0.001$ and 9.96±1.10 in 80 mg/kg group, $p<0.001$, 80 μm concentric circle: 5.96±0.78 in normal control vs.

7.88±0.89 in 20 mg/kg group, $p<0.01$, 10.15±0.52 in 40 mg/kg group, $p<0.001$ and 8.83±1.49 in 80 mg/kg group, $p<0.001$). In 20 and 100 μm concentric circles both BM 40 and 80 mg/kg groups showed significantly increased number of dendritic intersections (20 μm concentric circle: 2.97±0.62 in normal control vs. 5.12±0.88 in 40 mg/kg group, $p<0.001$ and 4.75±0.44 in 80 mg/kg group, $p<0.001$, 100 μm concentric circle: 3.83±1.02 in normal control vs. 6.03±1.12 in 40 mg/kg group, $p<0.01$ and 5.70±1.08 in 80 mg/kg group, $p<0.01$).

Basal dendritic branching points – At different concentric zones (Figure 11)

All the groups treated with BM showed a significant increase in dendritic branching points in 20–40 μm and 40–60 μm concentric zones (20–40 μm concentric zone: 2.78±0.45 in normal control vs. 3.93±0.41 in 20 mg/kg group, $p<0.01$, 4.81±0.74 in 40 mg/kg group, $p<0.001$ and 4.36±0.64 in 80 mg/kg group, $p<0.001$, 40–60 μm concentric zone: 1.77±0.35 in normal control vs. 2.67±0.19 in 20 mg/kg group, $p<0.01$, 3.67±0.46 in 40 mg/kg group, $p<0.001$ and 3.28±0.70 in 80 mg/kg group, $p<0.001$). In addition, BM 40 and 80 mg/kg groups also showed significantly increased number of dendritic branching points in 0–20, 60–80 and 80–100 μm concentric zones (0–20 μm concentric zone: 0.62±0.23 in normal control vs. 1.45±0.38 in 40 mg/kg group, $p<0.001$ and 1.28±0.39 in 80 mg/kg group, $p<0.01$, 60–80 μm concentric zone: 0.76±0.24 in normal control vs. 1.76±0.36 in 40 mg/kg group, $p<0.001$ and 1.55±0.33 in 80 mg/kg group, $p<0.001$, 80–100 μm concentric zone: 0.22±0.16 in normal control vs. 0.76±0.33 in 40 mg/kg group, $p<0.01$ and 0.66±0.41 in 80 mg/kg group, $p<0.05$).

Total number of branching points

Total number of branching points was found significantly increased in all the three groups treated with BM (20, 40 and 80 mg/kg) when compared to normal control group (6.13±0.53 in normal control vs. 9.21±0.43 in 20 mg/kg group, $p<0.001$, 12.46±1.47 in 40 mg/kg group, $p<0.001$ and 11.26±1.14 in 80 mg/kg group, $p<0.001$).

Discussion

Advanced neuroscience research has shown that the learning process is associated with alterations in the dendritic morphology of the hippocampal [24, 26–28] and amygdaloid [29–32] neurons. Rao BS *et al.* (1993) showed that self-stimulation rewarding experience promotes structural changes in pyramidal neurons of the CA3 region of the hippocampus in adult Wistar rats [24]. An operant conditioning study carried out by Mahajan DA and Desiraju T (1988) on Wistar rat pups of brain-growth-spurt age to assess the plasticity of apical dendritic branching of CA3 pyramidal neurons of the hippocampus revealed a significant increase in the number of branching points of the dendrites of the learning group compared with the other groups [26]. Environmental enrichment promotes structural and behavioral plasticity in the adult brain. Short-term

exposure to an enriched environment enhances dendritic branching points in the hippocampus of rats [27]. There are few reports in the literature correlating enhanced learning and memory with histological changes in brain. *Clitoria ternatea* (commonly called Shankapushpi) aqueous root extract treatment in rats has shown: (i) improved learning and memory (including passive avoidance learning) [33], (ii) increase in dendritic arborization of CA3 neurons of the hippocampus [34], (iii) an increase in the acetylcholine content of the hippocampus [35], and (iv) increase in dendritic arborization of amygdala neurons [31].

A few other herbal extracts have been reported to show similar effects. Fresh leaf extracts of medicinal herbs (reported in Ayurveda to have ‘Medhya’ properties) such as, *Centella asiatica* and *Ocimum sanctum* have been shown to improve learning and memory, correlated with an increase in dendritic arborization of amygdaloid neurons and hippocampal neurons with the former [36], and alterations in cytoarchitecture of neurons of the hippocampus and substantia nigra with the latter [37]. These studies suggest that dendritic arborizations are highly plastic in regions such as the hippocampus and amygdala and that they tend to bring about changes in learning and memory.

In the present study, treatment with all doses (BM 20, 40 and 80 mg/kg) for two weeks did not alter the dendritic arborization. However, treatment with BM 40 and 80 mg/kg for four weeks and with BM extract 20, 40 and 80 mg/kg for six weeks resulted in a significant increase in the dendritic length (dendritic intersections) and branching points. This may be the key neural basis for improved learning and memory in these rats.

Thus, dendritic structural reorganization is the key feature in learning and memory. Such structural alteration is induced by *Bacopa monniera* and a few other herbal extracts: (a) root powder extract of *Withania somnifera* has been shown to protect the hippocampal (CA2 and CA3) neurons undergoing neurodegeneration (due to stress exposure) to 80% [38, 39]; (b) methanol extracts from the dried roots of *Scutellaria baicalensis*, administered intraperitoneally significantly protected CA1 neurons against 10 mm transient forebrain ischemia [40]; (c) *Semecarpus anacardium* protects the hippocampal neurons from stress induced neurodegeneration [38]; (d) Oren-gedoku-to a traditional Chinese drug shown to work against impairment of learning and memory induced by transient cerebral ischemia [41]; (e) Biota, a traditional Chinese herbal medicine affects learning and memory process in the central nervous system and improves the impairment of memory, acquisition and retention disturbances produced by basal forebrain lesions [42]; (f) DX-9386, a traditional Chinese medicine consisting of *Ginseng*, *Polygala*, *Acorus* and *Hoelen* in the ratio of 1:1:25:50 potentiates LTP formation in the hippocampus and is useful in amelioration of learning deficits [43]; (g) *Clitoria ternatea* and jatamansi have also been reported to be excellent memory enhancers [33, 44].

Our findings with BM extract also suggest similar morphological changes in hippocampus. With BM

dendritic length was significantly increased in the hippocampus beyond 40 μm from the cell body both in apical and basal dendrites in the rats which received 40 and 80 mg/kg of BM daily for four and six weeks. However, no significant increase in dendritic length was observed in 20 mg/kg/day group at any time. This suggests that these doses of plant extract were adequate to induce structural changes in these neurons. Naturally, such changes will have a profound effect on the behavior [23] because of the additional dendrites, which are available on these neurons for the formation of new synapses [45]. From the results it can be noted that a significant number of additional dendritic branches are formed in CA3 neurons of BM extract treated rats. This result in more rapid and effective conduction of impulses, which may be one of the reasons for enhanced learning and memory in these rats, reported earlier [23].

Various molecular mechanisms have been proposed for the dendritic enhancement in different parts of the brain *in vivo* and *in vitro* [46–49]. Though we have not tested such a role of BM, it may possess such a stimulative property.

The beneficial effects of BM have been attributed to the active constituent saponin, as Bacoside A [50] and Bacoside B [51]. Bacosides A and B were found to facilitate the capacity for mental retention in rats and were active in both positive and negative reinforcement experiments [13]. The mixture of saponins Bacosides A and B in clinical trials showed facilitatory effect on both memory and learning [15]. The exact mechanism of action of BM could be attributed to a combination of cholinergic modulation [52] and antioxidant effects [53].

☐ Conclusions

The results of our experiment suggest that *Bacopa monniera* extract treatment in rats with higher doses for longer periods induce structural changes in hippocampal CA3 neurons, which improve their learning and memory.

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