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Research Article

PROTECTIVE EFFECT OF *LORANTHUS LONGIFLORUS* ON LEARNING AND MEMORY OF RATS EXPOSED TO ELECTROMAGNETIC RADIATION (EMR)

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ABSTRACT

The interaction of mobile phone radio-frequency electromagnetic radiation (RF-EMR) with the brain is a serious concern of our society. In this study, we aimed to experiment on the antioxidative property of a parasitic plant *Loranthus longiflorus* (Loranthaceae) to protect central nervous system against oxidative damages of mobile phone electromagnetic radiation (EMR). Healthy male albino wistar rats were exposed to RF-EMR by giving 5 min calling/5min interval for 1 hour per day for two month, Keeping a GSM (0.9 GHz/1.8 GHz) mobile phone in Silent mode (no ring tone) in the cage. After 15, 30, 45, 60 days exposure, three randomly picked animals from both groups were tested with using Morris water maze. Antioxidant compounds and their Neuroprotective action of *Loranthus longiflorus* bark sample collected from *Ficus religiosa* host trees were assessed. Rats which have been feed with Ethyl acetate Extract of *Loranthus longiflorus* (EAL) bark (700mg/kg body weight, p.o.)

Keywords: Mobile Phone, Electromagnetic radiation (EMR), Morris water maze, Loranthus longiflorus bark.

INTRODUCTION

As recent increase in the use of electromagnetic field producing equipments, such as mobile phones, both epidemiological and experimental studies have been motivated. Indisputable reports from harmful effects of these microwaves have been associated with growing concern and some alarms in our today society. In the year 1990, 12.4 million people worldwide had cellular subscriptions. By the end of 2009, only 20 years later, the number of mobile cellular subscriptions worldwide reached approximately 4.6 billion, 370 times the 1990 number, penetrating the developing economies and reaching the bottom of the economic pyramid. The interaction of mobile phone radiofrequency electromagnetic radiation (RF-EMR) with the brain is a serious concern of our society. Mobile telephones emit radiations that are intercepted in the proximity of the brain and cranial nerves. There is now an added worry if these radiations are carcinogenic or tumor promoter or have any other health implications¹. The use of mobile phones is increasing day by day, and it is estimated that approximately 500 million people worldwide are using mobile phones currently. A large proportion of users are made up of children and teenagers. Mobile phone has negative effects on sperm motility², antioxidant enzymes³ and sperm concentration⁴. Exposure to electromagnetic radiation (EMR) at even low frequencies (900 to 1800 Hz) causes some established pathologic consequences such as increased permeability of the Blood brain barrier, disturbed neurons function and alteration in electroencephalography (EEG) disturbed regional cerebral blood flow, oxidant and antioxidant balance, neurotransmitter imbalance and genomic responses^{5,6}. Penafiel et al. have shown that the radiation from TDMA digital cellular phones can cause significant changes in ornithine decarboxylase activity (ODC), which is essential for DNA synthesis'. Kolomytkin et al. studied specific receptor binding of three neurotransmitters: gammaaminobutyric acid (GABA), an inhibitory transmitter and

acetyl choline and glutamate, both excitatory to rat brain synaptosomes⁸. Experimental studies have shown that the radiofrequency electromagnetic radiation (RF-EMR) emitted from the mobile phones can affect the brain in various ways. These effects have been described in vitro and in vivo in a number of studies in particular, effects on cerebral blood flow9, blood-brain barrier permeability10, oxidant and antioxidant balance¹¹, Neurotransmitter balance¹², nerve cell damage¹³ and genomic responses¹⁴ have been reported. Antioxidative substances have to protect from central nervous system in front of oxidative effect of EMF. We have studied and use herbal extracts as antioxidative agents, because herbal-base medications are accompanied with lower imposed side effects and have more facing today society. Loranthus longiflorus (belongs to Loranthaceae family) is a perennial climbing woody parasitic plant. It is indigenous to tropical regions especially in India, Srilanka, Thailand, China, Australia, Bangladesh, Malaysia and Myanmar¹⁵ and widely distributed throughout in India¹⁶. It is a hemi parasitic plant whose whole plant is used in indigenous system of medicine as a potential medicinal agent like cooling, bitter, astringent, aphrodisiac, narcotic, diuretic, and useful in pulmonary tuberculosis, asthma, menstrual disorders, swelling wounds, ulcers, renal and vesical calculi and vitiated conditions of kapha and pitta¹⁵. Decoction of plant is used by women as an antifertility agent and also has anticancer activity^{17, 18}. Leaf paste is used in skin diseases, also applied on boils, setting dislocated bones and extracting pus. The plant has been scientifically proved to have antilithiatic, diuretic, cytotoxic and immunomodulatory activities¹⁹⁻²². Loranthus longiflorus also has Antioxidative and neuroprotective effects against oxidative stress in NG108-15 cells²³. Synonyms: Loranthus amplexifolius Desr., Loranthus bicolor Roxb., Loranthus falcatus L.f., Loranthus longiflorus Desr. The objective of this research was to see the effect of Loranthus longiflorus on Central nervous system that is induced by electromagnetic field.

MATERIALS AND METHODS Plant material

The selected plant, *Loranthus longiflorus*, a hemi parasite, was collected from the host tree *Ficus religiosa*, during the month of October, around Nagercoil town, Kanyakumari District, Tamil Nadu and identified based on the characters of Gamble Flora. The herbarium of the plant was prepared and preserved in the department of Botany, S.T. Hindu College, Nagercoil, Kanyakumai, District, Tamilnadu and India.

Plant extraction

The bark of *Loranthus longiflorus* collected from host tree were washed in freshwater to remove adhering dust and then dried under shade. The air dried, powdered bark of Loranthus was extracted at 20% (w/v) in Soxhlet extraction successively with ethyl Acetate. The successive extracts were evaporated to dryness and the stored residues were used for analyzing antioxidants and free radical scavenging activities.

Phytochemical screening

Preliminary chemical tests were done with individual extract for the presence of different group of chemicals i.e. Phenol, Tannins and flavonoids compounds.

Animals

Wistar albino rats weighing between 200-300g of either sex were used. All animals were housed in well ventilated polypropylene cages at 12:12 h light/dark schedule at 25±2°C and 55-65 RH with free access of food (standard laboratory rodent's chow) and water. All the animal experiments were approved by IAEC of Truba Institute of Pharmacy, Bhopal (M.P.) and the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

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Ethical clearance number to conduct the animal study: PIC/IAEC/2010/PN-04

Electromagnetic radiation Exposure Setup

A GSM (0.9 GHz/1.8 GHz) mobile phone in Silent mode (no ring tone) is kept in the cage $(36 \times 23 \times 21 \text{ cm})$. Animals exposed to RF-EMR by giving 5 min calling/ 5min interval for 1 hour per day for two month. Each call was of the duration of 5 min. Animals were free to move in the cage. The phone was kept in a small wood bottomed cage sized 12

 $cm \times 7 cm \times 7 cm$. The wire mesh on top of the wood bottom cage prevented the animals from contact with the phone. After 15, 30, 45, 60days exposure, three randomly picked animals from both groups were tested for using behavioral screening model of Central nervous system.²⁴

Experimental design

The animals were allocated into three experimental groups. Each group consisted of six animals.

I group (control) - Animals treated by vehicle, orally (p.o) applicated every day.

II group- Everyday exposed rats with mobile phone radiation. III group- Mobile phone radiation exposed rats treated with Extract of *Loranthus longiflorus* bark (700mg/kg, p.o.).

Morris water maze

This maze represents a more specific test of memory. The essential feature of the technique was that rats were placed in to a large circular pool of water and can escape onto a hidden platform. The platform was hidden by its placement just below the water surface and by opaque water. The Morris water maze consists of a large circular tank made of black opaque polyvinyl chloride or hard board coated with fiberglass and resin and then surface pointed white (1.8-2.0 m in diameter and 0.4-0.6 m height). The pool was filled with water (20-22°C) to a depth of 0.3-0.4 m, and rendered opaque by the addition of a small quantity of milk or nontoxic white color. An escape platform was made of Plexiglass with a 13 cm square platform attached to a 34 cm long clear plexiglass cylindrical pedestal (3 cm diameter) mounted on a 5 mm thick plexiglass base. For the hidden platform task, water was added to the circular tank to a level 2 cm above the top of the platform. For two platform task, the height of one platform was arranged to be 1-2 cm below or 1-2 cm above the water level so that one was hidden, the other visible. A third platform was floating triangular platform. This platform was anchored by means of nylon threads to a metal base which sits on the bottom of the pool.

Statistical analysis

All the data were given as means ±S.E.M. Data were analyzed by one-way ANOVA. Whenever ANOVA was significant, further comparisons between vehicle- and drug-treatment groups were performed using the Dunnett's test. The level of statistical significance adopted was P < 0.05 & P < 0.01.

 Table 1: Effect of 700mg/kg body weight Loranthus longiflorus bark extract and Mobile phone Electromagnetic radiation on Learning and Memory (After 15 and 30 days)

	Escape latency on the hidden platform (sec.)										
		After 15	5 Days		After 30 Days						
Groups	I trial	II trial	III trial	IV trial	I trial	II trial	III trial	IV trial			
I	32.34±2.68	18.62±3.58	9.32±2.35	6.75±1.32	30.41±2.67	18.42±2.13	9.15±1.57	6.21±1.67			
II	40.91±3.54	33.48±3.12	29.78±2.35	25.97±2.32	39.17±2.47	34.25±3.21	30.16±2.54	24.15±2.15			
III	30.21±3.54	19.43±3.24	7.54±2.34	5.87±1.25	31.15±2.54	19.12±2.43	7.31±1.32	5.43±1.21			

 Table 2: Effect of 700mg/kg body weight Loranthus longiflorus bark extract and Mobile phone Electromagnetic radiation on Learning and Memory (After 45 and 60 days)

	Escape latency on the hidden platform (sec.)										
		After 4	45 days		After 60 days						
Groups	I trial	II trial	III trial	IV trial	I trial	II trial	III trial	IV trial			
I	30.21±2.15	18.57±2.65	8.57±1.32	6.12±1.65	31.25±2.45	18.21±2.54	7.86±1.54	5.47±1.21			
П	40.15±3.54	36.25±2.35	31.54±2.56	26.47±3.54	40.67±2.35	35.28±2.65	32.38±2.58	28.54±2.24			
Ш	30.81±2.19	18.75±2.58	8.58±1.58	5.12±1.54	30.21±2.54	17.85±2.57	6.87±1.54	4.32±1.34			

RESULTS

Our experimental results revealed that EMR exposure affected Learning and memory. It significantly increases escape latency on the hidden platform in comparison to control group (P<0.01). *Loranthus longiflorus* (EAL) bark Extract feeding was not significantly effective to be protective in front of radiation after 15 days evaluation. After 45 and 60 days *Loranthus longiflorus* bark Extract could be effective in decreasing escape latency on the hidden platform (P<0.05). This result indicates the protective effect of *Loranthus longiflorus* bark against effect of EMR on memory (Table 1 and 2).

DISCUSSION

Plants and natural products are extensively used in several traditional systems of medicine, so screening these products for radio-protective compounds has several advantage, because they are usually considered non-toxic and widely accepted by humans. Many natural antioxidants consumed before or after radiation exposure indicated some level of radio-protection. The radiofrequency electromagnetic radiation (RF-EMR) emitted from the mobile phones can affect the brain in various ways. A previous study showed that EMF exposure immediately altered the metabolism of free radicals, decreased SOD activity in plasma²⁵. EMF is able to generate destructive reactive oxygen species including superoxide, hydrogen peroxide and hydroxyl radical and frequently used to produce oxidative and narcotic damages²⁶. Formation of reactive oxygen species (ROS) and increased oxidative stress may be involved in the action of microwave radiation on the biological system. ROS also cause injury by reacting with biomolecules such as lipids, proteins, and nucleic acid as well as by depleting enzymatic and no enzymatic anti-oxidants in the brain. Antioxidant treatments in animals and humans could be beneficial in preventing or reducing some complications of microwave radiation. Antioxidants play an important protective role against the reactive oxygen species²⁷. The present study has showed that Loranthus longiflorus bark extracts have had protective effect in exposed animals to EMR induced depression. We suppose protective property of Loranthus longiflorus against EMR is referred to its antioxidative potency and free radical scavenging activity. Some previous studies have investigated antioxidative property²³. The antioxidative effect is mainly due to phenolic components, such as flavonoids, phenolic acid and phenolic diterpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides²⁸. We have tried to study easy obtained herbal substance contain phenolic components and screening its protective efficacy in front of electromagnetic fields that affect everyday life. We cannot stop technology that emitted EMR, but we can protect ourselves, especially teenagers and young persons, against hazardous effects of radiations.

CONCLUSION

From the above findings it can be concluded that the Ethyl acetate extract of *Loranthus longiflorus* bark possesses protective effect on learning and memory against EMR exposure. The presence of tannins and phenolic compounds in the Ethyl acetate extract of the plant may be responsible for these activities.

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