

Histological Study of Cerebellar Structural affection by Chronic Microwave Radiation Exposure: A Possible Role for Prophylactic Administration of *Bacopa monniera*

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Abstract: Objectives: To evaluate the ameliorative effect of oral *Bacopa monniera* (BM) extract on cerebellar structural changes secondary to chronic microwave exposure. **Materials and Methods:** The study comprised 30 normal male albino rats equally divided into: Study group I exposed to microwave radiation for 30 minutes/day for 28 days, Study group II given standardized extract of BM in a dose of 40 mg/kg/day for 28 days and were exposed to microwave radiation for 30 minutes/day for 28 days and Control group without any exposure to radiation. Animals were exposed to power density of 170 mWatt/cm² at a fixed day-hour exposure distance of 20±5 cm. All animal were sacrificed by cervical dislocation and cerebellum was extracted, fixed and stained with hematoxylin-eosin (Hx&E) and silver for nerve fibers and cells. **Results:** The Hx & E section in Study group I showed marked areas of vacuolations with smaller, ill-defined orientation of stellate cells and basket cells in the molecular layer. The Purkinje cells are fewer, shrunken, degenerated with focal loss in some areas and replaced by vacuolations. Purkinje cells had dark pyknotic nuclei and surrounded by spaces with focal loss in some areas and vacuolation. The granular cells of the internal granular layer are widely separated, darkly stained, surrounded by astrocytic cells and contains vacuolation. The Golgi cells have pale nuclei and vacuolated cytoplasm. In BM-treated animals; Hx & E section showed that the molecular layer appeared reaching the pia mater and contains stellate and basket cells with minimal vacuolation. The Purkinje cells are absent in some areas, shrunken, vacuolated and degenerating with surrounding vacuolations. Purkinje cells have distorted nuclei, may be present in more than one row in some areas surrounded by spaces and vacuolation. The granular cells of the internal granular layer are dispersed, surrounded by astrocytic cells and contains vacuolation. **Conclusion:** Prophylactic administration of BM extract can ameliorate the cerebellar structural effect of equipments emitting microwaves especially mobile phones whose use became spreading and usually put in contact of the skull so exposing brain directly to its emitted microwaves.

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1.Introduction

The cerebellar cortex is built from four main types of neurons: granule cells; Purkinje cells and two types of inhibitory interneurons; the Golgi cells and the stellate/basket cells. The cortex receives three kinds of extra-cerebellar afferents: the mossy fibres, the climbing fibres, both of which are excitatory, and the diffusely organized mono-aminergic and cholinergic afferents (Smeets, 1998).

Granule cells are small, glutamatergic neurons and are the most numerous elements in the cerebellar cortex. Mossy-fibre terminals (rosettes) contact the short, claw-like dendrites of several granule cells in complex synapses (glomeruli). Axons of granule cells are unmyelinated and ascend towards the superficial, cell-poor molecular layer of the cerebellar cortex, where they typically bifurcate and terminate on dendrites of purkinje cells and interneurons (Mugnaini, 1997).

Purkinje cells are large, gabaergic neurons, which serve as the sole output of the cerebellar cortex. Their myelinated axons terminate on neurons of the cerebellar nuclei and certain brainstem nuclei. The initial ramifications of the purkinje-cell dendritic tree are relatively smooth; their distal branchlets are closely covered with spines. Parallel fibres terminate on the spines of these spiny branchlets. The proximal, smooth branches are innervated by multiple synapses from a single climbing fibre. The dendritic trees of Purkinje cells are flattened and oriented perpendicular to the parallel fibres. The 'lattice structure' of the cerebellar cortex is enhanced by the orientation of dendrites and axons of stellate/basket cells and the collateral plexus of the Purkinje-cell axons, which share the orientation of the purkinje-cell dendritic tree (Nieuwenhuys *et al.*, 1998; Voogd & Glickstein, 1998).

Bacopa monniera (BM, Brahmi: Scrophulariaceae), a traditional ayurvedic medicine, is reported to facilitate learning and memory in normal

rats and inhibit the amnesic effects of scopolamine, electroshock and immobilization stress. BM extracts have been reported to be non-toxic, non-teratogenic and non-mutagenic in rats and monkeys; single and multiple dosing studies in healthy human volunteers have not elicited adverse effects. Additionally, BM has a facilitatory effect on the capacity for mental retention and in treatments for a number of disorders, particularly disorders that involve anxiety, intellect and poor memory. Significant antidepressant activity comparable to that of imipramine has been observed with the Brahmi extract after five days of oral administration, using a rodent model of depression. An anticholinesterase activity has been also demonstrated (Sairam *et al.*, 2002, Saraf *et al.*, 2007, Sumathi *et al.*, 2012, Prisila Dulcy *et al.*, 2012, Sathyanarayanan *et al.*, 2013).

The use of mobile phones is increasing day by day, and a large proportion of users is made up of children and teenagers. Due to the wide and growing use of mobile communication, there is increasing concern about the interactions of electromagnetic radiation with the human organs and, in particular, with the brain. Experimental studies have shown that the radiofrequency electromagnetic radiation (RF-EMR) emitted from the mobile phones can affect the brain in various ways: in particular, effects on cerebral blood flow, blood-brain barrier permeability, oxidant and antioxidant balance, neurotransmitter balance, nerve cell damage, and genomic responses have been reported. There is some concern that short-term memory loss or other cognitive effects may be associated with the use of mobile telephones (Aalto *et al.*, 2006; Tamasidze & Nikolaishvili, 2007; Narayanan *et al.*, 2010, Hao *et al.*, 2013).

The current comparative study aimed to evaluate the effect of chronic microwave exposure on cerebellar structure and the possible ameliorative role of oral *Bacopa monniera* (BM) extract.

2. Materials and Methods

Animals

The study comprised 30 normal healthy growing male albino rats, weighing 100-200 gm. Rats were purchased from the laboratories of Ministry of Agriculture, and kept under standard conditions, temperature 20°C, humidity 60% and 12-hs day/night cycle, and maintained on standard diet and free water supply till the start of study regimens.

Bacopa monniera

Bacopa monniera was provided as BM extract (Becognize, 250 mg cap; (Swanson Superior Herbs Co.; supplied by American Wholesale Co., USA) with 45% bacoside concentration. Dose was calculated according to weight of animal as 40 mg/kg/day of the alcoholic extract of BM (Singh & Dhawan, 1982). The

calculated dose of BM extract was administered orally enveloped by 5% gum acacia, using an oral feeding tube and syringe.

Study Protocol

The animals were divided into the following groups (each in a separate cage) according to diet regimen used:

1. Control group included 10 rats kept on normal diet without any exposure to microwave radiation.
2. Study group I included 10 rats which were kept on normal diet and were exposed to microwave radiation for 30 minutes/day for 28 consecutive days.
3. Study group II (BM group) included 10 rats which were kept on normal diet and given standardized extract of BM in a dose of 40 mg/kg/day for 28 days and were exposed to microwave radiation for 30 minutes/day for 28 consecutive days.

Technique of exposure

Animals were exposed to the transmitter at a fixed day-hour to avoid diurnal variation. The forward power from the transmitter was monitored during the experiment and the standard gain horn was used at the end of the wave guide to keep animals under exposure distance of 20±5 cm. All animals were exposed to power density of 170 mWatt/cm² (Walters *et al.*, 1998).

All animal were sacrificed by cervical dislocation and cerebellum was extracted within 5 minutes and fixed in 10% buffered formalin, (pH 7.8) and, then thin sections (4 µm) were stained with hematoxylin-eosin (HE) (Lamberg & Rothesten, 1978), silver stain for nerve fibers and cells according to Hirano method (Lee & Luna, 1968).

3. Results

There were no gross anatomical changes detected in study groups compared to control group; the cerebellum consisted of two hemispheres and the surface contains many folds which have a core of white matter covered by grey matter.

In Control group; Hx & E section of the cerebellar cortex showed the molecular layer contains basket cells in its lower zone. The Purkinje cells are uniform with the characteristic flask-shaped appearance, well-apparent nuclei and branched dendritic tree directed towards the molecular layer. The cells of the internal granular layer showed evident cerebellar glomeruli with Golgi cells are present at the bases of the Purkinje cells and in the upper surface of the internal granular layer (Fig. 1). Silver stain section for examination of nerve fibers and cells showed the molecular layer contains the stellate and basket cells, and well defined nerve fibers. The Purkinje cell layer consisted of a single row of cells. The internal granular

layer contains densely populated cells with the characteristic cerebellar glomeruli and Golgi cells in its upper zone, (Fig. 2).

In Study group I; Hx & E section of the cerebellar cortex showed marked areas of vacuolations with smaller, ill-defined orientation of stellate cells and basket cells in the molecular layer. The Purkinje cells are fewer, shrunken, degenerated with focal loss in some areas and replaced by vacuolations. The cells of the internal granular layer are smaller and darkly stained with loss of cerebellar glomeruli (Fig. 3). Higher magnification showed that the Purkinje cells have dark pyknotic nucleus, surrounded by spaces and focal loss in some areas and vacuolation. The granular cells of the internal granular layer are widely separated, darkly stained, surrounded by astrocytic cells and contains vacuolation. The Golgi cells have pale nuclei and vacuolated cytoplasm (Fig. 4).

Silver stain section of study group I showed marked areas of vacuolations and ill defined nerve fibers in the molecular layer. The Purkinje cells are present in more than one row, not uniform, shrunken, surrounded by spaces and areas of marked vacuolation with ill-defined nerve processes are seen (Fig. 5).

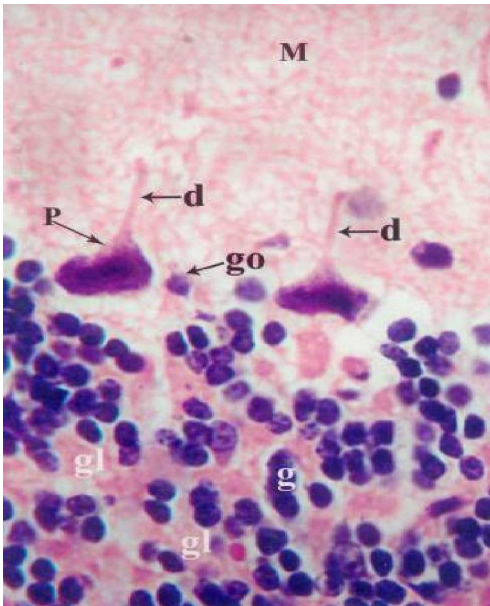


Fig. (1): A light micrograph of a section of the cerebellar cortex of Control group specimens showed the molecular layer (M) contains basket cells in its lower zone. The Purkinje cells (P) are uniform with the characteristic flask-shaped appearance, well-apparent nuclei and branched dendritic (d) tree directed towards the molecular layer. The cells of the internal granular layer showed evident cerebellar glomeruli (gl) with Golgi (go) cells are present at the bases of the Purkinje cells and in the upper surface of the internal granular layer. (Hx & E, 200).

In Study group II (BM group); Hx & E section showed that the molecular layer appeared reaching the pia mater and contains stellate and basket cells with minimal vacuolation. The Purkinje cells are absent in some areas, shrunken, vacuolated and degenerating with surrounding vacuolations. The cells of the internal granular layer are smaller and darkly stained with minimal loss of cerebellar glomeruli (Fig. 6). Higher magnification showed the Purkinje cells have distorted nucleus, may be present in more than one row in some areas surrounded by spaces and vacuolation. The granular cells of the internal granular layer are dispersed, surrounded by astrocytic cells and contains vacuolation (Fig. 7).

Silver stain section showed the molecular layer contains sporadic small areas of vacuolation with defined nerve fibers. The Purkinje cells are present in one row in some areas but in more than one row in other areas. Some Purkinje cells were not uniform, vacuolated and degenerated but others appeared normal with fusiform shape. The cells of the internal granular layer are darkly stained, more dispersed with decreased glomerular pattern (Fig. 8).

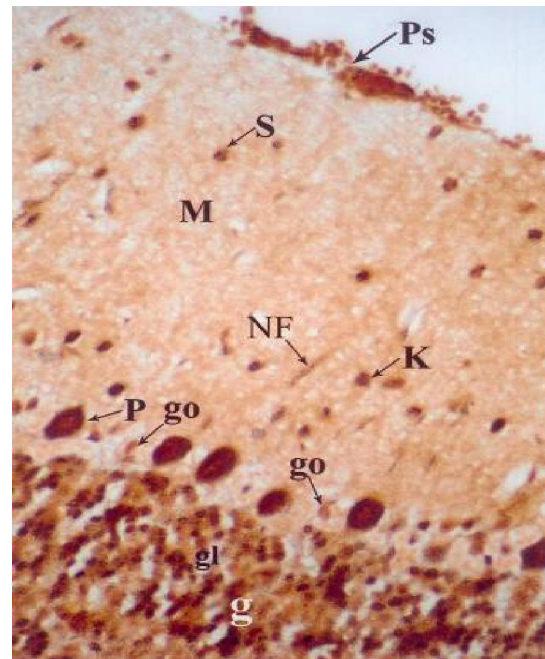


Fig. (2): A light micrograph of a section of the cerebellar cortex of control group showing the molecular layer (M) contains the stellate cells (S), the basket cells (K), and well defined nerve fibers (NF). The Purkinje cell layer (P) consisted of a single row of cells. The internal granular layer (g) contains densely populated cells with the characteristic cerebellar glomeruli (gl) and Golgi cells (Go) in its upper zone. The pial sheath (Ps) is noticed (Silver stain x400).

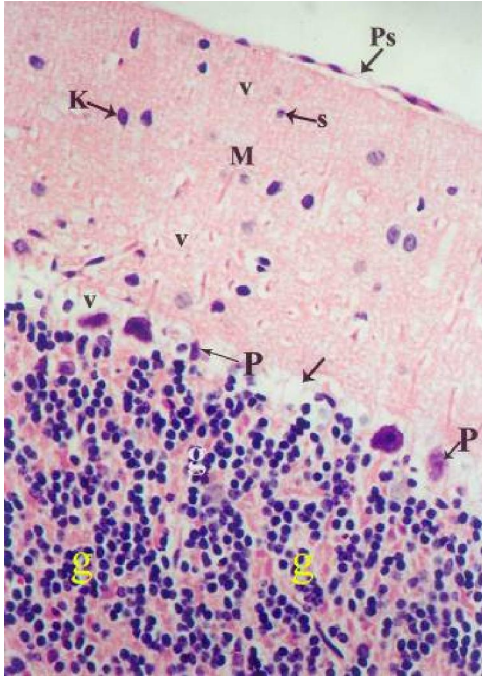


Fig. (3): A light micrograph of a section of the cerebellar cortex of Study group I specimens showed the molecular layer (M) contains marked areas of vacuolations (v) smaller, ill-defined orientation of stellate cells (s) and basket cells (K). The Purkinje cells (P) are fewer, shrunken, degenerated with focal loss in some areas and replaced by vacuolations (v). The cells of the internal granular layer (g) are smaller and darkly stained with loss of cerebellar glomeruli. The pial sheath is vascular (Ps). (Hx & E x200).

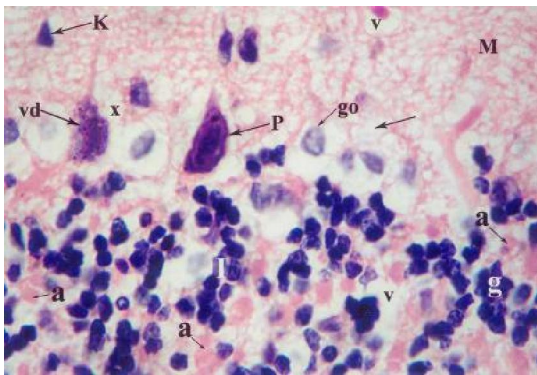


Fig. (4): A light micrograph of a section of the cerebellar cortex of Study group I, the molecular layer (M) contains areas of marked vacuolation (v) and basket cells (K). The Purkinje cells (P) have dark pyknotic nucleus (vd), surrounded by spaces (x) and focal loss in some areas (arrows) and surrounded by vacuolation (v). The granular cells of the internal granular layer (g) are widely separated, darkly stained, surrounded by astrocytic cells (a) and contains vacuolation (v). The Golgi cells (go) have pale nuclei and vacuolated cytoplasm (Hx & E x1000).

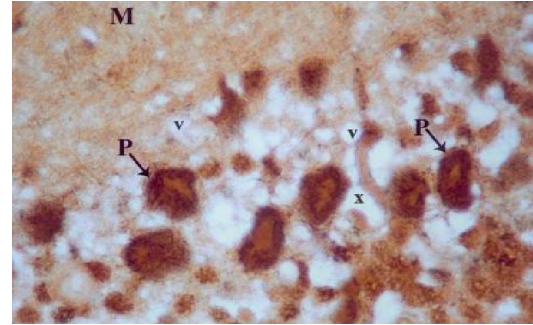


Fig. (5): A light micrograph of a section of the cerebellar cortex of Study group I specimens showed the molecular layer (M) contains marked areas of vacuolations (v) and ill defined nerve fibers. The Purkinje cells (P) are present in more than one row, not uniform, shrunken, surrounded by spaces (x) and areas of marked vacuolation (v) with ill-defined nerve processes are seen. (Silver stain x400).

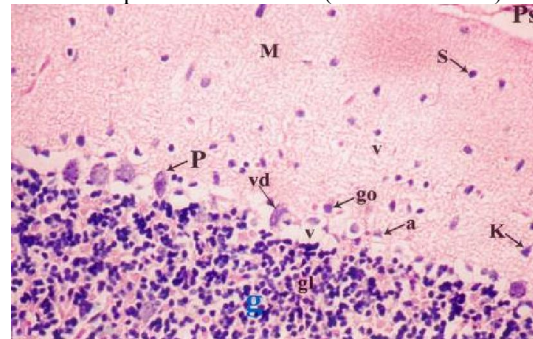


Fig. (6): A light micrograph of a section of the cerebellar cortex of Study group II (BM treated animals) specimens showed the molecular layer (M) contains stellate cells (S), basket cells (K), vacuolations and reaching the pia mater (Ps). The Purkinje cells (P) are absent in some areas, shrunken, vacuolated and degenerating (vd), and surrounded by vacuolations (v). The cells of the internal granular layer (g) are smaller and darkly stained with minimal loss of cerebellar glomeruli (gl) (Hx & E x400).

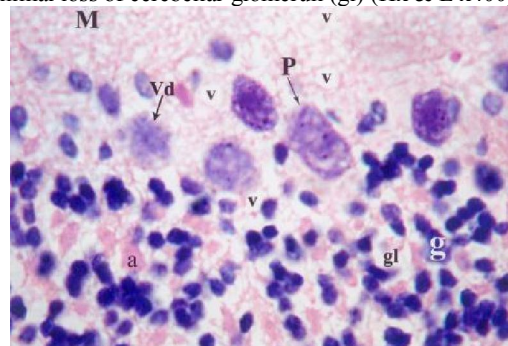


Fig. (7): A light micrograph of a section of the cerebellar cortex of Study group II (BM treated animals) specimens showed the molecular layer (M) contains areas of vacuolation (v). The Purkinje cells (P) have distorted nucleus (vd), may be present in more than one row in some areas surrounded by spaces (x) and vacuolation (v). The granular cells of the internal granular layer (g) are dispersed, surrounded by astrocytic cells (a) and contains vacuolation (v) (Hx & E x1000).

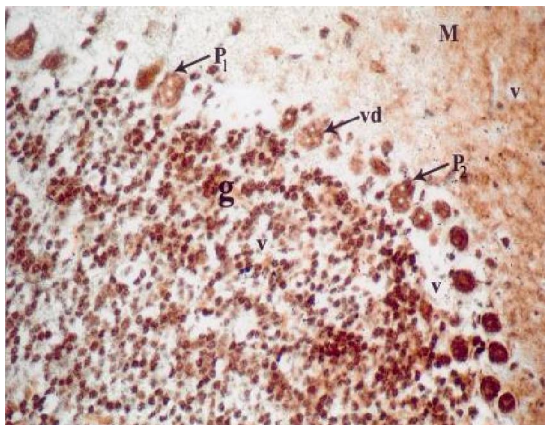


Fig. (8): A light micrograph of a section of the cerebellar cortex of Study group II (BM treated animals) specimens showed the molecular layer (M) contains sporadic small areas of vacuolation (v) with defined nerve fibers. The Purkinje cells (P1) are present in one row in some areas but not in more than one row in other areas (P2). Some Purkinje cells were not uniform, vacuolated and degenerated but others appeared normal with fusiform shape. The cells of the internal granular layer (g) are darkly stained, more dispersed with decreased glomerular pattern (Silver stain x400).

4. Discussion

The current work detected multiple structural changes secondary to chronic microwave exposure in study group I animals; however, the picture was more pronounced in the Purkinje cell layer which showed severe disturbance of arrangement, disappearance of cells in some areas, decrease of cells size in other areas. On contrary, the picture was ameliorated in specimens of BM treated animals (study group II) wherein the molecular layer appeared reaching the pia mater and contains stellate and basket cells with minimal vacuolation. The Purkinje cells are absent in some areas, have distorted nucleus, may be present in more than one row in some areas surrounded by spaces and vacuolation. Some Purkinje cells were not uniform, vacuolated and degenerated but others appeared normal with fusiform shape.

These findings indicated an ameliorative effect of BM extract on the cerebellar structural changes secondary to chronic microwave exposure and go in line with that previously reported in literature concerning similar effects of BM on neurotoxins on different brain regions. **Vollala et al. (2011a)** investigated the effect of BM on the dendritic morphology of neurons in the basolateral amygdala and showed a significant increase in dendritic length and the number of dendritic branching points along the length of the dendrites of the basolateral amygdaloid neurons of rats treated with 40 and 80 mg/kg of BM for 4 and 6 weeks and concluded that

constituents present in BM extract have neuronal dendritic growth-stimulating properties.

Vollala et al. (2011b) showed a significant increase in dendritic length and dendritic branching points along the length of dendrites of the amygdaloid neurons of rats treated with all doses of BM for 4 and 6 weeks and concluded that constituents present in BM extract have neuronal dendritic growth stimulating properties. **Tripathi et al. (2011)** assessed the comparative effects of BM (40 mg/kg body weight) and donepezil (2.5 mg/kg b. wt.) on aluminum (100 mg/kg b. wt. of AlCl₃) mediated oxidative damage in the cerebellum of aged rats and reported that light microscopic and ultrastructural studies detected damaged Purkinje's neurons and altered granular cell layer along with increased accumulation of lipofuscin granules in aluminum treated animals; however, these changes were quite less pronounced in BM group than that of donepezil and this may be due to the reduction of excess iron content by BM.

Balaji et al. (2012) reported that co-administration of BM significantly improved the memory, choline-esterase levels, and antioxidant enzymes but failed to alter the fluoride levels in sodium fluoride-treated mice, and histopathological studies revealed that BM protected the neuropathological alterations induced by sodium fluoride. **Sandhya et al. (2012)** found that induction of autism significantly affected normal behavior, increased oxidative stress and serotonin level, and altered histo-architecture of cerebellum with decreased number of Purkinje cells, neuronal degeneration and chromatolysis; treatment with BM significantly improved behavioral alterations, decreased oxidative stress markers and restored histoarchitecture of cerebellum.

These beneficial effects of BM extract could be attributed to its anti-oxidant and anti-inflammatory effect; **Mohanty et al. (2010)** reported that in ischemia-reperfusion injury BM restored the antioxidant network of the myocardium and reduced myocardial apoptosis, caspase 3 and Bax protein expression. **Viji et al. (2010)** investigated the anti-inflammatory function and mechanism(s) of action of an active component-betulinic acid isolated from BM and found that betulinic acid inhibited IL-6 production by preventing NF-kappaB nuclear translocation. **Saraf et al. (2010)** detected neuroprotective and antioxidant activity of BM on ischemia induced brain injury.

Tomlinson et al. (2013) reported that analysis of clinical and neuroimaging research revealed that behavioral effects across learning, memory, cognition, emotional processing, perception and timing, though the results were not sufficiently

similar as to offer a definitive statement of the cerebellum's role. **Lu et al. (2013)**, found that spiking activity in medial deep cerebellar nucleus neurons is correlated with respiratory and orofacial behaviors, including whisking and fluid licking and almost half of the recorded neurons showed activity correlated with more than one behavior, suggesting that these neurons may in fact modulate multiple brainstem substrates, and concluded that collectively, these results describe a potential pathway through which the cerebellum could modulate and coordinate respiratory and orofacial behaviors. Considering the main actions of BM extract are concerned with memory-enhancing and learning, these data supported the target of the current work for evaluation of a protective effect of BM on the cerebellar effects of chronic microwave exposure.

Thus, the obtained results and review of literature allowed concluding that prophylactic administration of *Bacopa monniera* extract can ameliorate the cerebellar structural effects of equipments emitting microwaves especially mobile phones whose use became spreading and usually put in contact of the skull so directly exposing brain to its emitted microwaves.

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