Evaluation of *Aegle marmelos* L. Fruit Extract in Reduction of Mobile Phone Induced Oxidative Stress in Mice, *Mus musculus*

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**ABSTRACT**

The extravagant use of mobile phones in recent years has attained the focus on research activities by many scientists to determine the effects of exposure to electromagnetic radiation (EMR) produced by mobile phones. In present study, we investigated the effect of *Aegle marmelos* (Am) on Mobile Phone induced oxidative stress in brain and testis of Mice. Mice were exposed to 900-1800 MHz EMR from Mobile phones for 7 days (1 h/day). In the EMR+Am groups, mice were exposed to EMR and pretreated with Am. In the control and Am groups’ mice were kept without exposure. Subsequently, lipid peroxidation and pathological changes in tissues were examined for each group. Oxidative damage was prevented by Am treatment, Furthermore, Am prevented the Mobile phone induced tissue damage in both the tissues histopathologically. The protective activities of *Aegle marmelos* which is caused by EMR radiations may have potential implication in the future for prevention/protection against oxidative stress induced by mobile phones.

**Keywords:** *Aegle marmelos*, EMR, Mobile phone, Oxidative stress.

**INTRODUCTION**

Mobile Phones produce radiations are categorized under Electromagnetic radiations (EMR). EMR is nothing but the energy radiates as a result of the motion of electric charges in the form of the waves. In the 1930s, scientists began to postulate that high frequency electromagnetic field (EMFs) might cause health problems, this thinking laid to start Debate regarding EMR and health hazard (Vocht et al., 2011). The activation of cellular stress response might affect variety of physiological processes, like brain tumor development and blood brain barrier permeability and is induced by of mobile phone radiations. The study showed that the mobile phone radiation exposure of cell for 1 hour, leads to increase in the expression of hsp27 (Leszczynski, 2014). However, in case of the normal cell, even the incubation...
at 43°C for 1 hour does not show any effect, but to increase the expression of hsp27 by heat shock requires 3 hrs of incubation of cells at 43°C.

The microwave may induce cancer and may also shows the symptoms associated with their use include electroencephalographic activity, memory problem, sleep disturbance, headaches, changes in the permeability of the blood brain barrier, nausea, dizziness and blood pressure also have been reported (Talib et al., 2010).

The hazards of electromagnetic radiations by mobile phone can be divided into two parts – i.e. thermal health hazard and non-thermal health hazard. In the thermal effect, the induction of polar molecules by electromagnetic field of radio waves, generate dielectric heat resulted into death of live tissue. For example, while receiving the massage through radio waves, some parts of head may experience increased temperature and results in damage of nerve fibers. The development of cataract as cornea lacks the thermoregulatory mechanism due to effects of mobile phones. Laboratory studies from different research groups show that Possible non thermal effects is after short periods of exposure to radiations of mobile phone are related to genotoxic effects i.e DNA strand can be broken and there are effects on gene expression. Phone radiations are capable of disturbing the DNA repair mechanism and this can continue for several hours after phone is used (Talib et al., 2010).

Studies have conclusively shown that the mobile phones are aid in introduction of oxidative stress which is resulted because of excess production of free radicals like superoxide anion, Singlet oxygen, hydrogen peroxide, hydroxyl radicals and peroxy nitrile. The free radicals generated cause peroxidation of polyunsatura-ted lipids and produce a range of end products that possesses DNA damaging potential. These includes lipid peroxides and various species that contains unpaired electrons, such that alkoxyl and peroxyl radicals. In addition, a carbonyl containing compounds like malon-dialdehyde-a genotoxic agent, various 4-hydroxy 2-alkenals and a number of 2-alkanals are also formed (Burcham, 1998).

Several studies have revealed the degradation of sperm with regard to their number, motility, viability, morphology and DNA exposure is due to mobile phone radiations. These effects are related to the duration and frequency of mobile phone use (Kesari and Behari, 2010; Kesari et al., 2011). The study proves that long term exposure to mobile phone radiation resulted into reduction in serum testosterone level. As testosterone is a primary male gender hormone, any change in the normal level may be disturbing for reproduction and general health. (Meo, 2010)

**Aegle marmelos** -

*Aegle marmelos* commonly known as 'Bael' belonging to the family Rutaceae. Due to its various medicinal properties, it has been widely used in indigenous systems of Indian medicine. For the treatment of various diseases in traditional medicinal system, fruit, leaf, bark and decoction of bark have been used. The Bael tree is originate from Eastern Ghat and central India. It is found mainly in tropical and subtropical regions and also in Indian subcontinent. *A. marmelos* is medium sized tree, up to 12 to 15 meter tall, slow growing have short trunk, thick, soft, flashing barks and sometimes spiny branches. The investigative study of different parts of *A. marmelos* revealed that there is presence of varied classes of compounds viz. alkaloid, coumarins, terpenoids, fatty acids and amino acids (Chakraborty et al., 2015).

It also possess potential anxiolytic and antidepressant activities. The leaf extract of *A. marmelos* significantly inhibits growth of all dermatophytic fungi. An aqueous extract of *A. marmelos* has also lipid lowering property known as hypolipidemic activity. The hypoglycemic activity was also shown by aqueous extract of the fruits of *A. marmelos*. During study in diabetic rats, it is shown that aqueous seed extract of *A. marmelos* possesses antidiabetic and hypolipidemic effects. During the study of anti-fertility activity of *A. marmelos* has shown effects on male rat reproduction affecting the sexual behaviour and epididymal sperm concentration (Patel et al., 2012). The Bael sherbet is a popular drink of bael fruit in India. Fruits serve as a source of freshness and energy. It is used as carminative and astringent. It finds good utility in thyroid related disorder. The fine therapeutic uses reported in cardiac stimulant, swollen joints, pregnancy trouble, typhoid and coma (Chakraborty et al., 2015).

In Ayurvedic system of medicines bael fruits are considered as an excellent remedy for many diseases. The analysis of bael fruit shows that, there is presence of various alkaloids, flavonoids and volatile oils like marmelisin, luvangetin, aurapten, psoralen, marmelide, tannin (Dhankhar et al., 2011). The *A. marmelos* has been used since ancient times for treating various diseases; some of them are now known to be the result of oxidative stress. Since decades, it has been observed that *A.
Marmelos helps to prevent radiation induced ill effects and increase survival. These studies indicate that A. marmelos is be an effective, nontoxic radio protective agent. Elevation in glutathione scavenges free radicals and arrests lipid peroxidation (Baliga et al., 2011).

The intraperitoneal administration of hydroalkoholic extract of the fruit pulp (20mg/kg body wt.) for five consecutive days before exposure to different doses of γ radiation shows preventive activity against radiation induced lipid peroxidation in the liver, kidney, intestine and spleen of mice (Jagetia et al., 2004).

In the present study, we have described A. marmelos helps to reduce mobile phone (EMR) induced oxidative stress in brain and testis of mice, Mus musculus.

**MATERIALS AND METHODS**

**Swiss albino mice:**
Swiss albino Mus musculus are used for the present study. 24 male mice weighing 25-30 grams are selected. The clean and dry plastic cages bedded with rice husk were used to keep mice. The 12 hours light–12 hour dark cycle at standard environmental conditions are maintained at the departmental animal house (Regd. No. 233/CPCSEA) where the mice are kept. Animals were provided with Amrut rat feed (Pranav Agro Company limited. Sangli, Maharashtra, India)

**Preparation of plant extract:**
The fruit powder of Aegle marmelos were purchased from the market. The powder was then subjected to aqueous extraction Soxhlet apparatus for 12-14 hour. Crude extract then passed through whatman No. 1 filter paper and concentrated by a rotary evaporator under low pressure. Darken brown colored residue is obtained and stored in glass bottle at room temperature.

**Exposure system:**
Exposure system consists of ventilated plastic cages. Un-anaesthetized mice were confined in the cage. Mice are kept in the EMR of the mobile phones. In present study, a 900 MHz electromagnetic near field signals for the GSM (Global System for mobile communication at 900 MHz, continuous waves, analog phone) system was used. The peak specific absorption rate (SAR) of the brain was 2W/kg and the average SAR of the whole body was 0.25W/kg

The animals were divided into following four groups (Four mice in each group).

**Group I:** Control Mice: Mice were placed as it is in the cage without exposure to EMR and without plant extract.

**Group II:** EMR induced mice: these mice were exposed to the EMR in the above-referred condition for 1 hour per day for 7 days.

**Group III:** EMR and Plant treated mice: The mice were exposed to EMR in the above referred conditions for 1 hour/day for 7 days along with Aegle marmelos fruit’s aqueous extract mixed with sugar syrup at the dose fed orally.

**Group IV:** A. marmelos treated mice: Mice were kept as it is without exposure to the EMR along with A. marmelos extract mixed with sugar syrup fed orally.

**Exposure periods:**
One week exposure period for 1 hour daily was conducted for each group. Mobile phones were activated by calling each other. All the groups were exposed to system at 7.00 am to 8.00 am daily.

**Biochemical evaluation:**
The biological effects of the EMR exposure from mobile phone to the brain and the efficacy of Aegle marmelos, the antioxidant enzyme activity and radio protective activity to the brain tissue against EMR was evaluated. After the last exposure on the 7th day, EMR group and control group animals were sacrificed by cervical dislocation, Brain and testis was removed, weighed, freezed and homogenized in respective homogenization media. Total lipid peroxidation was done by Will's method (1966). EMR + AM group animals were sacrificed after 7th day of last exposure to EMR.

**Will's method:**
The reaction mixture contained 0.2ml homogenate, 1.8ml distilled water, 1ml 20% TCA and 2ml 0.67%TBA. The reaction mixture was kept in boiling water bath for 10 min. and absorbance was measured on spectrophotometer at 532nm. Amount of MDA per mg tissue was calculated using following formula.

\[
\text{LIPID PEROXIDATION (X)} = \frac{\text{O.D. of Sample} \times 3 \times 6}{0.156 \times 02}
\]

Where:

- X = Amount of MDA in homogenate nM MDA/ mg tissue.
- 3 = Volume or sample taken for photometric observation.
- 6 = Scaling factor for conversion to per hour.
0.156= Absorbance for 1 hour mole solution of MDA measured in thick cell at 532nm.
0.2= Volume of sample.

**Histopathological demonstration:**

**Brain:**

After removing the brain from skull, brains were sectioned sagitally. Right hemisphere were removed and fixed with a CAF solution for 24 hours and embedded in paraffin. Tissues were then sectioned at 6µm, stained with Haematoxylin and Eosin (HE) and examined for histopathological changes of brain with using light microscope. The occurrence of dark neurons was judged semiquantitatively as 0 (no or occasional dark neurons), 1 (moderate occurrence of dark neurons) or 2 (abundant occurrence of dark neuron). The microscopical analysis was performed blind to the test situations (Fig.2).

**Testis**

After removing the testis from scrotal sacs one of testis was fixed with a CAF solutions for 24 hours and embedded in paraffin. Seminiferous tubules were evaluated using 6µm thick sections stained with Haematoxylin and Eosin. The shape of 10 randomly selected, essentially round seminiferous tubules from each animal, were observed and classified into one of three different grades I: grade 0 normal intact seminiferous epithelia, grade 2 seminiferous epithelium with pyknotic cells and desquamation or focal vaculation, grade 1 seminiferous epithelia intermediate between grade 0 and 2 (Fig.2).

**Haematoxylin and Eosin Staining**

The sections were deparaffinised and hydrated through descending alcohol grades and stained in Haematoxylin for 3 min., rinsed in distilled water. The sections are stained in Eosin for 5 sec and again rinsed with distilled water. The sections are dehydrated through ascending alcoholic grades and slides were mounted in DPX.

**RESULTS AND DISCUSSION**

**Lipid peroxidation:**

EMR exposure produces a significant increase in the brain tissue MDA content (1.065 ± 0.113 nMol/mg protein), an index for lipid peroxidation when compared with control (0.513 ± 0.039 nMol/mg protein) and Am group (0.410 ± 0.80 nMol/mg protein). As shown in fig. EMR-induced increments in MDA content of the brain were significantly prevented by *A. marmelos* treatment. The tissue MDA content in this group was remained to be 0.635 ± 0.075 nMol/mg protein (p<0.0001).

EMR exposure produces a significant increase in the testis tissue MDA content (1.32 ± 0.13 nMol/mg protein), an index for lipid peroxidation when compared with control (0.487 ± 0.125 nMol/mg protein) and Am group (0.602 ± 0.058 nMol/mg protein). There was significant decrease in MDA content of the testes and was 0.615 ± 0.048 nMol/mg protein. As mentioned in table of group of EMR and *A. marmelos*. Induced increment in MDA content of the testes was significantly prevented by *A. marmelos*.

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<td>EMR+Am</td>
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Figure 1: Effect of A. marmelos fruit extract on the brain and Testis tissue level of peroxidation (Mean ±SD), n=6

Figure 2: Damage to the Brain and Testis: Grade 0- Normal tissue, Grade I- Moderate damage, Grade II- High Damage

Histopathology:

Brain:
The pathological examining revealed scattered and grouped dark neurons, which were often shrunken and dark staining, homogenized with loss of discernible internal cell structures. Dark neuron were seen in all locations. The occurrence of dark neuron belongs to each group is shoen in Table-1 which shows a significant positive relation between EMR exposure and number of dark neurons. EMR group has more Grade-I and Grade-II scores while Control, Am and EMR+Am groups have more Grade-0 scores which shows positive relation between EMR exposure and number of dark neurons.

Testis:
The pathological examining revealed rounded and damaged seminiferous tubules, homogenized with loss of discernible internal cell structures. The occurrence of
seminiferous tubules belongs to each group is shown in Table -2. EMR group has more Grade-I and Grade-II scores while control, Am and EMR-Am group have more Grade-0 scores which shows positive relation between EMR exposure and damaged seminiferous tubules.

DISCUSSION:

The study of the experimental exposure of EMR to male mice provides following findings related to oxidative damage. Firstly, we demonstrated that due to mobile phone radiation there is increasing level of MDA in brain and testis in mice which is exposed to EMR. Secondly, the antioxidant A. marmelos extract given by oral treatment having potent free radical scavenger agent significantly helps to prevent oxidant damage in the brain and testis. In third, the mice are exposed to EMR along with oral treatment of A. marmelos extract having intermediate value of MDA content.

The recent studies show that free radicals that are derived from oxygen cause many disorders such as Neurodegenerative disorders like Alzheimer’s disease, Parkinson’s disease, (Ozgen et al., 2006) multiple sclerosis, amyotrophic lateral sclerosis, memory loss and depression. Cardiovascular disease like atherosclerosis, ischemic heart disease, cardiac hypertrophy, hypertension, shock and trauma (Bohorun et al., 2006), Inflammation (Neumann, 2001), AIDS (Westendorp et al., 1995), multiple sclerosis (Smith and McDonald, 1999), carcinogenesis (Valko et al., 2006) carciogenesis (Poul森 et al., 2011), in physiological ageing (Oliver et al., 1987) and infertility (Kesari et al., 2013).

Generally, mobile phones are used very close to the head, where the emitted radiations are absorbed by the brain (Ismail and Ibrahim, 2002). Some of the reports showed that free radicals are involved in EMR induced tissue injury (Irmak et al., 2002, 2003; Ilhan et al., 2004). Protein conformations and binding properties are also changed by EMR and an increase in the production of reactive oxygen species (ROS) that may lead to DNA damage (Challis, 2005; La Vignera et al., 2012). Reactive oxygen species are the most powerful and ubiquitous threats faced by cells. The free radical reactions with biomolecules, such as proteins and lipid membranes, are responsible for the functional worsening related to aging (Harman, 1969). The free radicals may be converted in to longer live toxic species that can migrate to sites distant from where they were produced. These toxic species and free radicals are collectively called reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS can modify proteins, lipids, and nucleic acids and develop or enhance age related manifestations. The oxidative stress disturbs the pro-oxidant: antioxidant balance and it has been implicated in several biological and pathological processes (Trachootham et al., 2008). Oxidative stress is described as the state of imbalance resulting from the production of ROS and RNS that exceed the capacity of the antioxidant defense systems. The oxidative stress has more adverse effects on CNS than other tissues. Free radical-associated oxidative stress induces physiological alterations in the CNS and this consequently causes age related dementia and neurodegenerative disease in aged animals, as the CNS possesses high content of peroxidizable unsaturated fatty acids high oxygen consumption per unit weight high content of lipid Peroxidation key ingredients: iron and ascorbate, and the scarcity of antioxidant defenses systems (Floyd, 1999). There are two major groups of endogenous antioxidants are low molecular weight antioxidants compounds (e.g., vitamins C and E, lipoic acid and Ubiquinone), and antioxidant enzymes (e.g., superoxide dismutase [SOD], superoxide reductase [SOR], catalase, glutathione peroxidase [GPx], and many heat-shock proteins (Gilgun et al., 2001). The free radical effect results into lipid Peroxidation.

Malondialdehyde (MDA) is formed after peroxidation of amino acids (Esterbauer, 1993), this MDA is undergo hydrolysis by lysine or arginine to form formic acid or acetaldehyde (Nair et al., 1998). MDA reacts with nucleic acids and induces DNA damage reacting with nucleic acids to form adducts that destabilize DNA base pairing (Agarwal and Drapper, 1992). The quantitative analysis of MDA is done by TBARS assay. MDA reacts with thiobarbituric acid (TBA) to form thiobarbituric acid reacting substances (TBARS).

The overall observations revealed that, the oxidative stress produced by EMR can causes different diseases. The effect of oxidative stress on brain cells may lead to neurodegenerative diseases and various disorders related to reproductive system. Due to the excess of free radicals in cells, in human being cause diseases like Parkinson’s disease, Alzheimer’s disease, amyotrophic lateral sclerosis and decrease fertilizing potential of spermatozoa, decrease in the level of testosterone, male infertility, reduce sperm count and motility etc. From
our experimental findings, we concluded that the damage induced by EMR is more in brain than in testis. Induction of Mobile phone radiation resulted into increase in the formation of reactive oxygen species in brain cells and testis (Rahal et al., 2014). Elder people must be more precautious for EMR-induced oxidative damage, because the reactive oxygen species may causes early ageing. For the users of mobile phones the major precautions are that they may restrict their own EMR exposure by limiting the length of calls, or by using "hands-free" devices to keep mobile phones away from the head and body. As a result, mobile phone users are exposed to limited EMR and may less harmful to human health. Because of the close proximity of mobile phones to the head has possibility that the power absorption may become locally excessive. Therefore, it is extremely important to establish an experimental evaluation procedure regarding the SAR for the human head for a safety point of view. The duration of exposure to cellular phones is an important factor for production of reactive oxidative species or oxidative stress, but the exact duration differs from individual to individual. Hence, setting the duration for experimental conditions is a complicated matter. In our experiment, we chose the 1 h/day exposure because it is considered to be the mean exposure period of mobile phone use by most individuals (Vocht et al., 2011).

*Aegle marmelos* is reported as it contain various biochemical constituents like Alkaloids, Coumarins, Phenylpropanoids Terpenoids, Carotenoid, Tannins, Flavonoids, steroids Fatty acids and Amino acids. The *Aegle marmelos* fruit contains Marmelosin, Luvengetin, Aurapten, Psoralen, Marmelide and Tannin (Baliga et al., 2011, Dhankar et al., 2011). *A. marmelos* fruit giving its antioxidant activity, radioprotective activity, antibacterial activity, hypoglycaemic activity, and possible protection against the damage caused by free radicals (Patel et al., 2012, Dhankar et al., 2011). These compounds from *A. marmelos* fruit extract have the free radical scavenging effect and destroy free radicals (Baliga et al., 2011). The hydro-alcoholic extract of *A. marmelos* has potent dose dependent anticancer activity (Dhankar et al., 2011). In the present study, the treatment of *A. marmelos* produced a significant reduction of MDA in the brain tissue and testis induced by EMR.

In conclusion, the mobile phone EMR produces ROS, which have adverse effect. The antioxidant property of *A. marmelos* decrease the oxidative stress and MDA level in Brain and Testis.

**Conflicts of interest:** The authors stated that no conflicts of interest.

**REFERENCES**


