AMELIORATING EFFECT OF THYMOQUINONE ON THE DELETERIOUS EFFECTS OF MOBILE PHONE RADIATION IN THE RAT BRAIN

BY

El Sayed K. El Sayed, Amany A.E. Ahmed, Engy M. El Morsy

FROM

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Helwan University, Ein Helwan, Cairo, Egypt.

ABSTRACT

Tremendous concerns have been raised about the possibility that exposure to electromagnetic radiation (EMR) from mobile phones could affect people's health. Many experimental studies suggest the possible role of reactive oxygen species (ROS) in EMR-induced damage in brain tissues with subsequent neurobehavioral changes.

The aim of the present study was to investigate the protective effect of thymoquinone (TQ) on EMR-induced oxidative damage in brain tissue of rats. Results of the current study show that exposure to EMR decreases the time spent in target quadrant of Morris water maze and the percentage time spent in open arms of elevated plus maze, and increases significantly the percentage time spent in closed arms of elevated plus maze, malondialdehyde (MDA), nitric oxide (NO), and norepinephrine (NE) contents in brain tissue. Furthermore, a significant decrease in brain catalase (CAT) activity, reduced glutathione (GSH) contents, serotonin (5-HT) and dopamine (DA) contents were also observed. These alterations were prevented by TQ (5, 10 mg/kg). The observed neuroprotective effects of TQ were attributed to its powerful antioxidant activities.

Key words Mobile phones radiation; Thymoquinone; Brain monoamines; Oxidative stress; Morris maze; Elevated plus maze.

Introduction

Electromagnetic Radiation (EMR) is a form of energy emitted and absorbed by charged particles, which exhibits wave-like behavior as it travels through space. It can be classified into non-ionizing radiation and ionizing radiation Figure (I). The non-ionizing radiation could be divided in term of frequency to extremely low frequency (ELF), radio frequency (RF) and microwaves (MW) frequencies (Consales et al., 2012).

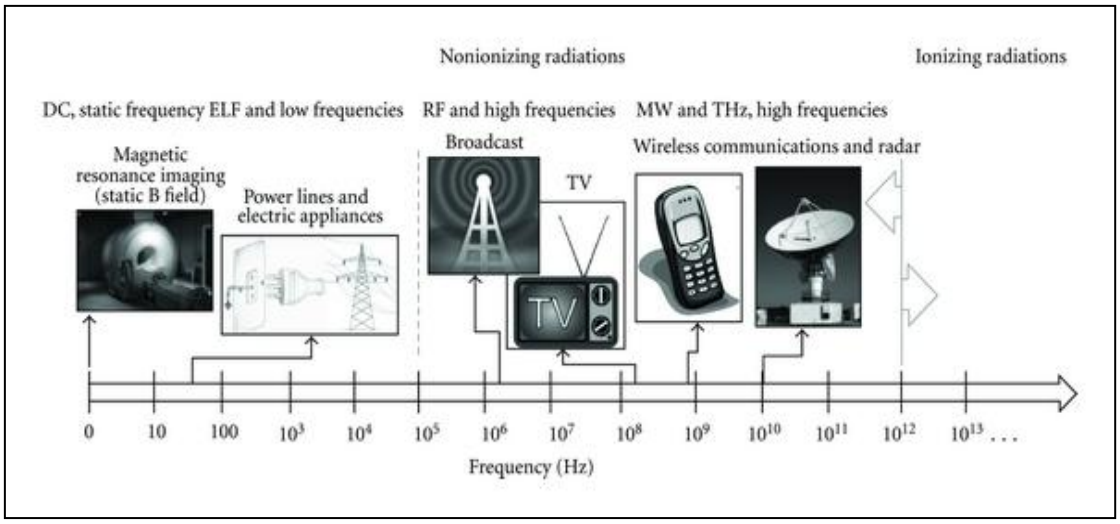

Figure (I): types of electromagnetic radiation.

The quantity used to measure how much EMR is actually absorbed by the body is called the Specific Absorption Rate (SAR). The SAR is usually expressed in units of watts per kilogram (W/Kg) or milliwatts per gram (mW/g) (ICNIRP, 2009). The international 900 MHz exposure guidelines limit level reported for mobile phone as maximum SAR of 2 W/Kg (ICNIRP, 1998) or 1.6 W/Kg (IEEE, 1992).

A particular concern has been raised about the hazardous effect of exposure to electromagnetic radiation emitted by mobile phone, which caused many reports to demand further investigations on this possibility (Maes et al., 2001). Mobile phone operates on wireless technology, with communication typically occurring via a 900-1800 MHz. This signal carries essentially no power when the user is not talking or receiving, but when the user communicates; the power of this pulsed electromagnetic field reaches a maximum of 250 Mw (Croft et al., 2002).

Salford et al., 2003 first reported the evidence for neuronal damage caused by non-thermal EMR exposure. Mobile phones with emission of 900 MHz EMR could be sources of neuronal damage. The brain may absorb emitted EMR more than other internal organs because mobile phones are generally used near the brain (Sorce and Krause, 2009).

Free radical are substantial element that take part in proper function of metabolic pathways of human cells and tissues in hydrophobic as well as in hydrophilic environment (Gałecka et al., 2008). They are also introduced through external sources such as exposure to the sun, pollution, stress, as well as unhealthy style (Puizina-Ivić, 2008). Free radicals are continuously generated in variety of biological processes, of which reactive oxygen species (ROS) including superoxide anion (O2-), hydroxyl radical (•OH) and hydrogen peroxide (H2O2) are the most relevant (Droge, 2002). Their overproductions overwhelm the level of antioxidants causing oxidative stress (Di Giacomo et al., 2009).

Mobile phones-induced free radical formation in brain tissues has been reported previously (Kesari et al., 2012). According to previous studies, biological systems may interact with EMR but there is not yet any robust evidence to support this suggestion. Malondialdehyde (MDA), the final product of lipid peroxidation, is commonly known as a marker of oxidative stress and antioxidant status (Gaweł et al., 2004). The nervous

system is particularly vulnerable to ROS due to its high metabolic rate, its deficient oxidant defense mechanisms and its diminished cellular turn over (Sorce et al., 2004). A variety of endogenous antioxidant enzymes (superoxide dismutase, catalase and peroxidase) protects the body from oxidative injury, thereby, forming the first line of defense (Popet et al., 2006). Moreover, free radicals are quickly scavenged by natural protective molecules in the cell including glutathione (GSH) and glutathione-s-transferase (GST) (Goven and Kaya, 2005).

Thymoquinone (TQ), the main constituent of the volatile oil from Nigella sativa seeds, is reported to possess a strong antioxidant property (Houghton et al., 1995). Chemically, TQ belongs to 2,5-disubstituted benzoquinone, which belongs group of compounds that have methyl and isopropyl groups at C-2 and C-5 positions, respectively (Dockal et al., 1985). TQ possesses various pharmacological properties including antioxidant effect, analgesic and anti-inflammatory action, anticarcinogenic and mutagenic activity, antidiabetic action and antimicrobial actions (Ali and Blunden, 2003). In addition to that many studies reported the in *vitro* and in *vivo* protective effects of TQ against various neurotoxins (Al-Majed et al., 2006; Ullah et al., 2012; Alhebshi et al., 2013; Radad et al., 2014).

Accordingly, the goal of the study was aimed to investigate the role of TQ as a protective agent to decrease the incidence of oxidative stress states as well as it is ability to amendment animal behavior and cognition induced by EMR from mobile phone.

Materials and Methods

Chemicals and drugs

Thymoquinone (TQ) and other chemical reagents were purchased from Sigma Aldrich chemical co. (St. Louis, MO., USA).

Animals

Male Sprague–Dawley albino rats weighting 120-150 g were used in the present experiment. The animals were purchased from El Nile Company (Cairo, Egypt). The animals were randomized and housed (5 rats/cage) in stainless steel wire bottom cages in conditioned atmosphere at 22 ± 2 ◦C with 42 ± 5% relative humidity and had a 12-12-h light-dark cycle. The rats were given tap water and fed with standard commercial rat chow ad libitum, and left to accommodate for one week before the experiment. The animal experiments described later were approved by the Ethics Committee, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

Experimental design

Rats were divided into four groups (n=10) as follows:

Group I: Rats received 0.1 ml/kg corn oil, via oral gavage for 21 days.

Group II: Rats received 0.1 ml/kg corn oil, via oral gavage and exposed to EMR of mobile phone for 1 h/day for 21 days (Memduh and Nilgun, 2012).

Group III: Rats received TQ (5 mg/kg/day) via oral gavage for 21 days; TQ was freshly prepared daily by addition of corn oil (1:1 v/v) just before administration to the rats. One hour later, rats were exposed to EMR of mobile phone for 1 hour (Hosseinzadeh et al., 2007).

Group IV: Rats received TQ (10 mg/kg/day) via oral gavage for 21 days; TQ was freshly prepared daily by addition of corn oil (1:1 v/v) just before administration to the rats. One hour later, rats were exposed to EMR of mobile phone for 1 hour (Hosseinzadeh et al., 2007).

Behavioral experiments included Morris water maze test (at day fifteen from EMR exposure till day eighteen with continuous daily exposure), two days later the same animals were exposed to elevated plus maze test (at day twenty). At the end of the experiment the rats were decapitated at day 21 one hour after the last exposure to EMR. The brains were carefully excised on an ice cooled glass plate and sectioned sagittally as right and left hemispheres, then immediately weighted and homogenized to obtain a 10 % (w/v) homogenate. Right hemispheres were homogenized in Tris-HCl buffer (50 mmol/l, pH 7.4), and were used for estimation of MDA, GSH, NO content and CAT activity. Left hemispheres were homogenized in an ice cold acidified n-butanol solution and were used for estimation of brain monoamines (5-HT, NE, and dopamine) contents.

EMR exposure system

The system consisted of a restrainer plastic tube (length: 15 cm, diameter: 5 cm) with a cone (3 cm length) in which the rat head was inserted. The end of the cone was opened and the rocket body had holes to facilitate breathing, minimize elevation in body temperature and decrease the stress of the rat while in the tube. Solid plastic disks were placed at the back of the restrainers to minimize the movements of the animals. All tubes were mounted circularly. Radiation (900 MHz) was produced by a mobile phone (model NOKIA 1280 with specific absorption rate (SAR) 1.15 W/kg). Mobile phones were activated by calling each other from 10:00 a.m to 11:00 a.m. The rats in group I were also placed in the restrained tubes as rats in the other groups for the same time, but without exposure to EMR (mobile phone off).

Behavioral tests

Morris water maze test (MWM)

The Morris water maze test is a classic test of spatial learning in rodents (Morris, 1984). The water maze was a black circular tank 150 cm in diameter and 62.5 cm in height. The tank was filled with water maintained at (20 ± 1°C) to a depth of 40 cm. The maze was divided geographically into four quadrants: northeast (NE), northwest (NW), southeast (SE), southwest (SW), and starting positions: north (N), south (S), east (E), west (w) that were equally spaced around the perimeter of the pool. A hidden circular platform (diameter: 13 cm) was located in the center of the NW quadrant, one cm below the surface of the water. All animals received a training session consisting of four trials, from day 15 till day 18. In all four trials, the starting position was different. A trial began by releasing the rats into the maze facing towards the wall of the pool. The latency to find the escape platform was recorded to a maximum of one minute. If the rat did not escape onto the platform within this time, it was guided to the platform and was allowed to remain there for 20 sec. Two hours after the last training trial (the fourth trial of the fourth day), the animals were subjected to a memory probe trial during which the rats swam for 60 sec in the absence of the training platform. All rats started from the same position, opposite to the target quadrant (the quadrant where the escape platform had been positioned). The time spent in the probe trial in seconds was calculated for each rat (the time spent in the target quadrant).

Elevated plus maze test (EPM)

The elevated plus maze is widely used to test anxiety in animal model (Calabrese, 2008). Experiments were run based on a previous method (Pellow et al., 1985). The plus maze apparatus consisted of two open arms (without walls), 10 cm × 50 cm (length), and two closed arms, (10 cm × 50 cm (length) × 40 cm (height), extending from a common central platform (10 cm ×10 cm). The brightly illuminated maze was made of wood, elevated to 50 cm above the floor. Each rat was placed individually at the center of the elevated plus maze with its head facing towards an open arm and observed for a period of 5 min. In the elevated plus maze test, the percentage of time spent in the open or closed arms was determined as follows:

$$\%\ Of\ time\ spent\ in\ seconds = \frac{\text{Number of seconds spent in arm}}{\text{300 seconds (5 min of observation)}}\ x\ 100$$

Oxidative stress parameters

Determination of brain lipid peroxides level

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) level in the brain homogenates supernatants (Mihara and Uchiyama, 1978). The principle of the assay depends on a colorimetric determination of pink pigment product, resulting from the reaction of one molecule of MDA with two molecules of thiobarbituric acid (TBA) at low pH (2-3) at 95°C for 45 min. The resultant colored product was extracted by n-butanol and measured at 535 nm spectrophotometrically, and expressed as nmol/g wet tissue.

Determination of reduced glutathione (GSH)

GSH was estimated spectrophotometrically by the method of Ellman , 1959 . Protein in brain homogenates was precipitated with 10% trichloroacetic acid and the contents were centrifuged at 2000 rpm for 5min. An aliquot of the clear supernatant (0.1ml) was taken and mixed with 1.7 ml of 0.1mM potassium phosphate buffer (pH 8) and 0.1 ml of Ellman's reagent. The optical density was measured at 412 nm against a blank, and expressed as µmol/g wet tissue.

Determination of catalase (CAT) activity

CAT activity assay was performed according to Aebi ,1984. CAT induces decomposition of H_2O_2 into water and oxygen. The rate of decomposition is proportional to the CAT concentration. The reaction proceeds for one minute, at which time the CAT is quenched with sodium azide. The remaining H_2O_2 facilitates the coupling of 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) and 4-aminoantipyrine (AAP) to give a quinoneimine dye measured at 240 nm. One unit CAT is the amount of enzyme that decomposes 1 µmole of H_2O_2 per minute, and catalase activity was expressed as U/mg protein

Total protein

The protein content was measured using bovine serum albumin (BSA) as a standard according to the method of Lowry et al., 1951. Different concentrations of BSA (0.01 to 0.1 mg/ml) were used to plot a standard curve. The principle of the assay is based on the reaction of copper with protein molecules in alkaline medium followed by the reduction of Folin-ciocalteu reagents by the copper-protein complex resulting in the development of a blue purple colour at room temperature, which is measured

colorimetrically at 500 nm, the samples value of proteins was used for calculating the catalase activity.

Determination of nitric oxide (NO) content

Nitric oxide was assayed according to the method of Miranda et al., 2001. Where brain homogenates were de-proteinated with absolute ethanol for 48 h at 4 °C, and then centrifuged at 12,000 r.p.m. for 15 min at 4 °C. To an aliquot of the supernatant, vanadium trichloride (0.8% in 1 M HCl) was added for the reduction of nitrate to nitrite, followed by the rapid addition of Griess reagent (N-1-naphthyl ethylenediamine dihydrochloride) (0.1%) and sulfanilamide (2% in 5% HCl). The reaction mixture was incubated for 30 min at 37 °C. The absorbance was measured at 540 nm and expressed as µmol/g wet tissue.

Brain monoamines

Serotonin as well as norepinephrine and dopamine contents in rat brain homogenates were carried out by fluorometric assay according to the method of Ciarlone, 1978. The method is based on a fluorometric assay in which a fluorescent product results from the reaction with ortho-phthalaldehyde solution (in case of serotonin) and the reaction with a mixture of alkaline sulfite and iodine solution (in case of norepinephrine and dopamine). The produced fluorescence was measured at its specific wave length using a fluorometer, and the values were expressed as µg/g wet tissue.

Statistical analysis

All results were expressed as mean ± S.E.M. The difference between groups was compared using one way analysis of variance (ANOVA) followed by Tukey-Kramer as post hoc test. The statistical analyses were performed with the software Graph Pad Prism, version 3 for windows, Graph Pad Software (San Diego, CA, USA). A value of $P < 0.05$ was considered significant for comparison of all results.

Results

Exposure of rats to EMR from mobile phone significantly decreased the time spent in the target quadrant by 35% as compared to group I. Oral administration of TQ (5 or 10 mg/kg) prior to exposure to EMR significantly increased the time spent in target quadrant by 41% and 44% respectively as compared to group II (Figure 1).

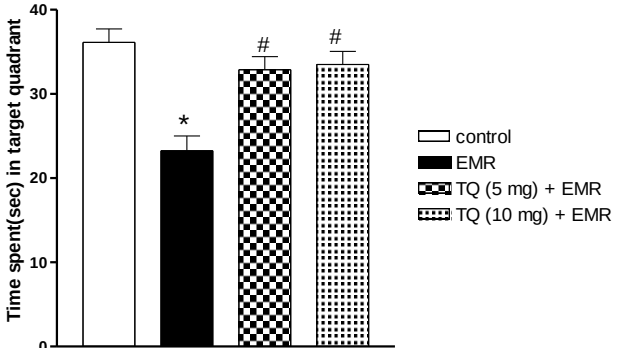

Figure 1: effect of oral administration of TQ (5 and 10 mg/kg) on the memory trial in rats exposed to EMR from mobile phone. Data is represented as mean±S.E.M. (n=10).
* significant difference at P < 0.05 from control group.
# significant difference at P < 0.05 from EMR group.

As illustrated in Figure (2a), EMR from mobile phone showed a significant decrease in the percentage of time spent in the open arm of EPM by 22% as compared to group I. While pretreatment with TQ (5 or 10 mg/kg) significantly increased the percentage of time spent in the open arm by 86% and 108% respectively as compared to group II. Exposure of the rats to mobile phone radiation showed a significant increase in the percentage of time spent in the closed arm of EPM by 8% as compared to group I. Moreover administration of TQ (5 or 10 mg/kg) prior to exposure to EMR significantly decreased that time by 36% and 41% respectively (Figure 2b).

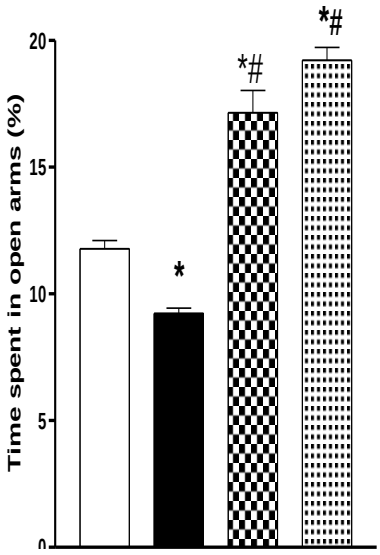

Figure 2a.

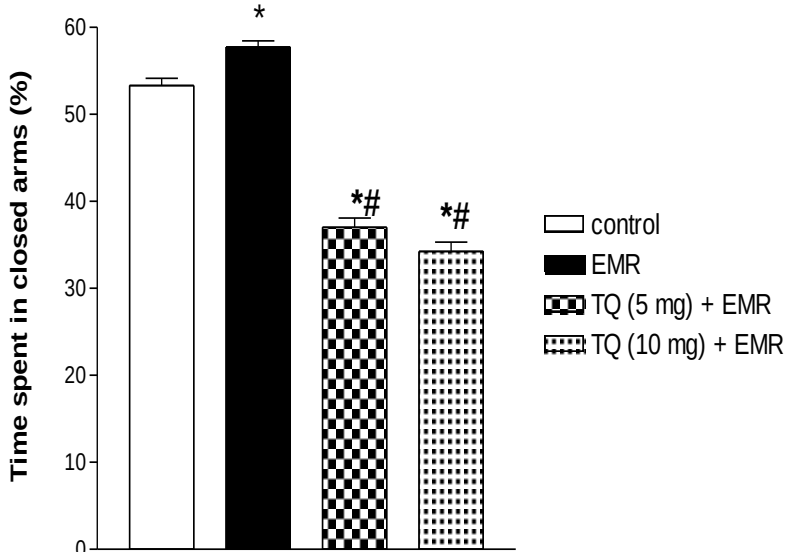

Figure 2b.

Figure 2a: effect of oral administration of TQ (5 and 10 mg/kg) on % time spent in the open arm in rats exposed to EMR from mobile phone. Figure 2b: effect of oral administration of TQ (5 and 10 mg/kg) on % time spent in the closed arm in rats exposed to EMR from mobile phone. Data is represented as mean±S.E.M. (n=10).

* significant difference at P < 0.05 from control group.

significant difference at P < 0.05 from EMR group.

Concerning the oxidative stress parameters the obtained results showed that EMR from mobile phone for 21 days produced a significant increase in brain lipid peroxides measured as MDA and NO contents by 36% and 48% respectively as compared to group I. However, pre-treatment with TQ (5 or 10 mg) normalized their levels (Table 1).

Table 1- Effects of TQ administration on malondialdehyde, reduced glutathione content, catalase activity and nitric oxide content in brain of EMR-exposed rats.

	Group I (Control)	Group II (EMR) `	Group III (TQ 5mg+EMR)	Group IV (TQ 10mg+EMR)
MDA (nmol/g tissue)	64.38 ± 1.81	87.63 ± 3.80*	67.38 ± 1.30#	64.88± 1.25#
GSH (µmol/g tissue)	0.75 ± 0.03	0.48 ± 0.03*	1.01± 0.04*#	1.23± 0.07*#@
CAT (U/mg protein)	9.40± 0.34	5.76 ± 0.28*	7. 86 ± 0.30#	8. 28 ± 0.35#
NO (µmol/g tissue)	5. 95 ± 0.25	8. 82 ± 0.34*	7. 35± 0.20#	6. 71± 0.24#

Data is represented as mean±S.E.M. (n=10).
* significant difference at P < 0.05 from group I
# significant difference at P < 0.05 from group II
@ significant difference at P < 0.05 from group III
ANOVA and Tukey-Kramer were used for statistical comparison of groups

Moreover, exposure of the rats to mobile phone for 21 days significantly decreased brain GSH content and CAT activity by 36% and 39% respectively as compared to group I. While administration of TQ (5 or 10 mg/kg) prior the exposure to EMR significantly reversed these levels. However administration of TQ (10 mg/kg) showed significant increase in GSH content by 22% as compared with group III (Table 1).

Table 2- Effect of TQ administration on serotonin, norepinephrine and dopamine contents in brain of EMR-exposed rats.

	Group I (Control)	Group II (EMR)	Group III (TQ 5mg+EMR)	Group IV (TQ 10mg+EMR)
5-HT (µg/g tissue)	0.67± 0.014	0.59± 0.004*	0.91 ± 0.012*#	1.00 ± 0.021*#@
NE (µg/g tissue)	0.53± 0.015	1.10± 0.014*	0.75 ± 0.029*#	0.64± 0.018*#@
DP (µg/g tissue)	1.96 ± 0.025	1.85 ± 0.021*	1.86 ± 0.016	1.90 ± 0.023

Data is represented as mean±S.E.M. (n=10).
* significant difference at P < 0.05 from group I
# significant difference at P < 0.05 from group II
@ significant difference at P < 0.05 from group III
ANOVA and Tukey-Kramer were used for statistical comparison of group

As given in Table (2), exposure to EMR from mobile phone significantly decreased both 5HT and DP brain contents, by 12% and 6% respectively while significantly elevated brain NE content by 108% as compared to group I. Pre-treatment with TQ (5 or 10 mg/kg) prior to EMR exposure caused a significant decrease in NE level by 32% and 42% respectively, accompanied by a significant increase in the brain 5HT content by 54% and 69% respectively as compared to group II. However, pre-treatment with TQ did not significantly affect the DP content as compared to group II. Furthermore pre-treatment with TQ (10 mg/kg) showed a significant increase in brain 5HT and a significant decrease in brain NE contents by 10% and 15% respectively as compared to group III.

Discussion

Morris water maze (MWM) is a test used to assess spatial memory and the ability to learn a specific task (Morris, 1984). In the current study, rats exposed to mobile phone radiation were unable to recall the exact position of the hidden platform on the memory probe trial. This, in turn, points to the poor spatial learning ability. These results are in agreement with Hao et al., 2013 who concluded that the cognitive function of rats exposed to mobile phone radiation is influenced by stress. MWM learning is aversely motivated behavior, acquiring this task will always be a stressful event for the animals involved. Moreover, the additional stress from EMR of mobile phone exposure may lead to profound effects on cognitive performance.

Both neurons and glial cells interact dynamically to enable information processing and behavior (Laming et al., 2000). Brillaud et al., 2007 reported that mobile phone exposure-induced glial reactivity in the rat brain may be attributed to the hypertrophy of glial cells and disruption of the integrity of blood-brain barrier (Finnie et al., 2002). The exposure of rats to mobile phone electromagnetic radiation resulted in neuronal damage in the brain cortex, hippocampus, and basal ganglia (Salford et al., 2003).

Oxidative stress may also contribute to the learning and memory deficits after exposure to EMR from mobile phone. Oxidative damage to the rat synapse in the cerebral cortex and hippocampus has been previously reported to contribute to the deficit of cognitive functions (Fukui et al., 2001).

On the other hand, elevated plus maze (EPM) test is an effective method to assess the neurobehavioral profile of animals under influence of anxiogenic/anxiolytic agents (Pellow, 1985). The present study indicates that exposure to EMR from mobile phones caused reduction in percent time spent in open arms and an increase in the percent time spent in closed arms. The current results reveal the anxiety behavior induced by EMR, which is in harmony with the previously reported findings of Kumar et al., 2009. EMR emitted by mobile phone can increase free radical production in the rat brain (Memduh and Nilgun, 2012). Oxidative stress is strongly correlated to anxiety behavior (Patki et al., 2013). Moreover, EMR can attenuate endogenous opioid peptides-induced analgesia in rodents (Del Seppia et al., 2007). The opioid system is well known to be involved in the regulation of anxiety behavior induced by variety of stressful stimuli (Kudryavtseva et al., 2004).

The animals treated with TQ showed a significant increase in the time spent in the target quadrant in MWM, which emphasizes the anxiolytic effect of TQ. This effect

can be attributed to the powerful antioxidant property of TQ that prevented the neurodegeneration and consequently improved the cognitive function in rats. Similarly, rats treated with TQ showed a significant increase in the time spent in open arms and significant decrease in the time spent in closed arms of EPM. Perveen et al., 2009 reported that repeated administration of *Nigella sativa* has an anxiolytic effect in rats.

In the current study, a significant increase in the brain level of lipid peroxidation was found in rats exposed to EMR. This result suggests that EMR from mobile phone can induce brain damage through increasing oxidative stress and lipid peroxidation. Similar findings were presented by Memduh and Nilgun, 2012. The observed increase in the level of MDA, accompanied by a significant decrease in brain GSH, can be attributed to GSH involvement in the detoxification of the increased free radicals produced within the cell in rat brain during mobile phone exposure. Friedman et al., 2007 reported that EMR from mobile phone can stimulate NADPH oxidase in the plasma membrane of mammalian cells, which rapidly generates ROS leading to cellular GSH depletion. On the other hand, catalase (CAT) is an endogenous antioxidant enzyme found in nearly all living organisms. It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Dasdag et al., 2008). CAT activity was significantly decreased in brain tissue of rats exposed to EMR from mobile phone. This finding is in accordance with that of Memduh and Nilgun, 2012. The decrease in brain CAT activity indicates a high degree of oxidative stress and increased H_2O_2 production after mobile phone exposure, and suggests an over consumption of the enzyme related to increased production of H_2O_2 in vivo.

The present investigation demonstrated that NO level was found to be increased following mobile phone exposure. These results are in agreement with the previous reports of Dasdag et al., 2008. The increased NO level may be related to mobile phone radiation-induced stress. NO induction was reported in various stressful conditions (Ozguner et al., 2005). It is likely that EMR-induced oxidative stress may lead to upregulation of iNOS and overproduction of NO which combines with ROS forming peroxynitrite, a potent oxidizing molecule capable of eliciting lipid peroxidation and cellular damage.

Treatment of rats with TQ prior to mobile phone exposure protected the brain tissues against oxidative stress produced by mobile phone radiation by decreasing MDA level and increasing GSH concentration. This decline in brain MDA demonstrates the antioxidant effect of TQ, and denotes its free radical scavenging activity. It is well documented that TQ acts as a scavenger of superoxide, hydroxyl radical and singlet molecular oxygen (Hosseinzadeh et al., 2007). Administration of TQ showed a significant rise in CAT activity in the brain of rats exposed to mobile phone radiation. Moreover TQ is reported to stimulate several important antioxidant enzymes like, CAT, SOD and GSH-Px (Ismail et al., 2010). These indirect antioxidative actions of TQ might explain its protective action in the brain tissue under conditions of high oxidative stress. The inhibitory effect of TQ against NO production has been reported previously. Where, TQ inhibited iNOS protein synthesis and iNOS mRNA expression in rat (El-Mahmoudy et al., 2002). Thus, TQ may reduce oxidative stress leading to the prevention of iNOS upregulation, decreased NO level and inhibition of peroxynitrite formation.

The current study showed a significant increase in the level of norepinephrine in the brains of rats exposed to EMR from mobile phones. The increased activity of NE in

the limbic system and decreased one in the cerebral cortex result in anxiety behavior (Rajendra et al., 2004). This type of anxiety may affect the circadian rhythm and also induce restlessness. The mechanism of increase in norepinephrine level can be attributed to enhaced activity of dopamine beta hydroxylase (DBH) enzyme, which converts dopamine to norepinephrine. The DBH requires two copper atoms per subunit for its activity (Klinman and Brenner, 1988). Copper is a paramagnetic metal and its role in the increasing activity of DBH may be altered by the external magnetic fields (Rajendra et al., 2004).

Serotonin plays an important role in modulation of various behaviors. Evidence supporting the involvement of central 5-HT in anxiety-related behavior and in the mechanism of action of anxiolytic agent was previously reported (Handley and McBlane, 1993). In the current study, exposure of rats to EMR from mobile phone results in behavioral changes which may be linked to decreased brain serotonin level. The observed decrease in brain serotonin level may be attributed to decreased release of 5-HT and deactivation of serotoninergic system (Kumar, 2002) to reduction of tryptophan level which is the rate-limiting step in the synthesis of serotonin (Kish, 2001), or to increased conversion of serotonin to 5- hydroxyindoleacetic acid by MAO-A (Siegel et al., 2007). Oral administration of TQ decreased NE level which may be explaining the anxiolytic effect of TQ (Rajendra et al., 2004). On the other hand the increased level of 5-HT observed on treatment with TQ is in accordance with the finding of Perveen et al., 2009 who observed that repeated administration of *N.sativa* oil increased brain tryptophan level in rats. It is well-documented that serotonin synthesis in brain depends upon the availability of the precursor amino acid tryptophan to serotonergic neurons (Leathwood, 1987).

Dopamine is known to be an efficient antioxidant and protects neurocytes from oxidative stress by scavenging free radicals, which may initiate oxidation of dopamine under oxidative stress condition (Iuga., 2011). In the present study, rats exposed to mobile phone radiation showed a significant decrease in dopamine level, which may be related to the oxidation of dopamine by free radical and ROS produced by electromagnetic radiation from mobile phone. However, TQ administration at the doses used did not affect dopamine levels.

Conclusion

The current study provides two important findings: Firstly, it demonstrated that mobile phones caused oxidative damage and alteration in brain monoamines which was accompanied with neurobehavioral changes. Secondary, concomitant administration of thymoquinone as an antioxidant, a potent free radical scavenger and an obvious modulator to the brain monoamines, significantly prevented the behavioral abnormalities and corrected the antioxidant status of the brain tissue in rats exposed to mobile phone radiation. It is greatly recommended to use thymoquinone concurrently with heavy exposure to mobile phone radiation. Further clinical studies are mandated to emphasize these results in human.

Declaration of interest

The authors declare that there are no conflicts of interest.

REFERENCES

Aebi H.(1984): Catalase in vitro. Methods Enzymol; 105: 121-126.

Alhebshi AH, Gotoh M, Suzuki I.(2013): Thymoquinone protects cultured rat primary neurons against amyloid β-induced neurotoxicity. Biochem Biophys Res Commun; 433: 362-367.

Ali, B.H. and Blunden, G. (2003). Pharmacological and toxicological properties of Nigella sativa. Phytother, Res. 17: 299–305.

Al-Majed AA, Al-Omar FA, Naqi MN.(2006): Neuroprotective effects of thymoquinone against transient forebrain ischemia in the rat hippocampus. Eur J Pharmacol; 543: 40–47.

Brillaud E, Piotrowski A, deSeze R.(2007): Effect of an acute 900MHz GSM exposure on glia in the rat brain: a time-dependent study. Toxicology; 238: 23-33.

Calabrese EJ.(2008): An assessment of anxiolytic drug screening tests: hormetic dose responses predominate. Crit Rev Toxicol; 38: 489–542.

Ciarlone AE.(1978): Further modification of a fluorometric method for analyzing brain amines. Microchemical J; 23: 9-12.

Consales, C, Merla, C, Marino, C and Benassi, B (2012). Electromagnetic field, oxidative stress, and neurodegeneration. Int J Cell Biol.2012; 683897.

Croft RJ, Chandler JS, Burgess AP, Barry RJ, Williams JD, Clark AR.(2002): Acute mobile phone operation affects neural function in humans. Clin Neurophysiol; 113: 1623-1632.

Dasdag S, Akdag MZ, Ulukaya E, Uzunlar AK, Ocak AR.(2009): Effect of mobile phone exposure on apoptotic glial cells and status of oxidative stress in rat brain. Electromagn Biol Med; 28: 342-354.

Dasdag S, Bilgin HM, Akdag H, Celik H, Aksen F.(2008): Effect of long term mobile phone exposure on oxidative-antioxidative processes and nitric oxide in rats. Biotechnol Biotechnol Eq; 22: 992 – 997.

Del Seppia C, Ghione S, Luschi P, Ossenkopp KP, Choleris E, Kavaliers M.(2007): Pain perception and electromagnetic fields. Neurosci Biobehav Rev; 31: 619-642.

Di Giacomo, C., Acquaviva, R., Sorrenti, V., Vanella, A., Grasso, S., Barcellona, M.L., Galvano, F., Vanella, L. and Renis, M. (2009). Oxidative and antioxidant status in plasma runners: effect of oral supplementation with natural antioxidants. J Med Food, 12: 145-150.

Dockal, E.R., Cass,Q.B., Brocksom, T.J., Brocksomand, U. and Corrěa, A.G.(1985). A Simple and Efficient Synthesis of Thymoquinone and Methyl P-Benzoquinone. Synth Commun, 15:1033 - 1036.

Drog, W. (2002). Aging-related changes in thiol/disulfide redox state: implications for the use of thiol antioxidants. Exp Gerontol, 37: 1333-1345.

Ellman GL.(1959): Tissue sulfhydryl groups. Arch Biochem Biophys; 82: 70-77.

El-Mahmoudy A, Matsuyama H, Brogan MA, Shimizu Y, El-Sayed MG, Minamoto N, Takewaki T.(2002): Thymoquinone suppresses expression of inducible nitric oxide synthase in rat macrophages. Int Immunopharmacol; 11: 1603–1611.

Finnie JW, Blumbergs PC, Manavis J, Utteridge TD, Gebski V, Davies RA, Vernon-Roberts B, Kuchel TR.(2002): Effect of long-term mobile communication microwave exposure on vascular permeability in mouse brain. Pathology; 34: 344-347.

Friedman J, Kraus S, Hauptman Y, Schiff Y, Seger R.(2007): Mechanism of short-term ERK activation by electromagnetic fields at mobile phone frequencies. Biochem J; 405: 559-568.

Fukui K, Onodera K, Shinkai T, Suzuki S, Urano S.(2001): Impairment of learning and memory in rats caused by oxidative stress and aging, and changes in antioxidative defense systems. Ann N Y Acad Sci; 928: 168–175.

Gałecka, E., Mrowicka, M., Malinowska, K. and Gałecki, P. (2008). Chosen non-enzymatic substances that participate in a protection against overproduction of free radicals. Pol Merkur Lekarski, 25: 269-272.

Gaweł S, Wardas M, Niedworok E, Wardas P.(2004): Malondialdehyde (MDA) as a lipid peroxidation marker. Wiad Lek; 57: 453- 455.

Goven A, Kaya N.(2005): Determination of reduced glutathione, glutathione-S-transferase and selenium levels in goose liver cells with damage induced by carbon tetrachloride and ethanol. Tur J Vet Anim Sci; 29: 1233–1238.

Handley SL, McBlane JW.(1993): 5HT drugs in animal models of anxiety. Psychopharmacology; 112: 13-20.

Hao D, Yang L, Chen S, Tong J, Tian Y, Su B, Wu S, Zeng Y.(2013): Effects of long-term electromagnetic field exposure on spatial learning and memory in rats. Neurol Sci; 34: 157-164.

Hosseinzadeh H, Parvardeh S, Asl MN, Sadeghnia HR, Ziaee T.(2007): Effect of thymoquinone and Nigella sativa seeds oil on lipid peroxidation level during global cerebral ischemia-reperfusion injury in rat hippocampus. Phytomedicine; 14: 621-627.

Houghton PJ, Zarka R, De las Heras B, Hoult JR.(1995): Fixed oil of Nigella sativa and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med; 61: 33–36.

ICNIRP. (1998). International Commission on Non-Ionizing Radiation Protection. Guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields up to 300 GHz. Health physics, 74: 494-522.

ICNIRP. (2009). International Commission on Non-Ionizing Radiation Protection. Exposure to high frequency electromagnetic fields, biological effects and health consequences (100 kHz –300 GHz)—review of the scientific evidence and health consequences.

IEEE. (1992). Institute of Electrical and Electronics Engineers Standard for safety with Respect to Human Exposure to Radio Frequency Electromagnetic fields, 3 kHz to 300 GHz, The IEEE Inc., New York, pp. 1-76.

Ismail M, Al-Nageep G, Chan KW.(2010): Nigella sativa thymoquinone-rich fraction greatly improves plasma antioxidant capacity and expression of antioxidant genes in hypercholesterolemic rats. Free Radic Biol Med; 48: 664–672.

Iuga C, Alvarez-Idaboy JR,(2011): Vivier-Bunge A. ROS initiated oxidation of dopamine under oxidative stress conditions in aqueous and lipidic environments. J Phys Chem B; 115: 12234-12246.

Kesari KK, Kumar S, Behari J.(2012): Pathophysiology of microwave radiation: effect on rat brain. Appl biochem biotechnol; 166: 379-388.

Kish SJ, Kalasinsky KS, Derkach P, Schmunk GA, Guttman M, Ang L, Adams V, Furukawa Y, Haycock JW.(2001): Striatal dopaminergic and serotonergic markers in human heroin users. Neuropsychopharmacology; 24: 561-567.

Klinman JP, Brenner M.(1988): Role of copper and catalytic mechanism in the copper monooxygenase, dopamine beta-hydroxylase (D beta H). Prog Clin Biol Res; 274: 227-248.

Kudryavtseva NN, Gerrits MA, Avgustinovich DF, Tenditnik MV, Van Ree JM.(2004): Modulation of anxiety-related behaviors by mu- and kappa-opioid receptor agonists depends on the social status of mice. Peptides; 25: 1355-1363.

Kumar RS, Sareesh NN, Nayak S, Mailankot M.(2009): Hypoactivity of Wistar rats exposed to mobile phone on elevated plus maze. Indian J Physiol Pharmacol; 53: 283-286.

Kumar S.(2002): Aluminium-induced changes in the rat brain serotonin system. Food Chem Toxicol; 40: 1875–1880.

Laming PR, Kimelberg H, Robinson S, Salm A, Hawrylak N, Müller C, Roots B, Ng K.(2000): Neuronal-glial interactions and behaviour. Neurosci Biobehav Rev; 24: 295-340.

Leathwood PD. (1987): Tryptophan availability and serotonin synthesis. Proc Nutr Soc; 46: 143-156.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ.(1951): Protein measurement with the Folin phenol reagent. J Biol Chem; 193: 265-275.

Maes A, Collier M, Verschaeve L.(2001): Cytogenetic effects of 900 MHz (GSM) microwaves on human lymphocytes. Bioelectromagnetics; 22: 91-96.

Memduh K, Nilgun S.(2012): Oxidative stress in hippocampus induced by 900 MHz electromagnetic field emitting mobile phone: Protection by melatonin. Biomedical Research; 23: 147-151.

Mihara M, Uchiyama M.(1978): Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem; 86: 271-278.

Miranda KM, Espey MG, Wink DA. A rapid,(2001): simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide; 5: 62–71.

Morris R. (1984): Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods; 11: 47–60.

Ozguner F, Altinbas A, Ozaydin M, Dogan A, Vural H, Kisioglu AN, Cesur G, Yildirim NG.(2005): Mobile phone-induced myocardial oxidative stress: protection by a novel antioxidant agent caffeic acid phenethyl ester. Toxicol Ind Health; 21: 223-230.

Patki G, Solanki N, Atrooz F, Allam F, Salim S.(2013): Depression, anxiety-like behavior and memory impairment are associated with increased oxidative stress and inflammation in a rat model of social stress. Brain Res; 1539: 73-86.

Pellow S, Chopin P, File SE, Briley M.(1985): Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods; 14: 149–167.

Perveen T, Haider S, Kanwal S, Haleem DJ.(2009): Repeated administration of Nigella sativa decreases 5-HT turnover and produces anxiolytic effects in rats. Pak J Pharm Sci; 22: 139-144.

Popet L, Lacrama AM, Isvoran A, Ostafe V.(2006): The effect of microwave radiation on catalase extracted from taraxacum roots. Ann West Univ Timisoara Ser Chem; 15: 76–86.

Puizina-Ivić, N. (2008). Skin aging. Acta Dematovenerol Alp Panonica Adriat, 17: 47-54.

Radad K, Hassanein K, Al-Shraim M, Moldzio R, Rausch WD.(2014): Thymoquinone ameliorates lead-induced brain damage in Sprague Dawley rats. Exp Toxicol Pathol; 66: 13-17.

Rajendra P, Sujatha H, Devendranath D, Gunasekaran B, Sashidhar R, Subramanyam C,(2004): Channakeshava Biological effects of power

frequency magnetic fields: Neurochemical and toxicological changes in developing chick embryos. Biomagn Res Technol; 2: 1.

Salford LG, Brun AE, Eberhardt JL, Malmgren L, Persson BR.(2003): Nerve cell damage in mammalian brain after exposure to microwaves from GSM mobile phones. Environ Health Perspect; 111: 881–883.

Siegel A, Bhatt S, Bhatt R, Zalcman SS.(2007): The neurobiological bases for development of pharmacological treatments of aggressive disorders. Curr Neuropharmacol; 5: 135-147.

Sorce S, Krause KH.(2009): NOX enzymes in the central nervous system: from signaling to disease. Antioxid Redox Signal; 11: 2481-2504.

Ullah I, Ullah N, Naseer MI, Lee HY, Kim MO.(2012): Neuroprotection with metformin and thymoquinone against ethanol-induced apoptotic neurodegeneration in prenatal rat cortical neurons. BMC Neurosci; 13: 11.

التأثير التحسيني لثيموكينون على التأثيرات الضارة للموجات المنبعثة من الهاتف المحمول في مخ الجرذان

للسادة الدكاترة

د/ اماني علي عيسى ، د/ انجي محمد المرسي ، د/ السيد كمال السيد

مـــــــــــــن

قسم الادوية والسموم كلية الصيدلة جامعة حلوان عين حلوان القاهرة - مصر

أثيرت مخاوف هائلة حول أن التعرض للأشعة الكهرومغناطيسية المنبعثة من الهواتف المحمولة قد تؤثر على صحة البشر، وتشير العديد من الدراسات التجريبية حول الدور المحتمل لشقائق الأكسجين الحرة الناتجة عن الأشعة الكهرومغناطيسية في تدمير أنسجة المخ و حدوث تغيرات سلوكية عصبية، وكان الهدف من الدراسة الحالية بحث الأثر الوقائي لمادة ثيموكينون على الضرر التأكسدي الناتج عن هذه الأشعة في أنسجة مخ الجرذان. تشير نتائج هذة الدراسة إلى أن التعرض للأشعة الكهرومغناطيسية يقلل من الوقت الذي تمكثه الجرذان في الربع المحدد داخل إختبار المتاهة المائية و نسبة الوقت الذي تمكثه في الاذرع المغلقة داخل متاهة الصليب المرتفع بالإضافة الى زيادة نسبة الوقت الذي تمكثه في الاذرع المفتوحة داخل متاهة الصليب المرتفع بالاضافة الى زيادة فى محتوى المخ من مالونديالديهايد وأكسيد النيتروجين ونوراينفرين وقد تزامن ذلك مع إنخفاض نشاط إنزيم الكتالاز و محتوى المخ من جليوتاثيون وكذلك مستوى كل من سريتونين ودوبامين، وقد أدى حقن الجرذان بمادة ثيموكينون (٥ و ١٠ مليغرام/ كيلوغرام) الى منع حدوث هذه التغيرات و يعزى التأثير الوقائي العصبي لمادة ثيموكينون إلى قدرتها القوية كمضادة للأكسدة.