

Radiofrequency Radiation Free Radical Studies Updated March 2014

106 papers: **Effect=93 (88%); No Effect=13 (12%)**

(E) Abu Khadra KM, Khalil AM, Abu Samak M, Aljaberi A. Evaluation of selected biochemical parameters in the saliva of young males using mobile phones. Electromagn Biol Med. 2014 Feb 5. [Epub ahead of print]

Abstract The biochemical status in the saliva of 12 males before/after using mobile phone has been evaluated. Radio frequency signals of 1800 MHz (continuous wave transmission, 217 Hz modulate and Global System for Mobile Communications [GSM - non-DTX]) with 1.09 w/kg specific absorption rate (SAR) value were used for 15 and 30 min. Cell phone radiation induced a significant increase of superoxide dismutase (SOD); there was a statistically significant effect of talking time on the levels of SOD, $F(2, 33) = 8.084$, $p < 0.05$, $\omega = 0.53$. The trend analysis suggests a significant quadratic trend, $F(1, 33) = 4.891$, $p < 0.05$; indicating that after 15 min of talking the levels of SOD increased, but as talking time increased the SOD activity started to drop. In contrast to this, there was no statistically significant effect of talking time on the level of salivary albumin, cytochrome c, catalase or uric acid. Results suggest that exposure to electromagnetic radiation may exert an oxidative stress on human cells as evidenced by the increase in the concentration of the superoxide radical anion released in the saliva of cell phone users.

(E) Achudume A, Onibere B, Aina F, Tchokossa P. Induction of oxidative stress in male rats subchronically exposed to electromagnetic fields at non-thermal intensities. J Electromagnetic Analysis and Applications 2(8), 482-487, 2010. (LI)

To investigate the oxidative stress-inducing potential of non-thermal electromagnetic fields in rats. Male Wister rats were exposed to electrical field intensity of $2.3 \pm 0.82 \mu\text{V/m}$. Exposure was in three forms: continuous waves, or modulated at 900 MHz or modulated GSM-nonDTX. The radio frequency radiation (RFR) was 1800 MHz, specific absorption radiation (SAR) (0.95-3.9 W/kg) for 40 and/or 60 days continuously. Control animals were located > 300 m from base station, while sham control animals were located in a similar environmental conditions, but in the vicinity of a non-functional base station. The rats were assessed for thiobarbituric and reactive species (TBARS), reduced glutathione (GSH) content, catalase activity, glutathione reductase (GR) and glucose residue after 40 and 60 days of exposure. At 40 days, electromagnetic radiation failed to induce any significant alterations. However, at 60 days of exposure various attributes evaluated decreased. The respective decreases in both nicotinamide adenine dinucleotide phosphate (NADPH) and Ascorbate- linked lipid peroxidation (LPO) with concomitant diminution in enzymatic antioxidative defense systems resulted in decreased glucose residue. The present studies showed some biochemical changes that may be associated with a prolong exposure to electromagnetic fields and its relationship to the activity of antioxidant system in rat Regular assessment and early detection of antioxidative defense system among people working around the base stations are recommended.

(E) Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, Sharma R. Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. Fertil Steril. 92(4) 1318-1325, 2009.

OBJECTIVE: To evaluate effects of cellular phone radiofrequency electromagnetic waves (RF-EMW) during talk mode on unprocessed (neat) ejaculated human semen. DESIGN: Prospective pilot study. SETTING: Center for reproductive medicine laboratory in tertiary hospital setting. SAMPLES: Neat semen samples from normal healthy donors (n = 23) and infertile patients (n = 9). INTERVENTION(S): After liquefaction, neat semen samples were divided into two aliquots. One aliquot (experimental) from each patient was exposed to cellular phone radiation (in talk mode) for 1 h, and the second aliquot (unexposed) served as the control sample under identical conditions. MAIN OUTCOME MEASURE(S): Evaluation of sperm parameters (motility, viability), reactive oxygen species (ROS), total antioxidant capacity (TAC) of semen, ROS-TAC score, and sperm DNA damage. RESULT(S): Samples exposed to RF-EMW showed a significant decrease in sperm motility and viability, increase in ROS level, and decrease in ROS-TAC score. Levels of TAC and DNA damage showed no significant differences from the unexposed group. CONCLUSION(S): Radiofrequency electromagnetic waves emitted from cell phones may lead to oxidative stress in human semen. We speculate that keeping the cell phone in a trouser pocket in talk mode may negatively affect spermatozoa and impair male fertility.

(E) Akoev IG, Pashovkina MS, Dolgacheva LP, Semenova TP, Kalmykov VL. [Enzymatic activity of some tissues and blood serum from animals and humans exposed to microwaves and hypothesis on the possible role of free radical processes in the nonlinear effects and modification of emotional behavior of animals]. [Article in Russian] Radiats Biol Radioecol. 42(3):322-330, 2002. (LI)

The dependence of activities of actomyosin ATPase, alkaline phosphatase, aspartataminotransferase, monoaminoxidase and that of affective rat behavior on frequency of modulation of microwaves (0.8-10 microW/cm²) was explored at short-time actions. Series of nonlinear phenomenons, inexplicable from positions of the energy approaches are revealed, The working hypothesis explaining opportunity of high performance of weak and super-weak microwaves and other revealed phenomena by resonance interaction of such electromagnetic radiofrequency radiation with paramagnetic molecules of biological tissues was proposed. This resonance interaction activate free radicals and initiate auto-supporting and auto-intensifying of chain chemical reactions. The spontaneous autocatalytic oxidation of catecholamines enlarges a common pool of free radicals, capable to participate in such enhanced generating. The protective role of monoaminoxidase is postulated. Monoaminoxidase is basically located on an outer surface of mitochondrias and it is deaminating monoamines. The deaminating prevents penetration of catecholamines inside of mitochondrias and their quinoid oxidation there with formation of free-radical semi-quinons, capable to destroy system of ATP synthesis. These inferences are obliquely confirmed by the experimentally revealed correlation between activity of monoaminoxidase and integrative activity of the rat brain.

Anghileri LJ, Mayayo E, Domingo JL. Iron-radiofrequency synergism in lymphomagenesis. Immunopharmacol Immunotoxicol. 28(1):175-183, 2006.

The parenteral **iron** administration effects on the acceleration of lymphomagenesis by radiofrequency exposure were investigated using an animal model that develops spontaneous lymphomas with ageing. Complementary studies of the in vivo uptake of ⁵⁹Fe-labeled ferric gluconate and ferric-ATP complex showed differences ob absorption and excretion between both **iron** compounds. In vitro assays of their

effects on calcium cellular uptake using a cell model and tissues homogenates showed a molecular structure-dependence. The current results (mortality, clinical and histopathological examinations) demonstrated a synergism between radiofrequency and ferric gluconate, and the increased risk of radiofrequency exposure when it is simultaneous to parenteral **iron** administration.

(E) *Atasoy HI, Gunal MY, Atasoy P, Elgun S, Bugdayci G. Immunohistopathologic demonstration of deleterious effects on growing rat testes of radiofrequency waves emitted from conventional Wi-Fi devices. J Pediatr Urol. 2012 Mar 30. [Epub ahead of print] (LI)

OBJECTIVE: To investigate effects on rat testes of radiofrequency radiation emitted from indoor Wi-Fi Internet access devices using 802.11.g wireless standards. METHODS: Ten Wistar albino male rats were divided into experimental and control groups, with five rats per group. Standard wireless gateways communicating at 2.437 GHz were used as radiofrequency wave sources. The experimental group was exposed to radiofrequency energy for 24 h a day for 20 weeks. The rats were sacrificed at the end of the study. Intracardiac blood was sampled for serum 8-hydroxy-2'-deoxyguanosine levels. Testes were removed and examined histologically and immunohistochemically. Testis tissues were analyzed for malondialdehyde levels and prooxidant-antioxidant enzyme activities. RESULTS: We observed significant increases in serum 8-hydroxy-2'-deoxyguanosine levels and 8-hydroxyguanosine staining in the testes of the experimental group indicating DNA damage due to exposure ($p < 0.05$). We also found decreased levels of catalase and glutathione peroxidase activity in the experimental group, which may have been due to radiofrequency effects on enzyme activity ($p < 0.05$).

CONCLUSIONS: These findings raise questions about the safety of radiofrequency exposure from Wi-Fi Internet access devices for growing organisms of reproductive age, with a potential effect on both fertility and the integrity of germ cells.

(E) *Avci B, Akar A, Bilgici B, Tunçel ÖK. Oxidative stress induced by 1.8 GHz radio frequency electromagnetic radiation and effects of garlic extract in rats. Int J Radiat Biol. 88(11):799-805, 2012.

PURPOSE: We aimed to study the oxidative damage induced by radiofrequency electromagnetic radiation (RF-EMR) emitted by mobile telephones and the protective effect of garlic extract used as an anti-oxidant against this damage. MATERIALS AND METHODS: A total of 66 albino Wistar rats were divided into three groups. The first group of rats was given 1.8 GHz, 0.4 W/kg specific absorption rate (SAR) for 1 h a day for three weeks. The second group was given 500 mg/kg garlic extract in addition to RF-EMR. The third group of rats was used as the control group. At the end of the study, blood and brain tissue samples were collected from the rats. RESULTS: After the RF-EMR exposed, the advanced oxidation protein product (AOPP) levels of brain tissue increased compared with the control group ($p < 0.001$). Garlic administration accompanying the RF-EMR, on the other hand, significantly reduced AOPP levels in brain tissue ($p < 0.001$). The serum nitric oxide (NO) levels significantly increased both in the first and second group ($p < 0.001$). However, in the group for which garlic administration accompanied that of RF-EMR, there was no difference in serum NO levels compared with the RF-EMR exposed group ($p > 0.05$). There was no significant difference among the groups with respect to malondialdehyde (MDA) levels in brain tissue and blood samples ($p > 0.05$). Similarly, no difference was detected among the groups regarding serum paroxonase (PON) levels ($p > 0.05$). We did not detect any PON levels in the brain tissue. CONCLUSIONS: The exposure of RF-EMR similar to 1.8 GHz Global system for mobile communication (GSM) leads to protein oxidation in brain tissue and an increase in serum NO. We observed that garlic administration reduced protein oxidation in brain tissue and that it did not have any effects on serum NO levels.

(E) Aweda MA, Gbeneditse S, Meidinyo RO. Effects of 2.45 GHz microwave exposures on the peroxidation status in Wistar rats. Niger Postgrad Med J. 10(4):243-246, 2003.

One of the consequences of exposures to microwave (MW) radiations is the enhanced production of free O₂, free radicals, peroxides and superoxides. The effects on the lipid peroxidation status (LPS) of whole body irradiation of 120 Wistar rats with 2.45 GHz MW at a power density of 6mWcm⁻² have been studied using the MW generator model ER6660E from Toshiba UK Ltd. The LPS in the rats was monitored for a period of 8 weeks post irradiation using thiobarbituric acid (TRA) method. The MW exposures caused an increase in the LPS from the mean control value of 4.18 x 10⁻⁶g l⁻¹ to a maximum of 6.50 x 10⁻⁶ g l⁻¹ within the first 24 hrs, and then gradually reduced to control value after about a week. 1mg kg⁻¹ of ascorbic acid administered before irradiation caused a decrease in the LPS from the control value to a minimum of 2.86 x 10⁻⁶g l⁻¹ within the first week. The value then gradually rose to a maximum of 3.96 x 10⁻⁶g l⁻¹ within the monitoring period. 1 mg kg⁻¹ of α-tocopherol also administered before irradiation also caused a decrease in the LPS from the control value to a minimum of 2.10 x 10⁻⁶ g l⁻¹ within the first week. The value then gradually rose to a maximum of 3.94 x 10⁻⁶ g l⁻¹ within the monitoring period. The results obtained from this study demonstrate that MW exposures cause significant increase in the LPS and there are protective effects of the anti-oxidants ascorbic acid and alpha-tocopherol.

(E) Ayata A, Mollaoglu H, Yilmaz HR, Akturk O, Ozguner F, Altuntas I. Oxidative stress-mediated skin damage in an experimental mobile phone model can be prevented by melatonin. J Dermatol. 31(11):878-883, 2004.

Most mobile phones emit 900 MHz of radiation that is mainly absorbed by the external organs. The effects of 900 MHz of radiation on fibrosis, lipid peroxidation, and anti-oxidant enzymes and the ameliorating effects of melatonin (Mel) were evaluated in rat skin. Thirty Wistar-Albino rats were used in the study. The experimental groups were the control group, the irradiated group (IR), and the irradiated+Mel treated group (IR+Mel). A dose of 900 MHz, 2 W radiation was applied to the IR group every day for 10 days (30 min/day). The IR+Mel group received 10 mg/kg/day melatonin in tap water for 10 days before the irradiation. At the end of the 10th day, a skin specimen was excised from the thoracoabdominal area. The levels of malondialdehyde (MDA) and hydroxyproline and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were studied in the skin samples. MDA and hydroxyproline levels and activities of CAT and GSH-Px were increased significantly in the IR group compared to the control group (p<0.05) and decreased significantly in the IR+Mel group (p<0.05). SOD activity was decreased significantly in the IR group and this decrease was not prevented by the Mel treatment. These results suggest that rats irradiated with 900 MHz suffer from increased fibrosis and lipid peroxidation (LPO). Mel treatment can reduce the fibrosis and LPO caused by radiation.

(E) *Aydin B, Akar A. Effects of a 900-MHz electromagnetic field on oxidative stress parameters in rat lymphoid organs, polymorphonuclear leukocytes and plasma. Arch Med Res. 42(4):261-267, 2011.

BACKGROUND AND AIMS: The present study investigated the effects of a 900-MHz electromagnetic field (EMF) for 2 h/day for 45 days on lymphoid organs (spleen, thymus, bone marrow), polymorphonuclear leukocytes (PMNs) and plasma of rats, focusing on changes in the enzymatic and nonenzymatic antioxidant system. We determined whether there is any difference between immature and mature rats in terms of oxidative damage caused by EMF and tested recovery groups to determine whether EMF-induced damage is reversible in immature and mature rats.

METHODS: Twenty four immature and 24 mature rats were divided randomly and equally into six

groups as follows: two control groups, immature (2 weeks old) and mature (10 weeks old); two groups were exposed to 900 MHz (28.2 ± 2.1 V/m) EMF for 2 h/day for 45 days. Two recovery groups were kept for 15 days after EMF exposure. RESULTS: Substantial, deleterious biochemical changes were observed in oxidative stress metabolism after EMF exposure. Antioxidant enzyme activity, glutathione levels in lymphoid organs and the antioxidant capacity of the plasma decreased, but lipid peroxidation and nitric oxide levels in PMNs and plasma and also myeloperoxidase activity in PMNs increased. Oxidative damage was tissue specific and improvements seen after the recovery period were limited, especially in immature rats. CONCLUSIONS: In the present study, much higher levels of irreversible oxidative damage were observed in the major lymphoid organs of immature rats than in mature rats.

(E) *Aynali G, Nazıroğlu M, Celik O, Doğan M, Yarıktaş M, Yasan H. Modulation of wireless (2.45 GHz)-induced oxidative toxicity in laryngotracheal mucosa of rat by melatonin. Eur Arch Otorhinolaryngol. 270(5):1695-1700, 2013. (LI)

It is well known that oxidative stress induces larynx cancer, although antioxidants induce modulator role on etiology of the cancer. It is well known that electromagnetic radiation (EMR) induces oxidative stress in different cell systems. The aim of this study was to investigate the possible protective role of melatonin on oxidative stress induced by Wi-Fi (2.45 GHz) EMR in laryngotracheal mucosa of rat. For this purpose, 32 male rats were equally categorized into four groups, namely controls, sham controls, EMR-exposed rats, EMR-exposed rats treated with melatonin at a dose of 10 mg/kg/day. Except for the controls and sham controls, the animals were exposed to 2.45 GHz radiation during 60 min/day for 28 days. The lipid peroxidation levels were significantly ($p < 0.05$) higher in the radiation-exposed groups than in the control and sham control groups. The lipid peroxidation level in the irradiated animals treated with melatonin was significantly ($p < 0.01$) lower than in those that were only exposed to Wi-Fi radiation. The activity of glutathione peroxidase was lower in the irradiated-only group relative to control and sham control groups but its activity was significantly ($p < 0.05$) increased in the groups treated with melatonin. The reduced glutathione levels in the mucosa of rat did not change in the four groups. There is an apparent protective effect of melatonin on the Wi-Fi-induced oxidative stress in the laryngotracheal mucosa of rats by inhibition of free radical formation and support of the glutathione peroxidase antioxidant system.

(E) Balci M, Devrim E, Durak I. Effects of mobile phones on oxidant/antioxidant balance in cornea and lens of rats. Curr Eye Res. 32(1):21-25, 2007.

Purpose: To investigate the effects of mobile-phone-emitted radiation on the oxidant/antioxidant balance in corneal and lens tissues and to observe any protective effects of vitamin C in this setting. Methods: Forty female albino Wistar rats were assigned to one of four groups containing 10 rats each. One group received a standardized daily dose of mobile phone radiation for 4 weeks. The second group received this same treatment along with a daily oral dose of vitamin C (250 mg/kg). The third group received this dose of vitamin C alone, while the fourth group received standard laboratory care and served as a control. In corneal and lens tissues, malondialdehyde (MDA) levels and activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were measured with spectrophotometric methods. Results: In corneal tissue, MDA level and CAT activity significantly increased in the mobile phone group compared with the mobile phone plus vitamin C group and the control group ($p < 0.05$), whereas SOD activity was significantly decreased ($p < 0.05$). In the lens tissues, only the MDA level significantly increased in the mobile phone group relative to mobile phone plus vitamin C group and the control groups ($p < 0.05$). In lens tissue, significant differences were not found between the groups in terms of SOD, GSH-Px, or CAT ($p > 0.05$). Conclusions: The results of this study

suggest that mobile telephone radiation leads to oxidative stress in corneal and lens tissues and that antioxidants such as vitamin C can help to prevent these effects.

(E) *Bilgici B, Akar A, Avci B, Tuncel OK. Effect of 900 MHz radiofrequency radiation on oxidative stress in rat brain and serum. Electromagn Biol Med. 32:20-29, 2013.

The increasing use of mobile telephones raises the question of possible adverse effects of the electromagnetic fields (EMF) that these phones produce. In this study, we examined the oxidative stress in the brain tissue and serum of rats that resulted from exposure to a 900-MHz EMF at a whole body average specific absorption rate (SAR) of 1.08 W/kg for 1 h/day for 3 weeks. We also examined the antioxidant effect of garlic powder (500 mg/kg/day) given orally to EMF-exposed rats. We found that malondialdehyde (MDA) ($p < 0.001$) and advanced oxidation protein product (AOPP) ($p < 0.05$) increased in rat brain tissue exposed to the EMF and that garlic reduced these effects ($p < 0.05$). There was no significant difference in the nitric oxide (NO) levels in the brain. Paraoxonase (PON) was not detected in the brain. There was a significant increase in the levels of NO ($p < 0.001$) detected in the serum after EMF exposure, and garlic intake did not affect this increase in NO. Our results suggest that there is a significant increase in brain lipid and protein oxidation after electromagnetic radiation (EMR) exposure and that garlic has a protective effect against this oxidative stress.

(E) *Bodera P, Stankiewicz W, Zawada K, Antkowiak B, Paluch M, Kieliszek J, Kalicki B, Bartosiński A, Wawer I. Changes in antioxidant capacity of blood due to mutual action of electromagnetic field (1800 MHz) and opioid drug (tramadol) in animal model of persistent inflammatory state. Pharmacol Rep. 65(2):421-428, 2013.

Background: The biological effects and health implications of electromagnetic field (EMF) associated with cellular mobile telephones and related wireless systems and devices have become a focus of international scientific interest and world-wide public concern. It has also been proved that EMF influences the production of reactive oxygen species (ROS) in different tissues. Methods: Experiments were performed in healthy rats and in rats with persistent inflammatory state induced by Complete Freund's Adjuvant (CFA) injection, which was given 24 h before EMF exposure and drug application. Rats were injected with CFA or the same volume of paraffin oil into the plantar surface of the left hind paw. Animals were exposed to the far-field range of an antenna at 1800 MHz with the additional modulation which was identical to that generated by mobile phone GSM 1800. Rats were given 15 min exposure, or were sham-exposed with no voltage applied to the field generator in control groups. Immediately before EMF exposure, rats were injected intraperitoneally with tramadol in the 20 mg/kg dose or vehicle in the 1 ml/kg volume. Results: Our study revealed that single EMF exposure in 1800 MHz frequency significantly reduced antioxidant capacity both in healthy animals and those with paw inflammation. A certain synergic mode of action between applied electromagnetic fields and administered tramadol in rats treated with CFA was observed. Conclusions: The aim of the study was to examine the possible, parallel/combined effects of electromagnetic radiation, artificially induced inflammation and a centrally-acting synthetic opioid analgesic drug, tramadol, (used in the treatment of severe pain) on the antioxidant capacity of blood of rats. The antioxidant capacity of blood of healthy rats was higher than that of rats which received only tramadol and were exposed to electromagnetic fields.

(NE) Brescia F, Sarti M, Massa R, Calabrese ML, Sannino A, Scarfi MR. Reactive oxygen species formation is not enhanced by exposure to UMTS 1950 MHz radiation and co-exposure to ferrous ions in Jurkat cells. Bioelectromagnetics. 30:525-535, 2009.

This study was designed to assess if radiofrequency (RF) radiation induces oxidative stress in cultured mammalian cells when given alone or in combination with ferrous ions (FeSO₄). For this purpose the production of reactive oxygen species (ROS) was measured by flow cytometry in human lymphoblastoid cells exposed to 1950 MHz signal used by the third generation wireless technology of the Universal Mobile Telecommunication System (UMTS) at Specific Absorption Rate of 0.5 and 2.0 W/kg. Short (5-60 min) or long (24 h) duration exposures were carried out in a waveguide system under strictly controlled conditions of both dosimetry and environment. Cell viability was also measured after 24 h RF exposure using the Resazurin and Neutral Red assays. Several co-exposure protocols were applied to test if RF radiation is able to alter ROS formation induced by FeSO₄ (RF given before or concurrently to FeSO₄). The results obtained indicate that non-thermal RF exposures do not increase spontaneous ROS formation in any of the experimental conditions investigated. Consistent with the lack of ROS production, no change in cell viability was observed in Jurkat cells exposed to RF radiation for 24 h. Similar results were obtained when co-exposures were considered: combined exposures to RF radiation and FeSO₄ did not increase ROS formation induced by the chemical treatment alone. In contrast, in cultures treated with FeSO₄ as positive control, a dose-dependent increase in ROS formation was recorded, validating the sensitivity of the method employed.

(E) *Burlaka A, Tsybulin O, Sidorik E, Lukin S, Polishuk V, Tsehmistrenko S, Yakymenko I. Overproduction of free radical species in embryonal cells exposed to low intensity radiofrequency radiation. Exp Oncol. 35(3):219-225, 2013. (LI)

Aim: Long-term exposure of humans to low intensity radiofrequency electromagnetic radiation (RF-EMR) leads to a statistically significant increase in tumor incidence. Mechanisms of such the effects are unclear, but features of oxidative stress in living cells under RF-EMR exposure were previously reported. Our study aims to assess a production of initial free radical species, which lead to oxidative stress in the cell. Materials and Methods: Embryos of Japanese quails were exposed in ovo to extremely low intensity RF-EMR of GSM 900 MHz (0.25 μ W/cm²) during 158-360 h discontinuously (48 c - ON, 12 c - OFF) before and in the initial stages of development. The levels of superoxide (O₂⁻), nitrogen oxide (NO[·]), thiobarbituric acid reactive substances (TBARS), 8-oxo-2'-deoxyguanosine (8-oxo-dG) and antioxidant enzymes' activities were assessed in cells/tissues of 38-h, 5- and 10-day RF-EMR exposed and unexposed embryos. Results: The exposure resulted in a significant persistent overproduction of superoxide and nitrogen oxide in embryo cells during all period of analyses. As a result, significantly increased levels of TBARS and 8-oxo-dG followed by significantly decreased levels of superoxide dismutase and catalase activities were developed in the exposed embryo cells. Conclusion: Exposure of developing quail embryos to extremely low intensity RF-EMR of GSM 900 MHz during at least one hundred and fifty-eight hours leads to a significant overproduction of free radicals/reactive oxygen species and oxidative damage of DNA in embryo cells. These oxidative changes may lead to pathologies up to oncogenic transformation of cells.

(E) Campisi A, Gulino M, Acquaviva R, Bellia P, Raciti G, Grasso R, Musumeci F, Vanella A, Triglia A. Reactive oxygen species levels and DNA fragmentation on astrocytes in primary culture after acute exposure to low intensity microwave electromagnetic field. Neurosci Lett. 473(1):52-55, 2010.

The exposure of primary rat neocortical astroglial cell cultures to acute electromagnetic fields (EMF) in the microwave range was studied. Differentiated astroglial cell cultures at 14 days in vitro were

exposed for 5, 10, or 20min to either 900MHz continuous waves or 900MHz waves modulated in amplitude at 50Hz using a sinusoidal waveform and 100% modulation index. The strength of the electric field (rms value) at the sample position was 10V/m. No change in cellular viability evaluated by MTT test and lactate dehydrogenase release was observed. A significant increase in ROS levels and DNA fragmentation was found only after exposure of the astrocytes to modulated EMF for 20min. No evident effects were detected when shorter time intervals or continuous waves were used. The irradiation conditions allowed the exclusion of any possible thermal effect. Our data demonstrate, for the first time, that even acute exposure to low intensity EMF induces ROS production and DNA fragmentation in astrocytes in primary cultures, which also represent the principal target of modulated EMF. Our findings also suggest the hypothesis that the effects could be due to hyperstimulation of the glutamate receptors, which play a crucial role in acute and chronic brain damage. Furthermore, the results show the importance of the amplitude modulation in the interaction between EMF and neocortical astrocytes.

(E) Cetin H, Naziroğlu M, Celik O, Yüksel M, Pastacı N, Ozkaya MO. Liver antioxidant stores protect the brain from electromagnetic radiation (900 and 1800 MHz)-induced oxidative stress in rats during pregnancy and the development of offspring. J Matern Fetal Neonatal Med. 2014 Mar 3. [Epub ahead of print]

Objectives: The present study determined the effects of mobile phone (900 and 1800 MHz)-induced electromagnetic radiation (EMR) exposure on oxidative stress in the brain and liver as well as the element levels in growing rats from pregnancy to 6 weeks of age. Methods: Thirty-two rats and their offspring were equally divided into 3 different groups: the control, 900 MHz, and 1800 MHz groups. The 900 MHz and 1800 MHz groups were exposed to EMR for 60 min/day during pregnancy and neonatal development. At the 4th, 5th, and 6th weeks of the experiment, brain samples were obtained. Results: Brain and liver glutathione peroxidase (GSH-Px) activities, as well as liver vitamin A and β -carotene concentrations decreased in the EMR groups, although brain iron, vitamin A, and β -carotene concentrations increased in the EMR groups. In the 6th week, selenium concentrations in the brain decreased in the EMR groups. There were no statistically significant differences in glutathione, vitamin E, chromium, copper, magnesium, manganese, and zinc concentrations between the 3 groups. Conclusion: EMR-induced oxidative stress in the brain and liver was reduced during the development of offspring. Mobile phone-induced EMR could be considered as a cause of oxidative brain and liver injury in growing rats.

(E) *Ceyhan AM, Akkaya VB, Güleçol ŞC, Ceyhan BM, Özgüner F, Chen W. Protective effects of β -glucan against oxidative injury induced by 2.45-GHz electromagnetic radiation in the skin tissue of rats. Arch Dermatol Res. 304(7):521-527, 2012.

In recent times, there is widespread use of 2.45-GHz irradiation-emitting devices in industrial, medical, military and domestic application. The aim of the present study was to investigate the effect of 2.45-GHz electromagnetic radiation (EMR) on the oxidant and antioxidant status of skin and to examine the possible protective effects of β -glucans against the oxidative injury. Thirty-two male Wistar albino rats were randomly divided into four equal groups: control; sham exposed; EMR; and EMR + β -glucan. A 2.45-GHz EMR emitted device from the experimental exposure was applied to the EMR group and EMR + β -glucan group for 60 min daily, respectively, for 4 weeks. β -glucan was administered via gavage at a dose of 50 mg/kg/day before each exposure to radiation in the treatment group. The activities of antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), as well as the concentration of malondialdehyde (MDA) were measured in tissue homogenates of the skin. Exposure to 2.45-GHz EMR caused a significant increase in MDA levels and CAT activity, while

the activities of SOD and GSH-Px decreased in skin tissues. Systemic β -glucan significantly reversed the elevation of MDA levels and the reduction of SOD activities. β -glucan treatment also slightly enhanced the activity of CAT and prevented the depletion of GSH-Px activity caused by EMR, but not statistically significantly. The present study demonstrated the role of oxidative mechanisms in EMR-induced skin tissue damages and that β -glucan could ameliorate oxidative skin injury via its antioxidant properties.

(E) *Chen YB, Li J, Liu JY, Zeng LH, Wan Y, Li YR, Ren D, Guo GZ. Effect of Electromagnetic Pulses (EMP) on associative learning in mice and a preliminary study of mechanism. Int J Radiat Biol. 87(12):1147-1154, 2011.

PURPOSE: To investigate the effects of electromagnetic pulses (EMP) on associative learning in mice and test a preliminary mechanism for these effects. **MATERIALS AND METHODS:** A tapered parallel plate gigahertz transverse electromagnetic (GTEM) cell with a flared rectangular coaxial transmission line was used to expose male BALB/c mice to EMP (peak-intensity 400 kV/m, rise-time 10 ns, pulse-width 350 ns, 0.5 Hz and total 200 pulses). Concurrent sham-exposed mice were used as a control. Associative learning, oxidative stress in the brain, serum chemistry and the protective action of tocopherol monoglucoside (TMG) in mice were measured, respectively. **RESULTS:** (1) Twelve hour and 1 day post EMP exposure associative learning was reduced significantly compared with sham control ($p < 0.05$) but recovered at 2 d post EMP exposure. (2) Compared with the sham control, lipid peroxidation of brain tissue and chemiluminescence (CL) intensity increased significantly ($p < 0.05$), while the activity of the antioxidant enzymes Superoxide Dismutase [SOD], Glutathione [GSH], Glutathione Peroxidase [GSH-Px], Catalase [CAT] decreased significantly ($p < 0.05$) at 3 h, 6 h, 12 h and 1 d post EMP exposure. All these parameters recovered at 2 d post EMP exposure. (3) No significant differences between the sham control group and EMP exposed group were observed in serum cholesterol and triglycerides. (4) Pretreatment of mice with TMG showed protective effects to EMP exposure. **CONCLUSIONS:** EMP exposure significantly decreased associative learning in mice and TMG acted as an effective protective agent from EMP exposure. This mechanism could involve an increase of oxidative stress in brain by EMP exposure.

(E) Dasdag S, Akdag MZ, Ulukaya E, Uzunlar AK, Ocak AR. Effect of mobile phone exposure on apoptotic glial cells and status of oxidative stress in rat brain. Electromagn Biol Med. 28(4):342-354, 2009.

The aim of this study was to investigate the effects of mobile phone exposure on glial cells in brain. The study carried out on 31 Wistar Albino adult male rats. The rat heads in a carousel exposed to 900 MHz microwave. For the study group (n:14), rats exposed to the radiation 2 h per day (7 days in a week) for 10 months. For the sham group (n:7), rats were placed into the carousel and the same procedure was applied except that the generator was turned off. For the cage control (n:10), nothing applied to rats in this group. In this study, rats were euthanized after 10 months of exposure periods and brains were removed. Brain tissues were immunohistochemically stained for the active (cleaved) caspase-3, which is a well-known apoptosis marker, and p53. The expression of the proteins was evaluated by a semi-quantitative scoring system. However, total antioxidative capacity (TAC), catalase, total oxidant status (TOS), and oxidative stress index were measured in rat brain. Final score for apoptosis in the exposed group was significantly lower than the sham ($p < 0.001$) and the cage control groups ($p < 0.01$). p53 was not significantly changed by the exposure ($p > 0.05$). The total antioxidant capacity and catalase in the experimental group was found higher than that in the sham group ($p < 0.001$, $p < 0.05$). In terms of the TOS and oxidative stress index, there was no statistically significant difference between exposure and sham groups ($p > 0.05$). In conclusion, the final score for apoptosis, total

antioxidant capacity and catalase in rat brain might be altered by 900 MHz radiation produced by a generator to represent exposure of global systems for mobile communication (GSM) cellular phones.

(E) De Iuliis GN, Newey RJ, King BV, Aitken RJ (2009) Mobile Phone Radiation Induces Reactive Oxygen Species Production and DNA Damage in Human Spermatozoa In Vitro. PLoS ONE 4(7): e6446. doi:10.1371/journal.pone.0006446

Background: In recent times there has been some controversy over the impact of electromagnetic radiation on human health. The significance of mobile phone radiation on male reproduction is a key element of this debate since several studies have suggested a relationship between mobile phone use and semen quality. The potential mechanisms involved have not been established, however, human spermatozoa are known to be particularly vulnerable to oxidative stress by virtue of the abundant availability of substrates for free radical attack and the lack of cytoplasmic space to accommodate antioxidant enzymes. Moreover, the induction of oxidative stress in these cells not only perturbs their capacity for fertilization but also contributes to sperm DNA damage. The latter has, in turn, been linked with poor fertility, an increased incidence of miscarriage and morbidity in the offspring, including childhood cancer. In light of these associations, we have analyzed the influence of RF-EMR on the cell biology of human spermatozoa in vitro. Principal Findings: Purified human spermatozoa were exposed to radio-frequency electromagnetic radiation (RF-EMR) tuned to 1.8 GHz and covering a range of specific absorption rates (SAR) from 0.4 W/kg to 27.5 W/kg. In step with increasing SAR, motility and vitality were significantly reduced after RF-EMR exposure, while the mitochondrial generation of reactive oxygen species and DNA fragmentation were significantly elevated (P,0.001). Furthermore, we also observed highly significant relationships between SAR, the oxidative DNA damage bio-marker, 8-OH-dG, and DNA fragmentation after RF-EMR exposure. Conclusions: RF-EMR in both the power density and frequency range of mobile phones enhances mitochondrial reactive oxygen species generation by human spermatozoa, decreasing the motility and vitality of these cells while stimulating DNA base adduct formation and, ultimately DNA fragmentation. These findings have clear implications for the safety of extensive mobile phone use by males of reproductive age, potentially affecting both their fertility and the health and wellbeing of their offspring.

(NE) *Demirel S, Doganay S, Turkoz Y, Dogan Z, Turan B, Firat PG. Effects of third generation mobile phone-emitted electromagnetic radiation on oxidative stress parameters in eye tissue and blood of rats. Cutan Ocul Toxicol. 31(2):89-94, 2012.

Purpose: To investigate the effects of electromagnetic radiation (EMR) emitted by a third generation (3G) mobile phone on the antioxidant and oxidative stress parameters in eye tissue and blood of rats. Methods: Eighteen Wistar albino rats were randomly assigned into two groups: Group I (n = 9) received a standardized a daily dose of 3G mobile phone EMR for 20 days, and Group II served as the control group (n = 9), receiving no exposure to EMR. Glutathione peroxidase (GSH-Px) and catalase (CAT) levels were measured in eye tissues; in addition, malondialdehyde (MDA) and reduced GSH levels were measured in blood. Results: There was no significant difference between groups in GSH-Px (p = 0.99) and CAT (p = 0.18) activity in eye tissue. There was no significant difference between groups in MDA (p = 0.69) and GSH levels (p = 0.83) in blood. Conclusions: The results of this study suggest that under a short period of exposure, 3G mobile phone radiation does not lead to harmful effects on eye tissue and blood in rats.

(E) *Deshmukh PS, Banerjee BD, Abegaonkar MP, Megha K, Ahmed RS, Tripathi AK, Mediratta PK. Effect of low level microwave radiation exposure on cognitive function and oxidative stress in rats. Indian J Biochem Biophys. 50(2):114-119, 2013. (LI)

Use of wireless communicating devices is increasing at an exponential rate in present time and is raising serious concerns about possible adverse effects of microwave (MW) radiation emitted from these devices on human health. The present study aimed to evaluate the effects of 900 MHz MW radiation exposure on cognitive function and oxidative stress in blood of Fischer rats. Animals were divided into two groups (6 animals/group): Group I (MW-exposed) and Group II (Sham-exposed). Animals were subjected to MW exposure (Frequency 900 MHz; specific absorption rate 8.4738×10^{-5} W/kg) in Gigahertz transverse electromagnetic cell (GTEM) for 30 days (2 h/day, 5 days/week). Subsequently, cognitive function and oxidative stress parameters were examined for each group. Results showed significant impairment in cognitive function and increase in oxidative stress, as evidenced by the increase in levels of MDA (a marker of lipid peroxidation) and protein carbonyl (a marker of protein oxidation) and unaltered GSH content in blood. Thus, the study demonstrated that low level MW radiation had significant effect on cognitive function and was also capable of leading to oxidative stress.

(E) Devrim E, Ergüder IB, Kılıçoğlu B, Yaykaşlı E, Cetin R, Durak I. Effects of Electromagnetic Radiation Use on Oxidant/Antioxidant Status and DNA Turn-over Enzyme Activities in Erythrocytes and Heart, Kidney, Liver, and Ovary Tissues From Rats: Possible Protective Role of Vitamin C. Toxicol Mech Methods. 18(9):679-683, 2008.

ABSTRACT In this study, the aim was to investigate possible effects of Electromagnetic Radiation (EMR) use on oxidant and antioxidant status in erythrocytes and kidney, heart, liver, and ovary tissues from rats, and possible protective role of vitamin C. For this aim, 40 Wistar albino female rats were used throughout the study. The treatment group was exposed to EMR in a frequency of 900 MHz, the EMR plus vitamin C group was exposed to the same EMR frequency and given vitamin C (250 mg/kg/day) orally for 4 weeks. There were 10 animals in each group including control and vitamin C groups. At the end of the study period, blood samples were obtained from the animals to get erythrocyte sediments. Then the animals were sacrificed and heart, kidney, liver, and ovary tissues were removed. Malondialdehyde (MDA) levels and superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), xanthine oxidase (XO), and adenosine deaminase (ADA) enzyme activities were measured in the tissues and erythrocytes. It was observed that MDA level, XO, and GSH-Px activities significantly increased in the EMR group as compared with those of the control group in the erythrocytes. In the kidney tissues, it was found that MDA level and CAT activity significantly increased, whereas XO and ADA activities decreased in the cellular phone group as compared with those of the control group. However, in the heart tissues it was observed that MDA level, ADA, and XO activities significantly decreased in the cellular phone group as compared with those of the control group. The results suggest that EMR at the frequency generated by a cell phone causes oxidative stress and peroxidation in the erythrocytes and kidney tissues from rats. In the erythrocytes, vitamin C seems to make partial protection against the oxidant stress.

(E) Elhag MA, Nabil GM, Attia AM. Effects of electromagnetic field produced by mobile phones on the oxidant and antioxidant status of rats. Pak J Biol Sci. 10(23):4271-4274, 2007.

This study was designed to investigate the effect of EMR produced by GSM Mobile Phones (MP) on the oxidant and antioxidant status in rats. Rats were divided into three groups: (1) controls, (2) rats exposed to a fractionated dose of EMR (15 min day⁻¹ for four days) (EMR-F) and (3) rats exposed to

an acute dose of EMR (EMR-A). A net drop in the plasma concentration of vitamin C (-47 and -59.8%) was observed in EMR-F and EMR-A groups, respectively, when compared to controls. While, a significant decrease in the levels of lipophilic antioxidant vitamins: vitamin E (-33 and -65.8%), vitamin A (-44.4 and -46.8%) was observed in EMR-F and EMR-A groups, respectively, when compared to controls. A net drop in plasma level of reduced glutathione (GSH) (-19.8 and -35.3%) was observed in EMR-F and EMR-A groups, respectively. EMR exposure of rats produced a significant decrease in catalase (CAT) and superoxide dismutase (SOD) activities, with the values of these activities for EMR-A group is significantly lower than those of EMR-F. These results indicate that the effects of acute doses of EMR produced by mobile phones on the rat's antioxidant status is significantly higher than those of fractionated doses of the same type of radiation. On the basis of present results, it can be concluded that exposure to acute doses of EMR produced by mobile phones is more hazardous than that produced by fractionated doses of the same type of radiation.

(E) *Eser O, Songur A, Aktas C, Karavelioglu E, Caglar V, Aylak F, Ozguner F, Kanter M. The effect of electromagnetic radiation on the rat brain: an experimental study.

AIM: The aim of this study is to determine the structural changes of electromagnetic waves in the frontal cortex, brain stem and cerebellum. MATERIAL and METHODS: 24 Wistar Albino adult male rats were randomly divided into four groups: group I consisted of control rats, and groups II-IV comprised electromagnetically irradiated (EMR) with 900, 1800 and 2450 MHz. The heads of the rats were exposed to 900, 1800 and 2450 MHz microwaves irradiation for 1h per day for 2 months. RESULTS: While the histopathological changes in the frontal cortex and brain stem were normal in the control group, there were severe degenerative changes, shrunken cytoplasm and extensively dark pyknotic nuclei in the EMR groups. Biochemical analysis demonstrated that the Total Antioxidative Capacity level was significantly decreased in the EMR groups and also Total Oxidative Capacity and Oxidative Stress Index levels were significantly increased in the frontal cortex, brain stem and cerebellum. IL-1 β level was significantly increased in the EMR groups in the brain stem. CONCLUSION: EMR causes to structural changes in the frontal cortex, brain stem and cerebellum and impair the oxidative stress and inflammatory cytokine system. This deterioration can cause to disease including loss of these areas function and cancer development.

(E) Esmekaya MA, Ozer C, Seyhan N. 900 MHz pulse-modulated radiofrequency radiation induces oxidative stress on heart, lung, testis and liver tissues. Gen Physiol Biophys. 30(1):84-89, 2011a.

Oxidative stress may affect many cellular and physiological processes including gene expression, cell growth, and cell death. In the recent study, we aimed to investigate whether 900 MHz pulse-modulated radiofrequency (RF) fields induce oxidative damage on lung, heart and liver tissues. We assessed oxidative damage by investigating lipid peroxidation (malondialdehyde, MDA), nitric oxide (NO x) and glutathione (GSH) levels which are the indicators of tissue toxicity. A total of 30 male Wistar albino rats were used in this study. Rats were divided randomly into three groups; control group (n = 10), sham group (device off, n = 10) and 900 MHz pulsed-modulated RF radiation group (n = 10). The RF rats were exposed to 900 MHz pulsed modulated RF radiation at a specific absorption rate (SAR) level of 1.20 W/kg 20 min/day for three weeks. MDA and NO x levels were increased significantly in liver, lung, testis and heart tissues of the exposed group compared to sham and control groups (p < 0.05). Conversely GSH levels were significantly lower in exposed rat tissues (p < 0.05). No significant difference was observed between sham and control groups. Results of our study showed that pulse-modulated RF radiation causes oxidative injury in liver, lung, testis and heart tissues mediated by lipid peroxidation, increased level of NO x and suppression of antioxidant defense mechanism.

(NE) Ferreira AR, Knakievicz T, de Bittencourt Pasquali MA, Gelain DP, Dal-Pizzol F, Fernandez CE, de Almeida de Salles AA, Ferreira HB, Moreira JC. Ultra high frequency-electromagnetic field irradiation during pregnancy leads to an increase in erythrocytes micronuclei incidence in rat offspring. Life Sci. 80(1)43-50, 2006.

Mobile telephones and their base stations are an important ultra high frequency-electromagnetic field (UHF-EMF) source and their utilization is increasing all over the world. Epidemiological studies suggested that low energy UHF-EMF emitted from a cellular telephone may cause biological effects, such as DNA damage and changes on oxidative metabolism. An in vivo mammalian cytogenetic test, the micronucleus (MN) assay, was used to investigate the occurrence of chromosomal damage in erythrocytes from rat offspring exposed to a non-thermal UHF-EMF from a cellular phone during their embryogenesis; the irradiated group showed a significant increase in MN occurrence. In order to investigate if UHF-EMF could also alter oxidative parameters in the peripheral blood and in the liver - an important hematopoietic tissue in rat embryos and newborns - we also measured the activity of antioxidant enzymes, quantified total sulfhydryl content, protein carbonyl groups, thiobarbituric acid-reactive species and total non-enzymatic antioxidant defense. No significant differences were found in any oxidative parameter of offspring blood and liver. The average number of pups in each litter has also not been significantly altered. Our results suggest that, under our experimental conditions, UHF-EMF is able to induce a genotoxic response in hematopoietic tissue during the embryogenesis through an unknown mechanism.

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

The exposure to non-thermal microwave electromagnetic field generated by mobile phones affects the expression of many proteins. This effect on transcription and protein stability can be mediated by the mitogen-activated protein kinase (MAPK) cascades, which serve as central signaling pathways, and govern essentially all stimulated cellular processes. Indeed, a long-term exposure of cells to mobile phone irradiation results in the activation of p38MAPKs as well as the ERK/MAPKs. Here we studied the immediate effect of irradiation on the MAPK cascades, and found that ERKs, but not stress related MAPKs are rapidly activated in response to various frequencies and intensities. Using signaling inhibitors we delineated the mechanism that is involved in this activation. We found that the first step is mediated in the plasma membrane by NADH oxidase, which rapidly generates reactive oxygen species (ROS). These ROS then directly stimulate matrix metalloproteinases and allow them to cleave and release heparin binding-EGF. This secreted factor, activates EGF receptor, which in turn further activates the ERK cascade. Thus, this study demonstrates for the first time a detailed molecular mechanism by which electromagnetic irradiation by mobile phones induces the activation of the ERK cascade and thereby induces transcription and other cellular processes.

(NE) *Furtado-Filho OV, Borba JB, Dallegrave A, Pizzolato TM, Henriques JA, Moreira JC, Saffi J. Effect of 950 MHz UHF electromagnetic radiation on biomarkers of oxidative damage, metabolism of UFA and antioxidants in the livers of young rats of different ages. Int J Radiat Biol. 2013 Jul 25. [Epub ahead of print]

Purpose: To assess the effect of 950 MHz ultra-high-frequency electromagnetic radiation (UHF EMR) on biomarkers of oxidative damage, as well as to verify the concentration of unsaturated fatty acids (UFA) and the expression of the catalase in the livers of rats of different ages. Materials and methods: Twelve rats were equally divided into two groups as controls (CR) and

exposed (ER), for each age (0, 6, 15 and 30 days). Radiation exposure lasted half an hour per day for up to 51 days (21 days of gestation and 6, 15 or 30 days of life outside the womb). The specific absorption rate (SAR) ranged from 1.3-1.0 W/kg. The damage to lipids, proteins and DNA was verified by thiobarbituric acid reactive substances (TBARS), protein carbonyls and comets, respectively. UFA were determined by gas chromatography with a flame ionization detector. The expression of catalase was by Western blotting. Results: The neonates had low levels of TBARS and concentrations of UFA after exposure. There was no age difference in the accumulation of protein carbonyls for any age. The DNA damage of ER 15 or 30 days was different. The exposed neonates exhibited lower expression of catalase. Conclusions: 950 MHz UHF EMR does not cause oxidative stress (OS), and it is not genotoxic to the livers of neonates or those of 6 and 15 day old rats, but it changes the concentrations of polyunsaturated fatty acid (PUFA) in neonates. For rats of 30 days, no OS, but it is genotoxic to the livers of ER to total body irradiation.

(E) Gajski G, Garaj-Vrhovac V. Radioprotective effects of honeybee venom (*Apis mellifera*) against 915-MHz microwave radiation-induced DNA damage in wistar rat lymphocytes: in vitro study. *Int J Toxicol.* 28(2):88-98, 2009.

The aim of this study is to investigate the radioprotective effect of bee venom against DNA damage induced by 915-MHz microwave radiation (specific absorption rate of 0.6 W/kg) in Wistar rats. Whole blood lymphocytes of Wistar rats are treated with 1 microg/mL bee venom 4 hours prior to and immediately before irradiation. Standard and formamidopyrimidine-DNA glycosylase (Fpg)-modified comet assays are used to assess basal and oxidative DNA damage produced by reactive oxygen species. Bee venom shows a decrease in DNA damage compared with irradiated samples. Parameters of Fpg-modified comet assay are statistically different from controls, making this assay more sensitive and suggesting that oxidative stress is a possible mechanism of DNA damage induction. Bee venom is demonstrated to have a radioprotective effect against basal and oxidative DNA damage. Furthermore, bee venom is not genotoxic and does not produce oxidative damage in the low concentrations used in this study.

(E) *Garaj-Vrhovac V, Gajski G, Pažanin S, Sarolić A, Domijan AM, Flajs D, Peraica M. Assessment of cytogenetic damage and oxidative stress in personnel occupationally exposed to the pulsed microwave radiation of marine radar equipment. *Int J Hyg Environ Health.* 4(1):59-65, 2011.

Due to increased usage of microwave radiation, there are concerns of its adverse effect in today's society. Keeping this in view, study was aimed at workers occupationally exposed to pulsed microwave radiation, originating from marine radars. Electromagnetic field strength was measured at assigned marine radar frequencies (3 GHz, 5.5 GHz and 9.4 GHz) and corresponding specific absorption rate values were determined. Parameters of the comet assay and micronucleus test were studied both in the exposed workers and in corresponding unexposed subjects. Differences between mean tail intensity (0.67 vs. 1.22) and moment (0.08 vs. 0.16) as comet assay parameters and micronucleus test parameters (micronuclei, nucleoplasmic bridges and nuclear buds) were statistically significant between the two examined groups, suggesting that cytogenetic alterations occurred after microwave exposure. Concentrations of glutathione and malondialdehyde were measured spectrophotometrically and using high performance liquid chromatography. The glutathione concentration in exposed group was significantly lower than in controls (1.24 vs. 0.53) whereas the concentration of malondialdehyde was significantly higher (1.74 vs. 3.17), indicating oxidative stress. Results suggests that pulsed microwaves from

working environment can be the cause of genetic and cell alterations and that oxidative stress can be one of the possible mechanisms of DNA and cell damage.

(E) *Ghanbari M¹, Mortazavi SB¹, Khavanin A¹, Khazaei M². The Effects of Cell Phone Waves (900 MHz-GSM Band) on Sperm Parameters and Total Antioxidant Capacity in Rats. Int J Fertil Steril. 7(1):21-28, 2013.

BACKGROUND: There is tremendous concern regarding the possible adverse effects of cell phone microwaves. Contradictory results, however, have been reported for the effects of these waves on the body. In the present study, the effect of cell phone microwaves on sperm parameters and total antioxidant capacity was investigated with regard to the duration of exposure and the frequency of these waves. MATERIALS AND METHODS: This experimental study was performed on 28 adult male Wistar rats (200-250 g). The animals were randomly assigned to four groups (n=7): i. control; ii. two-week exposure to cell phone-simulated waves; iii. three-week exposure to cell phonesimulated waves; and iv. two-week exposure to cell phone antenna waves. In all groups, sperm analysis was performed based on standard methods and we determined the mean sperm total antioxidant capacity according to the ferric reducing ability of plasma (FRAP) method. Data were analyzed by one-way ANOVA followed by Tukey's test using SPSS version 16 software. RESULTS: The results indicated that sperm viability, motility, and total antioxidant capacity in all exposure groups decreased significantly compared to the control group (p<0.05). Increasing the duration of exposure from 2 to 3 weeks caused a statistically significant decrease in sperm viability and motility (p<0.05). CONCLUSION: Exposure to cell phone waves can decrease sperm viability and motility in rats. These waves can also decrease sperm total antioxidant capacity in rats and result in oxidative stress.

(E) Guler G, Tomruk A, Ozgur E, Seyhan N. The effect of radiofrequency radiation on DNA and lipid damage in non-pregnant and pregnant rabbits and their newborns. Gen Physiol Biophys. 29(1):59-66, 2010.

The concerns of people on possible adverse health effects of radiofrequency radiation (RFR) generated from mobile phones as well as their supporting transmitters (base stations) have increased markedly. RFR effect on oversensitive people, such as pregnant women and their developing fetuses, and older people is another source of concern that should be considered. In this study, oxidative DNA damage and lipid peroxidation levels in the brain tissue of pregnant and non-pregnant New Zealand White rabbits and their newborns exposed to RFR were investigated. Thirteen-month-old rabbits were studied in four groups as non-pregnant-control, non-pregnant-RFR exposed, pregnant-control and pregnant-RFR exposed. They were exposed to RFR (1800 MHz GSM; 14 V/m as reference level) for 15 min/day during 7 days. Malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were analyzed. MDA and 8-OHdG levels of non-pregnant and pregnant-RFR exposed animals significantly increased with respect to controls (p < 0.001, Mann-Whitney test). No difference was found in the newborns (p > 0.05, Mann-Whitney). There exist very few experimental studies on the effects of RFR during pregnancy. It would be beneficial to increase the number of these studies in order to establish international standards for the protection of pregnant women from RFR.

(E) *Güler G, Tomruk A, Ozgur E, Sahin D, Sepici A, Altan N, Seyhan N. The effect of radiofrequency radiation on DNA and lipid damage in female and male infant rabbits. Int J Radiat Biol. 88(4):367-373, 2012.

PURPOSE: We aimed to design a prolonged radiofrequency (RF) radiation exposure and investigate in an animal model, possible bio-effects of RF radiation on the ongoing developmental stages of children

from conception to childhood. MATERIALS AND METHODS: A total of 72 New Zealand female and male white rabbits aged one month were used. Females were exposed to RF radiation for 15 min/day during 7 days, whereas males were exposed to the same level of radiation for 15 min/day during 14 days. Thirty-six female and 36 male infant rabbits were randomly divided into four groups: Group I [Intrauterine (IU) exposure (-); Extrauterine (EU) exposure (-)]: Sham exposure which means rabbits were exposed to 1800 MHz Global System for Mobile Telecommunication (GSM)-like RF signals neither in the IU nor in the EU periods. Group II [IU exposure (-); EU exposure (+)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals when they reached one month of age. Group III [IU exposure (+); EU exposure (-)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals in the IU period (between 15th and 22nd days of the gestational period). Group IV [IU exposure (+); EU exposure (+)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals both in the IU period (between 15th and 22nd days of the gestational period) and in the EU period when they reached one month of age. Biochemical analysis for lipid peroxidation and DNA damage were carried out in the livers of all rabbits. RESULTS: Lipid peroxidation levels in the liver tissues of female and male infant rabbits increased under RF radiation exposure. Liver 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels of female rabbits exposed to RF radiation were also found to increase when compared with the levels of non-exposed infants. However, there were no changes in liver 8-OHdG levels of male rabbits under RF exposure. CONCLUSION: Consequently, it can be concluded that GSM-like RF radiation may induce biochemical changes by increasing free radical attacks to structural biomolecules in the rabbit as an experimental animal model.

(E) Guney M, Ozguner F, Oral B, Karahan N, Mungan T. 900 MHz radiofrequency-induced histopathologic changes and oxidative stress in rat endometrium: protection by vitamins E and C. Toxicol Ind Health. 23(7):411-420, 2007.

There are numerous reports on the effects of electromagnetic radiation (EMR) in various cellular systems. Mechanisms of adverse effects of EMR indicate that reactive oxygen species (ROS) may play a role in the biological effects of this radiation. The aims of this study were to examine 900 MHz mobile phone-induced oxidative stress that promotes production of ROS and to investigate the role of vitamins E and C, which have antioxidant properties, on endometrial tissue against possible 900 MHz mobile phone-induced endometrial impairment in rats. The animals were randomly grouped (eight each) as follows: 1) Control group (without stress and EMR, Group I), 2) sham-operated rats stayed without exposure to EMR (exposure device off, Group II), 3) rats exposed to 900 MHz EMR (EMR group, Group III) and 4) a 900 MHz EMR exposed + vitamin-treated group (EMR + Vit group, Group IV). A 900 MHz EMR was applied to EMR and EMR + Vit group 30 min/day, for 30 days using an experimental exposure device. Endometrial levels of nitric oxide (NO, an oxidant product) and malondialdehyde (MDA, an index of lipid peroxidation), increased in EMR exposed rats while the combined vitamins E and C caused a significant reduction in the levels of NO and MDA. Likewise, endometrial superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities decreased in EMR exposed animals while vitamins E and C caused a significant increase in the activities of these antioxidant enzymes. In the EMR group histopathologic changes in endometrium, diffuse and severe apoptosis was present in the endometrial surface epithelial and glandular cells and the stromal cells. Diffuse eosinophilic leucocyte and lymphocyte infiltration were observed in the endometrial stroma whereas the combination of vitamins E and C caused a significant decrease in these effects of EMR. It is concluded that oxidative endometrial damage plays an important role in the 900 MHz mobile phone-induced endometrial impairment and the modulation of oxidative stress with vitamins E and C reduces the 900 MHz mobile phone-induced endometrial damage both at biochemical and histological levels.

(E) *Hamzany Y, Feinmesser R, Shpitzer T, Mizrachi A, Hilly O, Hod R, Bahar G, Otradnov I, Gavish M, Nagler RM. Is human saliva an indicator of the adverse health effects of using mobile phones? Antioxid Redox Signal. 18(6):622-627, 2013.

Increasing use of mobile phones creates growing concerns regarding harmful effects of radiofrequency nonionizing electromagnetic radiation on human tissues located close to the ear, where phones are commonly held for long periods of time. We studied 20 subjects in the mobile-phone group who had a mean duration of mobile phone use of 12.5 years (range 8-15) and a mean time use of 29.6 h per month (range 8-100). Deaf individuals served as controls. We compared salivary outcomes (secretion, oxidative damage indices, flow rate, and composition) between mobile phone users and nonusers. We report a significant increase in all salivary oxidative stress indices studied in mobile phone users. Salivary flow, total protein, albumin, and amylase activity were decreased in mobile phone users. These observations lead to the hypothesis that the use of mobile phones may cause oxidative stress and modify salivary function.

(E) *Hancı H, Odacı E, Kaya H, Aliyazıcıoğlu Y, Turan I, Demir S, Colakoğlu S. The effect of prenatal exposure to 900-MHz electromagnetic field on the 21-old-day rat testicle. Reprod Toxicol. 42:203-209, 2013.

The aim of this study was to investigate the effect of exposure to a 900-MHz electromagnetic field (EMF) in the prenatal term on the 21-old-day rat testicle. Pregnant rats were divided into control (CG) and EMF (EMFG) groups. EMFG was exposed to 900-MHz EMF during days 13-21 of pregnancy. Newborn CG rats were obtained from the CG and newborn EMFG (NEMFG) rats from the EMFG. Testicles were extracted at postnatal day 21. Lipid peroxidation and DNA oxidation levels, apoptotic index and histopathological damage scores were compared. NEMFG rats exhibited irregularities in seminiferous tubule basal membrane and epithelium, immature germ cells in the lumen, and a decreased diameter in seminiferous tubules and thickness of epithelium. Apoptotic index, lipid peroxidation and DNA oxidation were higher in NEMFG rats than in NCG. 21-day-old rat testicles exposed to 900-MHz EMF in the prenatal term may be adversely affected, and this effect persists after birth.

(E) Hässig M, Jud F, Naegeli H, Kupper J, Spiess BM. Prevalence of nuclear cataract in Swiss veal calves and its possible association with mobile telephone antenna base stations. Schweiz Arch Tierheilkd. 151(10):471-478, 2009. (LI)

The purpose of this study was to evaluate the prevalence of nuclear cataract in veal calves and to elucidate a possible impact by mobile phone base stations (MPBS). For this experiment a cohort study was conducted. A follow-up of the geographical location of each dam and its calf from conception through the fetal period up to slaughter was performed. The first trimester of gestation (organogenesis) was particularly emphasized. The activities of selected protective antioxidants (superoxide dismutase, catalase, glutathione peroxidase [GPx]) were assessed in aqueous humor of the eye to evaluate the redox status. Of 253 calves, 79 (32 %) had various degrees of nuclear cataract, but only 9 (3.6 %) calves had severe nuclear cataract. Results demonstrate a relation between the location of veal calves with nuclear cataracts in the first trimester of gestation and the strength of antennas. The number of antennas within 100 to 199 meters was associated with oxidative stress and there was an association between oxidative stress and the distance to the nearest MPBS. Oxidative stress was increased in eyes with cataract (OR per kilometer: 0.80, confidence interval 95 % 0.62,0.93). It has not been shown that the antennas actually affected stress. Hosmer-Lemeshow statistics showed an accuracy of 100 % in negative cases with low radiation, and only 11.11 % accuracy in positive cases with high radiation. This reflects, that there are a lot of other possibilities for nuclear cataract beside MPBS. Further studies on

the influence of electromagnetic fields during embryonic development animal or person at risk are indicated.

(NE) Hong MN, Kim BC, Ko YG, Lee YS, Hong SC, Kim T, Pack JK, Choi HD, Kim N, Lee JS. Effects of 837 and 1950 MHz radiofrequency radiation exposure alone or combined on oxidative stress in MCF10A cells. Bioelectromagnetics. 33(7):604-11, 2012.

The aim of this study was to determine whether the exposure to either single or multiple radio-frequency (RF) radiation frequencies could induce oxidative stress in cell cultures. Exposures of human MCF10A mammary epithelial cells to either a single frequency (837 MHz alone or 1950 MHz alone) or multiple frequencies (837 and 1950 MHz) were conducted at specific absorption rate (SAR) values of 4 W/kg for 2 h. During the exposure period, the temperature in the exposure chamber was maintained isothermally. Intracellular levels of reactive oxygen species (ROS), the antioxidant enzyme activity of superoxide dismutase (SOD), and the ratio of reduced/oxidized glutathione (GSH/GSSG) showed no statistically significant alterations as the result of either single or multiple RF radiation exposures. In contrast, ionizing radiation-exposed cells, used as a positive control, showed evident changes in all measured biological endpoints. These results indicate that single or multiple RF radiation exposure did not elicit oxidative stress in MCF10A cells under our exposure conditions.

(NE) Hook, G. J., Spitz, D. R., Sim, J. E., Higashikubo, R., Baty, J. D., Moros, E. G. and Roti Roti, J. L. Evaluation of Parameters of Oxidative Stress after In Vitro Exposure to FMCW- and CDMA-Modulated Radiofrequency Radiation Fields. Radiat. Res. 162, 497–504, 2004.

The goal of this study was to determine whether radiofrequency (RF) radiation is capable of inducing oxidative stress or affecting the response to oxidative stress in cultured mammalian cells. The two types of RF radiation investigated were frequency-modulated continuous-wave with a carrier frequency of 835.62 MHz (FMCW) and code division multiple access centered on 847.74 MHz (CDMA). To evaluate the effect of RF radiation on oxidative stress, J774.16 mouse macrophage cells were stimulated with γ -interferon (IFN) and bacterial lipopolysaccharide (LPS) prior to exposure. Cell cultures were exposed for 20–22 h to a specific absorption rate of 0.8 W/kg at a temperature of $37.0 \pm 0.3^\circ\text{C}$. Oxidative stress was evaluated by measuring oxidant levels, antioxidant levels, oxidative damage and nitric oxide production. Oxidation of thiols was measured by monitoring the accumulation of glutathione disulfide (GSSG). Cellular antioxidant defenses were evaluated by measuring superoxide dismutase activity (CuZnSOD and MnSOD) as well as catalase and glutathione peroxidase activity. The trypan blue dye exclusion assay was used to measure any changes in viability. The results of these studies indicated that FMCW- and CDMA-modulated RF radiation did not alter parameters indicative of oxidative stress in J774.16 cells. FMCW- and CDMA-modulated fields did not alter the level of intracellular oxidants, accumulation of GSSG or induction of antioxidant defenses in IFN/LPS-stimulated cells. Consistent with the lack of an effect on oxidative stress parameters, no change in toxicity was observed in J774.16 cells after either optimal (with or without inhibitors of nitric oxide synthase) or suboptimal stimulation.

(E) Hou Q, Wang M, Wu S, Ma X, An G, Liu H, Xie F. Oxidative changes and apoptosis induced by 1800-MHz electromagnetic radiation in NIH/3T3 cells. Electromagn Biol Med. 2014 Mar 25. [Epub ahead of print]

To investigate the potential adverse effects of mobile phone radiation, we studied reactive oxygen species (ROS), DNA damage and apoptosis in mouse embryonic fibroblasts (NIH/3T3) after intermittent exposure (5 min on/10 min off, for various durations from 0.5 to 8 h) to an 1800-MHz GSM-talk mode electromagnetic radiation (EMR) at an average specific absorption rate of 2 W/kg. A

2',7'-dichlorofluorescein diacetate fluorescence probe was used to detect intracellular ROS levels, immunofluorescence was used to detect γ H2AX foci as a marker for DNA damage, and flow cytometry was used to measure apoptosis. Our results showed a significant increase in intracellular ROS levels after EMR exposure and it reached the highest level at an exposure time of 1 h ($p < 0.05$) followed by a slight decrease when the exposure continued for as long as 8 h. No significant effect on the number of γ H2AX was detected after EMR exposure. The percentage of late-apoptotic cells in the EMR-exposed group was significantly higher than that in the sham-exposed groups ($p < 0.05$). These results indicate that an 1800-MHz EMR enhances ROS formation and promotes apoptosis in NIH/3T3 cells.

(E) Höytö A, Luukkonen J, Juutilainen J, Naarala J. Proliferation, oxidative stress and cell death in cells exposed to 872 MHz radiofrequency radiation and oxidants. Radiat. Res. 170(2):235-243, 2008. (WS)

Human SH-SY5Y neuroblastoma and mouse L929 fibroblast cells were exposed to 872 MHz radiofrequency (RF) radiation using continuous waves (CW) or a modulated signal similar to that emitted by GSM mobile phones at a specific absorption rate (SAR) of 5 W/kg in isothermal conditions. To investigate possible combined effects with other agents, menadione was used to induce reactive oxygen species, and tert-butylhydroperoxide (t-BOOH) was used to induce lipid peroxidation. After 1 or 24 h of exposure, reduced cellular glutathione levels, lipid peroxidation, proliferation, caspase 3 activity, DNA fragmentation and viability were measured. Two statistically significant differences related to RF radiation were observed: Lipid peroxidation induced by t-BOOH was increased in SH-SY5Y (but not in L929) cells, and menadione-induced caspase 3 activity was increased in L929 (but not in SH-SY5Y) cells. Both differences were statistically significant only for the GSM-modulated signal. The other end points were not significantly affected in any of the experimental conditions, and no effects were observed from exposure to RF radiation alone. The positive findings may be due to chance, but they may also reflect effects that occur only in cells sensitized by chemical stress. Further studies are required to investigate the reproducibility and dose response of the possible effects.

(E) Ilhan A, Gurel A, Armutcu F, Kamisli S, Iraz M, Akyol O, Ozen S. Ginkgo biloba prevents mobile phone-induced oxidative stress in rat brain. Clin Chim Acta. 340(1-2): 153-162, 2004.

BACKGROUND: The widespread use of mobile phones (MP) in recent years has raised the research activities in many countries to determine the consequences of exposure to the low-intensity electromagnetic radiation (EMR) of mobile phones. Since several experimental studies suggest a role of reactive oxygen species (ROS) in EMR-induced oxidative damage in tissues, in this study, we investigated the effect of Ginkgo biloba (Gb) on MP-induced oxidative damage in brain tissue of rats. METHODS: Rats (EMR+) were exposed to 900 MHz EMR from MP for 7 days (1 h/day). In the EMR+Gb groups, rats were exposed to EMR and pretreated with Gb. Control and Gb-administrated groups were produced by turning off the mobile phone while the animals were in the same exposure conditions. Subsequently, oxidative stress markers and pathological changes in brain tissue were examined for each group. RESULTS: Oxidative damage was evident by the: (i) increase in malondialdehyde (MDA) and nitric oxide (NO) levels in brain tissue, (ii) decrease in brain superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities and (iii) increase in brain xanthine oxidase (XO) and adenosine deaminase (ADA) activities. These alterations were prevented by Gb treatment. Furthermore, Gb prevented the MP-induced cellular injury in brain tissue histopathologically. CONCLUSION: Reactive oxygen species may play a role in the mechanism that has been proposed to explain the biological side effects of MP, and Gb prevents the MP-induced oxidative stress to preserve antioxidant enzymes activity in brain tissue.

(E) Imge EB, Kiliçoğlu B, Devrim E, Cetin R, Durak I. Effects of mobile phone use on brain tissue from the rat and a possible protective role of vitamin C - a preliminary study. Int J Radiat Biol. 86(12):1044-1049, 2010.

Purpose: To evaluate effects of mobile phone use on brain tissue and a possible protective role of vitamin C. Materials and methods: Forty female rats were divided into four groups randomly (Control, mobile phone, mobile phone plus vitamin C and, vitamin C alone). The mobile phone group was exposed to a mobile phone signal (900 MHz), the mobile phone plus vitamin C group was exposed to a mobile phone signal (900 MHz) and treated with vitamin C administered orally (per os). The vitamin C group was also treated with vitamin C per os for four weeks. Then, the animals were sacrificed and brain tissues were dissected to be used in the analyses of malondialdehyde (MDA), antioxidant potential (AOP), superoxide dismutase, catalase (CAT), glutathione peroxidase (GSH-Px), xanthine oxidase, adenosine deaminase (ADA) and 5'nucleotidase (5'-NT). Results: Mobile phone use caused an inhibition in 5'-NT and CAT activities as compared to the control group. GSH-Px activity and the MDA level were also found to be reduced in the mobile phone group but not significantly. Vitamin C caused a significant increase in the activity of GSH-Px and non-significant increase in the activities of 5'-NT, ADA and CAT enzymes. Conclusion: Our results suggest that vitamin C may play a protective role against detrimental effects of mobile phone radiation in brain tissue.

(E) Irmak MK, Fadillioglu E, Gulec M, Erdogan H, Yagmurca M, Akyol O. Effects of electromagnetic radiation from a cellular telephone on the oxidant and antioxidant levels in rabbits. Cell Biochem Funct. 20(4):279-283, 2002.

The number of reports on the effects induced by electromagnetic radiation (EMR) in various cellular systems is still increasing. Until now no satisfactory mechanism has been proposed to explain the biological effects of this radiation. Oxygen free radicals may play a role in mechanisms of adverse effects of EMR. This study was undertaken to investigate the influence of electromagnetic radiation of a digital GSM mobile telephone (900 MHz) on oxidant and antioxidant levels in rabbits. Adenosine deaminase, xanthine oxidase, catalase, myeloperoxidase, superoxide dismutase (SOD) and glutathione peroxidase activities as well as nitric oxide (NO) and malondialdehyde levels were measured in sera and brains of EMR-exposed and sham-exposed rabbits. Serum SOD activity increased, and serum NO levels decreased in EMR-exposed animals compared to the sham group. Other parameters were not changed in either group. This finding may indicate the possible role of increased oxidative stress in the pathophysiology of adverse effect of EMR. Decreased NO levels may also suggest a probable role of NO in the adverse effect.

(E) **Jelodar G, Akbari A, Nazifi S. The prophylactic effect of vitamin C on oxidative stress indexes in rat eyes following exposure to radiofrequency wave generated by a BTS antenna model. Int J Radiat Biol. 89(2):128-131, 2013.

Purpose: This study was conducted to evaluate the effect of radiofrequency wave (RFW)-induced oxidative stress in the eye and the prophylactic effect of vitamin C on this organ by measuring the antioxidant enzymes activity including: glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), and malondialdehyde (MDA). Materials and methods: Thirty-two adult male Sprague-Dawley rats were randomly divided into four experimental groups and treated daily for 45 days as follows: Control, vitamin C (L-ascorbic acid 200 mg/kg of body weight/day by gavage), test (exposed to 900 MHz RFW) and the treated group (received vitamin C in addition to exposure to RFW). At the end of the experiment all animals were sacrificed, their eyes were removed and were used for measurement of antioxidant enzymes and MDA activity. Results: The results indicate that

exposure to RFW in the test group decreased antioxidant enzymes activity and increased MDA compared with the control groups ($P < 0.05$). In the treated group vitamin C improved antioxidant enzymes activity and reduced MDA compared to the test group ($P < 0.05$). Conclusions: It can be concluded that RFW causes oxidative stress in the eyes and vitamin C improves the antioxidant enzymes activity and decreases MDA.

(E) *Jelodar G, Nazifi S, Akbari A. The prophylactic effect of vitamin C on induced oxidative stress in rat testis following exposure to 900 MHz radio frequency wave generated by a BTS antenna model. Electromagn Biol Med. 2013 Jan 16. [Epub ahead of print] Radio frequency wave (RFW) generated by base transceiver station (BTS) has been reported to make deleterious effects on reproduction, possibly through oxidative stress. This study was conducted to evaluate the effect of RFW generated by BTS on oxidative stress in testis and the prophylactic effect of vitamin C by measuring the antioxidant enzymes activity, including glutathione peroxidase, superoxide dismutase (SOD) and catalase, and malondialdehyde (MDA). Thirty-two adult male Sprague-Dawley rats were randomly divided into four experimental groups and treated daily for 45 days as follows: sham, sham+vitamin C (l-ascorbic acid 200 mg/kg of body weight/day by gavage), RFW (exposed to 900 MHz RFW) 'sham' and 'RFW' animals were given the vehicle, i.e., distilled water and the RFW+vitamin C group (received vitamin C in addition to exposure to RFW). At the end of the experiment, all the rats were sacrificed and their testes were removed and used for measurement of antioxidant enzymes and MDA activity. The results indicate that exposure to RFW in the test group decreased antioxidant enzymes activity and increased MDA compared with the control groups ($p < 0.05$). In the treated group, vitamin C improved antioxidant enzymes activity and reduced MDA compared with the test group ($p < 0.05$). It can be concluded that RFW causes oxidative stress in testis and vitamin C improves the antioxidant enzymes activity and decreases MDA.

(NE) *Kang KA, Lee HC, Lee JJ, Hong MN, Park MJ, Lee YS, Choi HD, Kim N, Ko YK, Lee JS. Effects of combined radiofrequency radiation exposure on levels of reactive oxygen species in neuronal cells. J Radiat Res. 2013 Oct 8. [Epub ahead of print]

The objective of this study was to investigate the effects of the combined RF radiation (837 MHz CDMA plus 1950 MHz WCDMA) signal on levels of intracellular reactive oxygen species (ROS) in neuronal cells. Exposure of the combined RF signal was conducted at specific absorption rate values of 2 W/kg of CDMA plus 2 W/kg of WCDMA for 2 h. Co-exposure to combined RF radiation with either H₂O₂ or menadione was also performed. The experimental exposure groups were incubator control, sham-exposed, combined RF radiation-exposed with or without either H₂O₂ or menadione groups. The intracellular ROS level was measured by flow cytometry using the fluorescent probe dichlorofluorescein diacetate. Intracellular ROS levels were not consistently affected by combined RF radiation exposure alone in a time-dependent manner in U87, PC12 or SH-SY5Y cells. In neuronal cells exposed to combined RF radiation with either H₂O₂ or menadione, intracellular ROS levels showed no statically significant alteration compared with exposure to menadione or H₂O₂ alone. These findings indicate that neither combined RF radiation alone nor combined RF radiation with menadione or H₂O₂ influences the intracellular ROS level in neuronal cells such as U87, PC12 or SH-SY5Y.

(E) *Kesari KK, Behari J. Microwave exposure affecting reproductive system in male rats. Appl Biochem Biotechnol 31:495-498, 2010. (LI)

The object of present study is to investigate the effects of 50 GHz microwave frequency electromagnetic fields on reproductive system of male rats. Male rats of Wistar strain were used in the study. Animals 60 days old were divided into two groups-group I sham exposed and group II experimental (microwave exposed). During exposure, rats were confined in Plexiglas cages with drilled

ventilation holes for 2 h a day for 45 days continuously at a specified specific absorption rate of 8.0×10^{-4} W/kg. After the last exposure, the rats were sacrificed immediately and sperms were collected. Antioxidant enzyme (superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase), histone kinase, apoptosis, and cell cycle were analyzed in sperm cells. Result shows a significant decrease in the level of sperm GPx and SOD activity ($p \leq 0.05$), whereas catalase shows significant increase in exposed group of sperm samples as compared with control ($p < 0.02$). We observed a statistically significant decrease in mean activity of histone kinase as compared to the control ($p < 0.016$). The percentage of cells dividing in a spermatogenesis was estimated by analyzing DNA per cell by flow cytometry. The percentage of apoptosis in electromagnetic field exposed group shows increased ratio as compared to sham exposed ($p < 0.004$). There were no significant differences in the G(0)/G phase; however, a significant decrease ($p < 0.026$) in S phase was obtained. Results also indicate a decrease in percentage of G/M transition phase of cell cycle in exposed group as compared to sham exposed ($p < 0.019$). We conclude that these radiations may have a significant effect on reproductive system of male rats, which may be an indication of male infertility.

(E) Kesari KK, Behari J. Evidence for mobile phone radiation exposure effects on reproductive pattern of male rats: role of ROS. Electromagn Biol Med. 31(3):213-22, 2012.

The relationship between radiofrequency electromagnetic fields emitted from mobile phone and infertility is a matter of continuing debate. It is postulated that these radiations may affect the reproduction pattern spell by targeting biochemistry of sperm. In an attempt to expedite the issue, 70 days old Wistar rats ($n = 6$) were exposed to mobile phone radiofrequency (RF) radiation for 2 h per day for 45 days and data compared with sham exposed ($n = 6$) group. A significant decrease ($P < 0.05$) in the level of testosterone and an increase in caspase-3 activity were found in the RF-exposed animals. Distortions in sperm head and mid piece of sperm mitochondrial sheath were also observed as captured by Transmission Electron Microscope (TEM). In addition, progeny from RF-exposed rats showed significant decreases in number and weight as compared with that of sham-exposed animals. A reduction in testosterone, an increase in caspase-3, and distortion in spermatozoa could be caused by overproduction of reactive oxygen species (ROS) in animals under mobile phone radiation exposure. Our findings on these biomarkers are clear indications of possible health implications of repeated exposure to mobile phone radiation.

(E) Kesari KK, Kumar S, Behari J. Mobile phone usage and male infertility in Wistar rats. Indian J Exp Biol. 48(10):987-992, 2010.

A significant decrease in protein kinase C and total sperm count along with increased apoptosis were observed in male Wistar rats exposed to mobile phone frequencies (2 h/day x 35 days at 0.9 W/kg specific absorption rate). The results suggest that a reduction in protein kinase activity may be related to overproduction of reactive oxygen species (ROS) under microwave field exposure. Decrease in sperm count and an increase in apoptosis may be causative factor due to mobile radiation exposure leading to infertility.

(E) Kesari KK, Kumar S, Behari J. Effects of Radiofrequency Electromagnetic Wave Exposure from Cellular Phones on the Reproductive Pattern in Male Wistar Rats. Appl Biochem Biotechnol. 164(4):546-559, 2011.

The present study investigates the effect of free radical formation due to mobile phone exposure and effect on fertility pattern in 70-day-old male Wistar rats (sham exposed and exposed). Exposure took place in Plexiglas cages for 2 h a day for 35 days to mobile phone frequency. The specific absorption rate was estimated to be 0.9 W/kg. An analysis of antioxidant enzymes

glutathione peroxidase ($P < 0.001$) and superoxide dismutase ($P < 0.007$) showed a decrease, while an increase in catalase ($P < 0.005$) was observed. Malondialdehyde ($P < 0.003$) showed an increase and histone kinase ($P = 0.006$) showed a significant decrease in the exposed group. Micronuclei also show a significant decrease ($P < 0.002$) in the exposed group. A significant change in sperm cell cycle of G(0)-G(1) ($P = 0.042$) and G(2)/M ($P = 0.022$) were recorded. Generation of free radicals was recorded to be significantly increased ($P = 0.035$). Our findings on antioxidant, malondialdehyde, histone kinase, micronuclei, and sperm cell cycle are clear indications of an infertility pattern, initiated due to an overproduction of reactive oxygen species. It is concluded that radiofrequency electromagnetic wave from commercially available cell phones might affect the fertilizing potential of spermatozoa.

(E) *Kesari KK, Kumar S, Behari J. 900-MHz microwave radiation promotes oxidation in rat brain. Electromagn Biol Med. 30(4):219-234, 2011.

Recently, there have been several reports referring to detrimental effects due to radio frequency electromagnetic fields (RF-EMF) exposure. Special attention was given to investigate the effect of mobile phone exposure on the rat brain. Since the integrative mechanism of the entire body lies in the brain, it is suggestive to analyze its biochemical aspects. For this, 35-day old Wistar rats were exposed to a mobile phone for 2 h per day for a duration of 45 days where specific absorption rate (SAR) was 0.9 W/Kg. Animals were divided in two groups: sham exposed ($n = 6$) and exposed group ($n = 6$). Our observations indicate a significant decrease ($P < 0.05$) in the level of glutathione peroxidase, superoxide dismutase, and an increase in catalase activity. Moreover, protein kinase shows a significant decrease in exposed group ($P < 0.05$) of hippocampus and whole brain. Also, a significant decrease ($P < 0.05$) in the level of pineal melatonin and a significant increase ($P < 0.05$) in creatine kinase and caspase 3 was observed in exposed group of whole brain as compared with sham exposed. Finally, a significant increase in the level of ROS (reactive oxygen species) ($P < 0.05$) was also recorded. The study concludes that a reduction or an increase in antioxidative enzyme activities, protein kinase C, melatonin, caspase 3, and creatine kinase are related to overproduction of reactive oxygen species (ROS) in animals under mobile phone radiation exposure. Our findings on these biomarkers are clear indications of possible health implications.

(E) *Khalil AM, Gagaa MH, Alshamali AM. 8-Oxo-7, 8-dihydro-2'-deoxyguanosine as a biomarker of DNA damage by mobile phone radiation. Hum Exp Toxicol. 31(7):734-740, 2012.

We examined the effect of exposure to mobile phone 1800 MHz radio frequency radiation (RFR) upon the urinary excretion of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodG), one major form of oxidative DNA damage, in adult male Sprague-Dawley rats. Twenty-four rats were used in three independent experiments (RFR exposed and control, 12 rats, each). The animals were exposed to RFR for 2 h from Global System for Mobile Communications (GSM) signal generator with whole-body-specific absorption rate of 1.0 W/kg. Urine samples were collected from the rat while housed in a metabolic cage during the exposure period over a 4-h period at 0.5, 1.0, 2.0 and 4.0 h from the beginning of exposure. In the control group, the signal generator was left in the turn-off position. The creatinine-standardized concentrations of 8-oxodG were measured. With the exception of the urine collected in the last half an hour of exposure, significant elevations were noticed in the levels of 8-oxodG in urine samples from rats exposed to RFR when compared to control animals. Significant differences were seen overall across time points of urine collection with a maximum at 1 h after exposure, suggesting repair of the DNA lesions leading to 8-oxodG formation.

(NE) *Khalil AM, Abu Khadra KM, Aljaberi AM, Gagaa MH, Issa HS. Assessment of oxidant/antioxidant status in saliva of cell phone users. Electromagn Biol Med. 2013 Jun 19. [Epub ahead of print]

Abstract Hazardous health effects resulting from exposure to radiofrequency electromagnetic radiation (RF-EMR) emitted from cell phones have been reported in the literature. However, the cellular and molecular targets of RF-EMR are still controversial. The aim of this study was to examine the oxidant/antioxidant status in saliva of cell phone users. Saliva samples collected before using a cell phone as well as at the end of 15 and 30 min calls were tested for two commonly used oxidative stress biomarkers: malondialdehyde (MDA) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-Oxo-dG). The 8-oxo-dG levels were determined by enzyme-linked immunosorbent (ELISA) competitive assay, while the MDA levels were measured using the OxiSelect MDA adduct ELISA Kit. The antioxidant capacity of the saliva was evaluated using the oxygen radical absorption capacity (ORAC) and the hydroxyl radical averting capacity (HORAC) assays according to the manufacture instructions. The mean 8-oxo-dG and the Bradford protein concentrations (ng/ml and mg/ml, respectively) peaked at 15 min. The levels of HORAC, ORAC and MDA progressively increased with time and reached maximum at 30 min. However, there was no significant effect of talking time on the levels of 8-OxodG and MDA. Similarly, there was no statistically significant effect of talking time on the oxygen and hydroxyl radicals averting capacities, (ORAC) and (HORAC), respectively. These findings suggest that there is no relationship between exposure to radio frequency radiation (RFR) and changes in the salivary oxidant/antioxidant profile.

(E) Kim MJ, Rhee SJ. Green tea catechins protect rats from microwave-induced oxidative damage to heart tissue. J Med Food. 7(3):299-304, 2004.

We investigated the effects of **green tea** catechin on oxidative damage in microwave-exposed rats. The microwave-exposed rats received one of three diets: catechin-free (MW-0C), 0.25% catechin (MW-0.25C), or 0.5% catechin (MW-0.5C). Rats were sacrificed 6 days after microwave irradiation (2.45 GHz, 15 minutes). Cytochrome P(450) levels in the MW-0C group was increased by 85% compared with normal, but was 11% and 14% lower in the MW-0.25C and MW-0.5C groups than in the MW-0C group. NADPH-cytochrome P(450) reductase activity in the MW-0C group was increased by 29%, compared with the normal group, but was significantly less in the MW-0.25C and MW-0.5C groups. Superoxide dismutase activity in the MW-0C group was decreased by 34%, compared with the normal group, but in the MW-0.25C and MW-0.5C groups was 19% and 25% higher. The activity of glutathione peroxidase in the MW-0C group was decreased by 28% but remained near normal with catechin supplements. Superoxide radical concentrations in the MW-0C group were increased by 35%, compared with the normal group. However, superoxide radicals in the MW-0.25C and MW-0.5C groups were 11% and 12% lower, respectively, compared with the MW-0C group. Microwave irradiation significantly increased levels of thiobarbituric acid-reactive substances, carbonyl values, and lipofuscin contents, but **green tea** catechin partially overcame the effects of the microwave irradiation. In conclusion, the mixed function oxidase system was activated, the formation of superoxide radical, lipid peroxide, oxidized protein, and lipofuscin was increased, and the antioxidative defense system was weakened in heart tissue of microwave-exposed rats, but the oxidative damage was significantly reduced by catechin supplementation.

(NE) *Kismali G, Ozgur E, Guler G, Akcay A, Sel T, Seyhan N. The influence of 1800 MHz GSM-like signals on blood chemistry and oxidative stress in non-pregnant and pregnant rabbits. Int J Radiat Biol. 88(5):414-419, 2012.

PURPOSE: Environmental electromagnetic fields originate from man-made sources, such as mobile phones and base stations, and have led to increasing public concern about their possible adverse health effects. We aimed to investigate the possible effects of radiofrequency radiation (RFR) generated from these devices on oversensitive animals, such as pregnant rabbits. MATERIALS AND METHODS: In the present study, the effects of whole body 1800 MHz Global System for Mobile Communications (GSM)-like RFR exposure for 15 min/day for seven days on blood chemistry and lipid peroxidation levels in both non-pregnant and pregnant New Zealand White rabbits were investigated. Thirteen-month-old rabbits were studied in the following four groups: Non-pregnant control, non-pregnant RFR-exposed, pregnant control and pregnant RFR-exposed. RESULTS: Lipid peroxidation, namely malondialdehyde (MDA) levels, did not change after RFR exposure. However, blood chemistry parameters, such as cholesterol (CHO), total protein (TP), albumin (ALB), uric acid, creatinin and creatine kinase (CK) and creatine kinase-myocardial band isoenzyme (CK-MB) changed due to both pregnancy and RFR exposure. CONCLUSION: Our investigations have been shown that no indication for oxidative stress was detected in the blood of pregnant rabbits upon RF exposure at specific conditions employed in the present study. Minor changes in some blood chemistry parameters were detected but CK-MB and CK increases were found remarkable. Studies on RFR exposure during pregnancy will help establish international standards for the protection of pregnant women from environmental RFR.

(E) Koylu H, Mollaoglu H, Ozguner F, Nazyroglu M, Delibab N. Melatonin modulates 900 Mhz microwave-induced lipid peroxidation changes in rat brain. Toxicol Ind Health. 22(5):211-216, 2006.

Microwaves (MW) from cellular phones may affect biological systems by increasing free radicals, which may enhance lipid peroxidation levels of the brain, thus leading to oxidative damage. Melatonin is synthesized in and secreted by the pineal gland at night and exhibits anti-oxidant properties. Several studies suggest that supplementation with anti-oxidant can influence MW-induced brain damage. The present study was designed to determine the effects of MW on the brain lipid peroxidation system, and the possible protective effects of melatonin on brain degeneration induced by MW. Twenty-eight Sprague-Dawley male rats were randomly divided into three groups as follows: (1) sham-operated control group (N = 8); (2) study 900-MHz MW-exposed group (N = 8); and (3) 900-MHz MW-exposed+melatonin (100 microg/kg sc before daily MW exposure treated group) (N = 10). Cortex brain and hippocampus tissues were removed to study the levels of lipid peroxidation as malonyl dialdehyde. The levels of lipid peroxidation in the brain cortex and hippocampus increased in the MW group compared with the control group, although the levels in the hippocampus were decreased by MW+melatonin administration. The brain cortex lipid peroxidation levels were unaffected by melatonin treatment. We conclude that melatonin may prevent MW-induced oxidative changes in the hippocampus by strengthening the anti-oxidant defense system, by reducing oxidative stress products.

(E) Lai, H, Singh, NP, Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells. Bioelectromagnetics 18(6):446-454, 1997.

Effects of in vivo microwave exposure on DNA strand breaks, a form of DNA damage, were investigated in rat brain cells. In previous research, we have found that acute (2 hours) exposure to

pulsed (2 microseconds pulses, 500 pps) 2450-MHz radiofrequency electromagnetic radiation (RFR) (power density 2 mW/cm², average whole body specific absorption rate 1.2 W/kg) caused an increase in DNA single- and double-strand breaks in brain cells of the rat when assayed 4 hours post exposure using a microgel electrophoresis assay. In the present study, we found that treatment of rats immediately before and after RFR exposure with either melatonin (1 mg/kg/injection, SC) or the spin-trap compound N-tert-butyl-alpha-phenylnitron (PBN) (100 mg/kg/injection, i.p.) blocks this effects of RFR. Since both melatonin and PBN are efficient free radical scavengers it is hypothesized that free radicals are involved in RFR-induced DNA damage in the brain cells of rats. Since cumulated DNA strand breaks in brain cells can lead to neurodegenerative diseases and cancer and an excess of free radicals in cells has been suggested to be the cause of various human diseases, data from this study could have important implications for the health effects of RFR exposure.

(NE) Lantow M, Schuderer J, Hartwig C, Simko M. Free Radical Release and HSP70 Expression in Two Human Immune-Relevant Cell Lines after Exposure to 1800 MHz Radiofrequency Radiation. Radiat Res. 165(1):88-94, 2006.

The goal of this study was to investigate whether radiofrequency (RF) electromagnetic-field (EMF) exposure at 1800 MHz causes production of free radicals and/or expression of heat-shock proteins (HSP70) in human immune-relevant cell systems. Human Mono Mac 6 and K562 cells were used to examine free radical release after exposure to incubator control, sham, RF EMFs, PMA, LPS, heat (40 degrees C) or co-exposure conditions. Several signals were used: continuous-wave, several typical modulations of the Global System for Mobile Communications (GSM): GSM-non DTX (speaking only), GSM-DTX (hearing only), GSM-Talk (34% speaking and 66% hearing) at specific absorption rates (SARs) of 0.5, 1.0, 1.5 and 2.0 W/kg. Heat and PMA treatment induced a significant increase in superoxide radical anions and in ROS production in the Mono Mac 6 cells when compared to sham and/ or incubator conditions. No significant differences in free radical production were detected after RF EMF exposure or in the respective controls, and no additional effects on superoxide radical anion production were detected after co-exposure to RF EMFs+PMA or RF EMFs+LPS. The GSM-DTX signal at 2 W/kg produced a significant difference in free radical production when the data were compared to sham because of the decreasing sham value. This difference disappeared when data were compared to the incubator controls. To determine the involvement of heat-shock proteins as a possible inhibitor of free radical production, we investigated the HSP70 expression level after different RF EMF exposures; no significant effects were detected.

(NE) Lantow M, Lupke M, Frahm J, Mattsson MO, Kuster N, Simko M. ROS release and Hsp70 expression after exposure to 1,800 MHz radiofrequency electromagnetic fields in primary human monocytes and lymphocytes. Radiat Environ Biophys. 45(1):55-62, 2006.

The aim of this study is to investigate if 1,800 MHz radiofrequency electromagnetic fields (RF-EMF) can induce reactive oxygen species (ROS) release and/or changes in heat shock protein 70 (Hsp70) expression in human blood cells, using different exposure and co-exposure conditions. Human umbilical cord blood-derived monocytes and lymphocytes were used to examine ROS release after exposure to continuous wave or different GSM signals (GSM-DTX and GSM-Talk) at 2 W/kg for 30 or 45 min of continuous or intermittent (5 min ON/5 min OFF) exposure. The cells were exposed to incubator conditions, to sham, to RF-EMF, or to chemicals in parallel. Cell stimulation with the phorbol ester phorbol-12-myristate-13-acetate (PMA; 1 µM) was used as positive control for ROS release. To investigate the effects on Hsp70 expression, the human monocytes were exposed to the GSM-DTX signal at 2 W/kg for 45 min, or to heat treatment (42 degrees C) as positive control. ROS production and Hsp70 expression were determined by flow cytometric analysis. The data were compared to sham and/or to control values and the statistical analysis was performed by the Student's t-test (P<0.05). The

PMA treatment induced a significant increase in ROS production in human monocytes and lymphocytes when the data were compared to sham or to incubator controls. After continuous or intermittent GSM-DTX signal exposure (2 W/kg), a significantly different ROS production was detected in human monocytes if the data were compared to sham. However, this significant difference appeared due to the lowered value of ROS release during sham exposure. In human lymphocytes, no differences could be detected if data were compared either to sham or to incubator control. The Hsp70 expression level after 0, 1, and 2 h post-exposure to GSM-DTX signal at 2 W/kg for 1 h did not show any differences compared to the incubator or to sham control.

(E) *Liu C, Duan W, Xu S, Chen C, He M, Zhang L, Yu Z, Zhou Z. Exposure to 1800 MHz radiofrequency electromagnetic radiation induces oxidative DNA base damage in a mouse spermatocyte-derived cell line. Toxicol Lett 218(1): 2-9, 2013.

Whether exposure to radiofrequency electromagnetic radiation (RF-EMR) emitted from mobile phones can induce DNA damage in male germ cells remains unclear. In this study, we conducted a 24 h intermittent exposure (5 min on and 10 min off) of a mouse spermatocyte-derived GC-2 cell line to 1800 MHz Global System for Mobile Communication (GSM) signals in GSM-Talk mode at specific absorption rates (SAR) of 1 W/kg, 2 W/kg or 4 W/kg. Subsequently, through the use of formamidopyrimidine DNA glycosylase (FPG) in a modified comet assay, we determined that the extent of DNA migration was significantly increased at a SAR of 4 W/kg. Flow cytometry analysis demonstrated that levels of the DNA adduct 8-oxoguanine (8-oxoG) were also increased at a SAR of 4 W/kg. These increases were concomitant with similar increases in the generation of reactive oxygen species (ROS); these phenomena were mitigated by co-treatment with the antioxidant α -tocopherol. However, no detectable DNA strand breakage was observed by the alkaline comet assay. Taking together, these findings may imply the novel possibility that RF-EMR with insufficient energy for the direct induction of DNA strand breaks may produce genotoxicity through oxidative DNA base damage in male germ cells.

(E) Lu YS, Huang BT, Huang YX. Reactive Oxygen Species Formation and Apoptosis in Human Peripheral Blood Mononuclear Cell Induced by 900 MHz Mobile Phone Radiation. Oxid Med Cell Longev. 2012:740280, 2012.

We demonstrate that reactive oxygen species (ROS) plays an important role in the process of apoptosis in human peripheral blood mononuclear cell (PBMC) which is induced by the radiation of 900 MHz radiofrequency electromagnetic field (RFEMF) at a specific absorption rate (SAR) of ~0.4 W/kg when the exposure lasts longer than two hours. The apoptosis is induced through the mitochondrial pathway and mediated by activating ROS and caspase-3, and decreasing the mitochondrial potential. The activation of ROS is triggered by the conformation disturbance of lipids, protein, and DNA induced by the exposure of GSM RFEMF. Although human PBMC was found to have a self-protection mechanism of releasing carotenoid in response to oxidative stress to lessen the further increase of ROS, the imbalance between the antioxidant defenses and ROS formation still results in an increase of cell death with the exposure time and can cause about 37% human PBMC death in eight hours.

(E) Luukkonen J, Hakulinen P, Mäki-Paakkanen J, Juutilainen J, Naarala J. Enhancement of chemically induced reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells by 872MHz radiofrequency radiation. Mutat Res. 662(1-2):54-58, 2009.

The objective of the study was to investigate effects of 872MHz radiofrequency (RF) radiation on intracellular reactive oxygen species (ROS) production and DNA damage at a relatively high SAR value (5W/kg). The experiments also involved combined exposure to RF radiation and menadione, a

chemical inducing intracellular ROS production and DNA damage. The production of ROS was measured using the fluorescent probe dichlorofluorescein and DNA damage was evaluated by the Comet assay. Human SH-SY5Y neuroblastoma cells were exposed to RF radiation for 1h with or without menadione. Control cultures were sham exposed. Both continuous waves (CW) and a pulsed signal similar to that used in global system for mobile communications (GSM) mobile phones were used. Exposure to the CW RF radiation increased DNA breakage ($p < 0.01$) in comparison to the cells exposed only to menadione. Comparison of the same groups also showed that ROS level was higher in cells exposed to CW RF radiation at 30 and 60min after the end of exposure ($p < 0.05$ and $p < 0.01$, respectively). No effects of the GSM signal were seen on either ROS production or DNA damage. The results of the present study suggest that 872MHz CW RF radiation at 5W/kg might enhance chemically induced ROS production and thus cause secondary DNA damage. However, there is no known mechanism that would explain such effects from CW RF radiation but not from GSM modulated RF radiation at identical SAR.

(NE) Luukkonen J, Juutilainen J, Naarala J. Combined effects of 872 MHz radiofrequency radiation and ferrous chloride on reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells. Bioelectromagnetics. 31(6):417-424, 2010.

The aim of the present study was to investigate possible cooperative effects of radiofrequency (RF) radiation and ferrous chloride (FeCl_2) on reactive oxygen species (ROS) production and DNA damage. In order to test intracellular ROS production as a possible underlying mechanism of DNA damage, we applied the fluorescent probe DCFH-DA. Integrity of DNA was quantified by alkaline comet assay. The exposures to 872 MHz RF radiation were conducted at a specific absorption rate (SAR) of 5 W/kg using continuous waves (CW) or a modulated signal similar to that used in Global System for Mobile Communications (GSM) phones. Four groups were included: (1) Sham exposure (control), (2) RF radiation, (3) Chemical treatment, (4) Chemical treatment, and RF radiation. In the ROS production experiments, human neuroblastoma (SH-SY5Y) cells were exposed to RF radiation and 10 $\mu\text{g/ml}$ FeCl_2 for 1 h. In the comet assay experiments, the exposure time was 3 h and an additional chemical (0.015% diethyl maleate) was used to make DNA damage level observable. The chemical treatments resulted in statistically significant responses, but no effects from either CW or modulated RF radiation were observed on ROS production, DNA damage or cell viability.

(E) *Maaroufi K, Save E, Poucet B, Sakly M, Abdelmelek H, Had-Aissouni L. Oxidative stress and prevention of the adaptive response to chronic iron overload in the brain of young adult rats exposed to a 150 kilohertz electromagnetic field. Neuroscience. 186:39-47, 2011.

Iron surcharge may induce an oxidative stress-based decline in several neurological functions. In addition, electromagnetic fields (EMF) of frequencies up to about 100 kHz, emitted by electric/electronic devices, have been suggested to enhance free radical production through an iron dependent pathway. The purpose of this study was therefore to determine a possible relationship between iron status, exposure to EMF, and brain oxidative stress in young adult rats. Samples were micro-dissected from prefrontal cortex, hippocampus, striatum, and cerebellum after chronic saline or iron overload (IO) as well as after chronic sham exposure or exposure to a 150 kHz EMF or after combining EMF exposure with IO. The brain samples were used to monitor oxidative stress-induced lipid peroxidation and activity of the antioxidant enzymes superoxide dismutase and catalase. While IO did not induce any oxidative stress in young adult rats, it stimulated antioxidant defenses in the cerebellum and prefrontal cortex in particular. On the contrary, EMF exposure stimulated lipid peroxidation mainly in the cerebellum, without affecting antioxidant defenses. When EMF was coapplied with IO, lipid peroxidation was further

increased as compared to EMF alone while the increase in antioxidant defenses triggered by the sole IO was abolished. These data suggest that EMF exposure may be harmful in young adults by impairing the antioxidant defenses directed at preventing iron-induced oxidative stress.

(E) Mailankot M, Kunnath AP, Jayalekshmi H, Koduru B, Valsalan R. Radio frequency electromagnetic radiation (RF-EMR) from GSM (0.9/1.8GHz) mobile phones induces oxidative stress and reduces sperm motility in rats. Clinics (Sao Paulo). 64(6):561-565, 2009.

INTRODUCTION: Mobile phones have become indispensable in the daily lives of men and women around the globe. As cell phone use has become more widespread, concerns have mounted regarding the potentially harmful effects of RF-EMR from these devices. OBJECTIVE: The present study was designed to evaluate the effects of RF-EMR from mobile phones on free radical metabolism and sperm quality. MATERIALS AND METHODS: Male albino Wistar rats (10-12 weeks old) were exposed to RF-EMR from an active GSM (0.9/1.8 GHz) mobile phone for 1 hour continuously per day for 28 days. Controls were exposed to a mobile phone without a battery for the same period. The phone was kept in a cage with a wooden bottom in order to address concerns that the effects of exposure to the phone could be due to heat emitted by the phone rather than to RF-EMR alone. Animals were sacrificed 24 hours after the last exposure and tissues of interest were harvested. RESULTS: One hour of exposure to the phone did not significantly change facial temperature in either group of rats. No significant difference was observed in total sperm count between controls and RF-EMR exposed groups. However, rats exposed to RF-EMR exhibited a significantly reduced percentage of motile sperm. Moreover, RF-EMR exposure resulted in a significant increase in lipid peroxidation and low GSH content in the testis and epididymis. CONCLUSION: Given the results of the present study, we speculate that RF-EMR from mobile phones negatively affects semen quality and may impair male fertility.

(E) *Manta AK, Stravopodis DJ, Papassideri IS, Margaritis LH. Reactive oxygen species elevation and recovery in Drosophila bodies and ovaries following short-term and long-term exposure to DECT base EMF. Electromagn Biol Med. 2013 Jun 19. [Epub ahead of print] (LI)

Abstract The objective of this study was to approach the basic mechanism(s) underlying reported ovarian apoptotic cell death and fecundity decrease induced by nonionizing radiation (NIR) in *Drosophila melanogaster*. ROS (Reactive Oxygen Species) levels were measured in the bodies and the ovaries of (sexually mature) 4-day-old flies, following exposure for 0.5, 1, 6, 24 and 96 h to a wireless DECT (Digital Enhanced Cordless Telephone) base radiation (1.88-1.90 GHz). Electrical field intensity was 2.7 V/m, measured within the fly vials and calculated SAR (Specific Absorption Rate) value = 0.009 W/Kg. Male and female bodies showed twofold increase in ROS levels ($p < 0.001$) after 6 h of exposure, slightly increasing with more irradiation (24 and 96 h). Ovaries of exposed females had a quick response in ROS increase after 0.5 h (1.5-fold, $p < 0.001$), reaching 2.5-fold after 1 h with no elevation thereafter at 6, 24 and 96 h. ROS levels returned to normal, in the male and the female bodies 24 h after 6 h of exposure of the flies ($p < 0.05$) and in the ovaries 4 h after 1 h exposure of the females ($p < 0.05$). It is postulated that the pulsed (at 100 Hz rate and 0.08 ms duration) idle state of the DECT base radiation is capable of inducing free radical formation albeit the very low SAR, leading rapidly to accumulation of ROS in a level-saturation manner under continuous exposure, or in a recovery manner after interruption of radiation, possibly due to activation of the antioxidant machinery of the organism.

(E) *Meena R, Kumari K, Kumar J, Rajamani P, Verma HN, Kesari KK. Therapeutic approaches of melatonin in microwave radiations-induced oxidative stress-mediated toxicity on male fertility pattern of Wistar rats Electromagn Biol Med. 2013 May 15. [Epub ahead of print]

Abstract Microwave (MW) radiation produced by wireless telecommunications and a number of electrical devices used in household or in healthcare institutions may adversely affects the reproductive pattern. Present study aimed to investigate the protective effects of melatonin (is well known antioxidant that protects DNA, lipids and proteins from free radical damage) against oxidative stress-mediated testicular impairment due to long-term exposure of MWs. For this, 70-day-old male Wistar rats were divided into four groups (n = 6/group): Sham exposed, Melatonin (Mel) treated (2 mg/kg), 2.45 GHz MWs exposed and MWs + Mel treated. Exposure took place in Plexiglas cages for 2 h a day for 45 days where, power density (0.21 mW/cm²) and specific absorption rate (SAR 0.14 W/Kg) were estimated. After the completion of exposure period, rats were sacrificed and various stress related parameters, that is LDH-X (lactate dehydrogenase isoenzyme) activity, xanthine oxidase (XO), ROS (reactive oxygen species), protein carbonyl content, DNA damage and MDA (malondialdehyde) were performed. Result shows that melatonin prevent oxidative damage biochemically by significant increase (p < 0.001) in the levels of testicular LDH-X, decreased (p < 0.001) levels of MDA and ROS in testis (p < 0.01). Meanwhile, it reversed the effects of MWs on XO, protein carbonyl content, sperm count, testosterone level and DNA fragmentation in testicular cells. These results concluded that the melatonin has strong antioxidative potential against MW induced oxidative stress mediated DNA damage in testicular cells.

(E) *Megha K, Deshmukh PS, Banerjee BD, Tripathi AK, Abegaonkar MP. Microwave radiation induced oxidative stress, cognitive impairment and inflammation in brain of Fischer rats. Indian J Exp Biol. 50(12):889-896, 2012. (LI)

Public concerns over possible adverse effects of microwave radiation emitted by mobile phones on health are increasing. To evaluate the intensity of oxidative stress, cognitive impairment and inflammation in brain of Fischer rats exposed to microwave radiation, male Fischer-344 rats were exposed to 900 MHz microwave radiation (SAR = 5.953 x 10⁽⁻⁴⁾ W/kg) and 1800 MHz microwave radiation (SAR = 5.835 x 10⁽⁻⁴⁾ W/kg) for 30 days (2 h/day). Significant impairment in cognitive function and induction of oxidative stress in brain tissues of microwave exposed rats were observed in comparison with sham exposed groups. Further, significant increase in level of cytokines (IL-6 and TNF-alpha) was also observed following microwave exposure. Results of the present study indicated that increased oxidative stress due to microwave exposure may contribute to cognitive impairment and inflammation in brain.

(E) Meral I, Mert H, Mert N, Deger Y, Yoruk I, Yetkin A, Keskin S. Effects of 900-MHz electromagnetic field emitted from cellular phone on brain oxidative stress and some vitamin levels of guinea pigs. Brain Res. 1169:120-124, 2007.

This study was designed to demonstrate the effects of 900-MHz electromagnetic field (EMF) emitted from cellular phone on brain tissue and also blood malondialdehyde (MDA), glutathione (GSH), retinol (vitamin A), vitamin D(3) and tocopherol (vitamin E) levels, and catalase (CAT) enzyme activity of guinea pigs. Fourteen male guinea pigs, weighing 500-800 g were randomly divided into one of two experimental groups: control and treatment (EMF-exposed), each containing seven animals. Animals in treatment group were exposed to 890- to 915-MHz EMF (217-Hz pulse rate, 2-W maximum peak power, SAR 0.95 w/kg) of a cellular phone for 12 h/day

(11-h 45-min stand-by and 15-min spiking mode) for 30 days. Control guinea pigs were housed in a separate room without exposing EMF of a cellular phone. Blood samples were collected through a cardiac puncture and brains were removed after decapitation for the biochemical analysis at the end of the 30 days of experimental period. It was found that the MDA level increased ($P < 0.05$), GSH level and CAT enzyme activity decreased ($P < 0.05$), and vitamins A, E and D(3) levels did not change ($P > 0.05$) in the brain tissues of EMF-exposed guinea pigs. In addition, MDA, vitamins A, D(3) and E levels, and CAT enzyme activity increased ($P < 0.05$), and GSH level decreased ($P < 0.05$) in the blood of EMF-exposed guinea pigs. It was concluded that electromagnetic field emitted from cellular phone might produce oxidative stress in brain tissue of guinea pigs. However, more studies are needed to demonstrate whether these effects are harmful or/and affect the neural functions.

(E) *Monselise EB, Levkovitz A, Gottlieb HE, Kost D. Bioassay for assessing cell stress in the vicinity of radio-frequency irradiating antennas. J Environ Monit. 13(7):1890-1896, 2011.

The 24 h exposure of water plants (etiolated duckweed) to RF-EMF between 7.8 V m⁻¹ and 1.8 V m⁻¹, generated by AM 1.287 MHz transmitting antennas, resulted in alanine accumulation in the plant cells, a phenomenon we have previously shown to be a universal stress signal. The magnitude of the effect corresponds qualitatively to the level of RF-EMF exposure. In the presence of 10 mM vitamin C, alanine accumulation is completely suppressed, suggesting the involvement of free radicals in the process. A unique biological connection has thus been made between exposure to RF-EMF and cell stress, in the vicinity of RF transmitting antennas. This simple test, which lasts only 24 h, constitutes a useful bioassay for the quick detection of biological cell stress caused in the vicinity of RF irradiating antennas.

(E) Moustafa YM, Moustafa RM, Belacy A, Abou-El-Ela SH, Ali FM. Effects of acute exposure to the radiofrequency fields of cellular phones on plasma lipid peroxide and antioxidase activities in human erythrocytes. J Pharm Biomed Anal 26(4):605-608, 2001.

Radiofrequency fields of cellular phones may affect biological systems by increasing free radicals, which appear mainly to enhance lipid peroxidation, and by changing the antioxidase activities of human blood thus leading to oxidative stress. To test this, we have investigated the effect of acute exposure to radiofrequency fields of commercially available cellular phones on some parameters indicative of oxidative stress in 12 healthy adult male volunteers. Each volunteer put the phone in his pocket in standby position with the keypad facing the body. The parameters measured were lipid peroxide and the activities of superoxide dismutase (SOD), total glutathione peroxidase (GSH-Px) and catalase. The results obtained showed that the plasma level of lipid peroxide was significantly increased after 1, 2 and 4 h of exposure to radiofrequency fields of the cellular phone in standby position. Moreover, the activities of SOD and GSH-Px in human erythrocytes showed significant reduction while the activity of catalase in human erythrocytes did not decrease significantly. These results indicate that acute exposure to radiofrequency fields of commercially available cellular phones may modulate the oxidative stress of free radicals by enhancing lipid peroxidation and reducing the activation of SOD and GSH-Px, which are free radical scavengers. Therefore, these results support the interaction of radiofrequency fields of cellular phones with biological systems.

(E) *Nazıroğlu M, Çiğ B, Doğan S, Uğuz AC, Dilek S, Faouzi D. 2.45-Gz wireless devices induce oxidative stress and proliferation through cytosolic Ca²⁺ influx in human leukemia cancer cells. Int J Radiat Biol. 88(6):449-456, 2012a.

PURPOSE: Electromagnetic radiation from wireless devices may affect biological systems by increasing free radicals. The present study was designed to determine the effects of 2.45 GHz radiation on the antioxidant redox system, calcium ion signaling, cell count and viability in human leukemia 60 cells. MATERIALS AND METHODS: Twelve cell cultures were equally divided into two main groups as controls (n = 6) and irradiated (n = 6) and then subdivided into four different subgroups depending on the duration of exposure, namely 1, 2, 12 and 24 hours. The samples were analyzed immediately after the experimental period. RESULTS: The extent of lipid peroxidation, cytosolic free Ca²⁺ and cell numbers were higher in 2.45 GHz groups than in the controls. The increase of cytosolic free Ca²⁺ concentrations was radiation time-dependent and was highest at 24-h exposure. The reduced glutathione, glutathione peroxidase, vitamin C and cell viability values did not show any changes in any of the experimental groups. 2-aminoethyl diphenylborinate inhibits Ca²⁺ ions influx by blockage of the transient receptor potential melastatin 2. CONCLUSIONS: 2.45 GHz electromagnetic radiation appears to induce proliferative effects through oxidative stress and Ca²⁺ influx although blocking of transient receptor potential melastatin 2 channels by 2-aminoethyl diphenylborinate seems to counteract the effects on Ca²⁺ ions influx.

(E) *Nazıroğlu M, Çelik Ö, Özgül C, Çiğ B, Doğan S, Bal R, Gümral N, Rodríguez AB, Pariente JA. Melatonin modulates wireless (2.45 GHz)-induced oxidative injury through TRPM2 and voltage gated Ca(2+) channels in brain and dorsal root ganglion in rat. Physiol Behav. 105(3):683-692, 2012b.

We aimed to investigate the protective effects of melatonin and 2.45 GHz electromagnetic radiation (EMR) on brain and dorsal root ganglion (DRG) neuron antioxidant redox system, Ca(2+) influx, cell viability and electroencephalography (EEG) records in the rat. Thirty two rats were equally divided into four different groups namely group A1: Cage control, group A2: Sham control, group B: 2.45 GHz EMR, group C: 2.45 GHz EMR+melatonin. Groups B and C were exposed to 2.45 GHz EMR during 60 min/day for 30 days. End of the experiments, EEG records and the brain cortex and DRG samples were taken. Lipid peroxidation (LP), cell viability and cytosolic Ca(2+) values in DRG neurons were higher in group B than in groups A1 and A2 although their concentrations were increased by melatonin, 2-aminoethyldiphenyl borinate (2-APB), diltiazem and verapamil supplementation. Spike numbers of EEG records in group C were lower than in group B. Brain cortex vitamin E concentration was higher in group C than in group B. In conclusion, Melatonin supplementation in DRG neurons and brain seems to have protective effects on the 2.45 GHz-induced increase Ca(2+) influx, EEG records and cell viability of the hormone through TRPM2 and voltage gated Ca(2+) channels.

(E) *Oksay T, Nazıroğlu M, Doğan S, Güzel A, Gümral N, Koşar PA. Protective effects of melatonin against oxidative injury in rat testis induced by wireless (2.45 GHz) devices. Andrologia. 2012 Nov 12. doi: 10.1111/and.12044. [Epub ahead of print]

Wireless devices have become part of everyday life and mostly located near reproductive organs while they are in use. The present study was designed to determine the possible protective effects of melatonin on oxidative stress-dependent testis injury induced by 2.45-GHz electromagnetic radiation (EMR). Thirty-two rats were equally divided into four different groups, namely cage control (A1), sham control (A2), 2.45-GHz EMR (B) and 2.45-GHz EMR+melatonin (C). Group

B and C were exposed to 2.45-GHz EMR during 60 min day⁻¹ for 30 days. Lipid peroxidation levels were higher in Group B than in Group A1 and A2. Melatonin treatment prevented the increase in the lipid peroxidation induced by EMR. Also reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) levels in Group D were higher than that of exposure group. Vitamin A and E concentrations decreased in exposure group, and melatonin prevented the decrease in vitamin E levels. In conclusion, wireless (2.45 GHz) EMR caused oxidative damage in testis by increasing the levels of lipid peroxidation and decreasing in vitamin A and E levels. Melatonin supplementation prevented oxidative damage induced by EMR and also supported the antioxidant redox system in the testis.

(E) Oktem F, Ozguner F, Mollaoglu H, Koyu A, Uz E. Oxidative Damage in the Kidney Induced by 900-MHz-Emitted Mobile Phone: Protection by Melatonin. Arch Med Res. 36(4):350-355, 2005.

BACKGROUND: The mobile phones emitting 900-MHz electromagnetic radiation (EMR) may be mainly absorbed by kidneys because they are often carried in belts. Melatonin, the chief secretory product of the pineal gland, was recently found to be a potent free radical scavenger and antioxidant. The aim of this study was to examine 900-MHz mobile phone-induced oxidative stress that promotes production of reactive oxygen species (ROS) on renal tubular damage and the role of melatonin on kidney tissue against possible oxidative damage in rats. **METHODS:** The animals were randomly grouped as follows: 1) sham-operated control group and 2) study groups: i) 900-MHz EMR exposed (30 min/day for 10 days) group and ii) 900-MHz EMR exposed+melatonin (100 µg kg⁻¹ s.c. before the daily EMR exposure) treated group. Malondialdehyde (MDA), an index of lipid peroxidation, and urine N-acetyl-beta-d-glucosaminidase (NAG), a marker of renal tubular damage were used as markers of oxidative stress-induced renal impairment. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate the changes of antioxidant status. **RESULTS:** In the EMR-exposed group, while tissue MDA and urine NAG levels increased, SOD, CAT, and GSH-Px activities were reduced. Melatonin treatment reversed these effects as well. In this study, the increase in MDA levels of renal tissue and in urine NAG and also the decrease in renal SOD, CAT, GSH-Px activities demonstrated the role of oxidative mechanism induced by 900-MHz mobile phone exposure, and melatonin, via its free radical scavenging and antioxidant properties, ameliorated oxidative tissue injury in rat kidney. **CONCLUSIONS:** These results show that melatonin may exhibit a protective effect on mobile phone-induced renal impairment in rats.

(E) Oral B, Guney M, Ozguner F, Karahan N, Mungan T, Comlekci S, Cesur G. Endometrial Apoptosis Induced by a 900-MHz Mobile Phone: Preventive Effects of Vitamins E and C. Adv Ther. 23(6):957-973, 2006.

Numerous reports have described the effects induced by an electromagnetic field (EMF) in various cellular systems. The purposes of this study were to examine oxidative stress that promotes production of reactive oxygen species induced by a 900-megahertz (MHz) mobile phone and the possible ameliorating effects of vitamins E and C on endometrial tissue against EMF-induced endometrial impairment and apoptosis in rats. Animals were randomly grouped as follows: (1) sham-operated control group (n=8), (2) 900 MHz EMF-exposed group (n=8; 30 min/d for 30 d), and (3) 900 MHz EMF-exposed group, treated with vitamins E and C (n=8; 50 mg/kg intramuscularly and 20 mg/kg body weight intraperitoneally before daily EMF exposure). Malondialdehyde (an index of lipid peroxidation) was used as a marker of oxidative stress-induced endometrial impairment; Bcl-2, Bax, caspase-3, and caspase-8 were assessed immunohistochemically. In this study, increased malondialdehyde levels in endometrial tissue and apoptosis illustrated the role of the oxidative mechanism induced by exposure to a 900-MHz mobile phone-like device and vitamins E and C; via free radical scavenging and antioxidant

properties, oxidative tissue injury and apoptosis were ameliorated in rat endometrium. In conclusion, exposure to 900-MHz radiation emitted by mobile phones may cause endometrial apoptosis and oxidative stress, but treatment with vitamins E and C can diminish these changes and may have a beneficial effect in preventing endometrial changes in rats.

(E) Ozguner F, Aydin G, Mollaoglu H, Gokalp O, Koyu A, Cesur G. Prevention of mobile phone induced skin tissue changes by melatonin in rat: an experimental study. Toxicol Ind Health. 20(6-10):133-139, 2004.

Most of the mobile phones in Turkey emit 900 MHz radiation which is mainly absorbed by the skin and, to a lesser extent, muscle. The aim of this study was to investigate the effects the 900 MHz electromagnetic irradiation emitted by these devices on the induction of histopathologic changes in skin and the effect of melatonin (Mel) on any of these changes. Thirty male Wistar-Albino rats were used in the study. The experimental groups were composed of: a nontreated control group, an irradiated group (IR) without Mel and an irradiated with Mel treatment group (IR + Mel). 900 MHz radiation was applied to IR group for 10 days (30 min/day). The IR + Mel group received 10 mg/kg per day melatonin in tap water for 10 days before irradiation. At the end of the tenth day, the skin graft was excized from the thoraco-abdominal area. Histopathologic changes in skin were analyzed. In the IR group, increased thickness of stratum corneum, atrophy of epidermis, papillomatosis, basal cell proliferation, increased granular cell layer (hypergranulosis) in epidermis and capillary proliferation, impairment in collagen tissue distribution and separation of collagen bundles in dermis were all observed compared to the control group. Most of these changes, except hypergranulosis, were prevented with melatonin treatment. In conclusion, exposure to 900 MHz radiation emitted by mobile phones caused mild skin changes. Furthermore, melatonin treatment can reduce these changes and may have a beneficial effect to prevent 900 MHz mobile phone-induced rat skin changes.

(E) *Ozgur E, Kismali G, Guler G, Akcay A, Ozkurt G, Sel T, Seyhan N. Effects of Prenatal and Postnatal Exposure to GSM-Like Radiofrequency on Blood Chemistry and Oxidative Stress in Infant Rabbits, an Experimental Study. Cell Biochem Biophys. 2013 Mar 24. [Epub ahead of print]

We aimed to investigate the potential hazardous effects of prenatal and/or postnatal exposure to 1800 MHz GSM-like radiofrequency radiation (RFR) on the blood chemistry and lipid peroxidation levels of infant rabbits. A total of 72 New Zealand female and male white rabbits aged 1-month were used. Thirty-six female and 36 male were divided into four groups which were composed of nine infants: (i) Group 1 were the sham exposure (control), (ii) Group 2 were exposed to RFR, 15 min daily for 7 days in the prenatal period (between 15th and 22nd days of the gestational period) (prenatal exposure group). (iii) Group 3 were exposed to RFR 15 min/day (14 days for male, whereas 7 days for female) after they reached 1-month of age (postnatal exposure group). (iv) Group 4 were exposed to RFR for 15 min daily during 7 days in the prenatal period (between 15th and 22nd days of the gestational period) and 15 min/day (14 days for male, whereas 7 days for female) after they reached 1-month of age (prenatal and postnatal exposure group). Results showed that serum lipid peroxidation level in both female and male rabbits changed due to the RFR exposure. However, different parameters of the blood biochemistry were affected by exposure in male and female infants. Consequently, the whole-body 1800 MHz GSM-like RFR exposure may lead to oxidative stress and changes on some blood chemistry parameters. Studies on RFR exposure during prenatal and postnatal periods will help to establish international standards for the protection of pregnant and newborns from environmental RFR.

(E) Ozguner F, Oktem F, Ayata A, Koyu A, Yilmaz HR. A novel antioxidant agent caffeic acid phenethyl ester prevents long-term mobile phone exposure-induced renal impairment in rat. Prognostic value of malondialdehyde, N-acetyl-beta-D-glucosaminidase and nitric oxide determination. Mol Cell Biochem. 277(1-2):73-80, 2005.

Caffeic acid phenethyl ester (CAPE), a flavonoid like compound, is one of the major components of honeybee propolis. It has been used in folk medicine for many years in Middle East countries. It was found to be a potent free radical scavenger and antioxidant recently. The aim of this study was to examine long-term applied 900 MHz emitting mobile phone-induced oxidative stress that promotes production of reactive oxygen species (ROS) and, was to investigate the role of CAPE on kidney tissue against the possible electromagnetic radiation (EMR)-induced renal impairment in rats. In particular, the ROS such as superoxide and nitric oxide (NO) may contribute to the pathophysiology of EMR-induced renal impairment. Malondialdehyde (MDA, an index of lipid peroxidation) levels, urinary N-acetyl-beta-D: -glucosaminidase (NAG, a marker of renal tubular injury) and nitric oxide (NO, an oxidant product) levels were used as markers of oxidative stress-induced renal impairment and the success of CAPE treatment. The activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) in renal tissue were determined to evaluate the changes of antioxidant status. The rats used in the study were randomly grouped (10 each) as follows: i) Control group (without stress and EMR), ii) Sham-operated rats stayed without exposure to EMR (exposure device off), iii) Rats exposed to 900 MHz EMR (EMR group), and iv) A 900 MHz EMR exposed + CAPE treated group (EMR + CAPE group). In the EMR exposed group, while tissue MDA, NO levels and urinary NAG levels increased ($p < 0.0001$), the activities of SOD, CAT, and GSH-Px in renal tissue were reduced ($p < 0.001$). CAPE treatment reversed these effects as well ($p < 0.0001$, $p < 0.001$ respectively). In conclusion, the increase in NO and MDA levels of renal tissue, and in urinary NAG with the decrease in renal SOD, CAT, GSH-Px activities demonstrate the role of oxidative mechanisms in 900 MHz mobile phone-induced renal tissue damage, and CAPE, via its free radical scavenging and antioxidant properties, ameliorates oxidative renal damage. These results strongly suggest that CAPE exhibits a protective effect on mobile phone-induced and free radical mediated oxidative renal impairment in rats.

(E) Ozguner F, Oktem F, Armagan A, Yilmaz R, Koyu A, Demirel R, Vural H, Uz E. Comparative analysis of the protective effects of melatonin and caffeic acid phenethyl ester (CAPE) on mobile phone-induced renal impairment in rat. Mol Cell Biochem. 276(1-2):31-37, 2005.

Melatonin and caffeic acid phenethyl ester (CAPE), a component of honeybee propolis, were recently found to be potent free radical scavengers and antioxidants. There are a number of reports on the effects induced by electromagnetic radiation (EMR) in various cellular systems. Mechanisms of adverse effects of EMR indicate that reactive oxygen species may play a role in the biological effects of this radiation. The present study was carried out to compare the protective effects of melatonin and CAPE against 900 MHz EMR emitted mobile phone-induced renal tubular injury. Melatonin was administered whereas CAPE was given for 10 days before the exposure. Urinary N-acetyl-beta-D-glucosaminidase (NAG, a marker of renal tubular injury) and malondialdehyde (MDA, an index of lipid peroxidation), were used as markers of oxidative stress-induced renal impairment in rats exposed to EMR. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate the changes of antioxidant status in renal tissue. Urinary NAG and renal MDA were increased in EMR exposed rats while both melatonin and CAPE caused a significant reduction in the levels of these parameters. Likewise, renal SOD and GSH-Px activities were decreased in EMR exposed animals while melatonin caused a significant increase in the activities of these antioxidant enzymes but CAPE did not. Melatonin caused a significant decrease in urinary NAG activity and MDA levels which were increased because of EMR exposure. CAPE also reduced elevated MDA levels in

EMR exposed renal tissue, but the effect of melatonin was more potent than that of CAPE. Furthermore, treatment of EMR exposed rats with melatonin increased activities of SOD and GSH-Px to higher levels than those of control rats. In conclusion, melatonin and CAPE prevent renal tubular injury by reducing oxidative stress and protect the kidney from oxidative damage induced by 900 MHz mobile phone. Nevertheless, melatonin seems to be a more potent antioxidant compared with CAPE in kidney.

(E) Ozguner F, Altinbas A, Ozaydin M, Dogan A, Vural H, Kisioglu AN, Cesur G, Yildirim NG. Mobile phone-induced myocardial oxidative stress: protection by a novel antioxidant agent caffeic acid phenethyl ester. Toxicol Ind Health. 21(9):223-230, 2005.

Electromagnetic radiation (EMR) or radiofrequency fields of cellular mobile phones may affect biological systems by increasing free radicals, which appear mainly to enhance lipid peroxidation, and by changing the antioxidant defense systems of human tissues, thus leading to oxidative stress. Mobile phones are used in close proximity to the heart, therefore 900 MHz EMR emitting mobile phones may be absorbed by the heart. Caffeic acid phenethyl ester (CAPE), one of the major components of honeybee propolis, was recently found to be a potent free radical scavenger and antioxidant, and is used in folk medicine. The aim of this study was to examine 900 MHz mobile phone-induced oxidative stress that promotes production of reactive oxygen species (ROS) and the role of CAPE on myocardial tissue against possible oxidative damage in rats. Thirty rats were used in the study. Animals were randomly grouped as follows: sham-operated control group (N: 10) and experimental groups: (a) group II: 900 MHz EMR exposed group (N: 10); and (b) group III: 900 MHz EMR exposed+CAPE-treated group (N: 10). A 900 MHz EMR radiation was applied to groups II and III 30 min/day, for 10 days using an experimental exposure device. Malondialdehyde (MDA, an index of lipid peroxidation), and nitric oxide (NO, a marker of oxidative stress) were used as markers of oxidative stress-induced heart impairment. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate the changes of antioxidant status. In the EMR exposed group, while tissue MDA and NO levels increased, SOD, CAT and GSH-Px activities were reduced. CAPE treatment in group III reversed these effects. In this study, the increased levels of MDA and NO and the decreased levels of myocardial SOD, CAT and GSH-Px activities demonstrate the role of oxidative mechanisms in 900 MHz mobile phone-induced heart tissue damage, and CAPE, via its free radical scavenging and antioxidant properties, ameliorates oxidative heart injury. These results show that CAPE exhibits a protective effect on mobile phone-induced and free radical mediated oxidative heart impairment in rats.

(E) Ozguner F, Bardak Y, Comlekci S. Protective effects of melatonin and caffeic acid phenethyl ester against retinal oxidative stress in long-term use of mobile phone: A comparative study. Mol Cell Biochem. 282(1-2):83-88, 2006.

There are numerous reports on the effects of electromagnetic radiation (EMR) in various cellular systems. Melatonin and caffeic acid phenethyl ester (CAPE), a component of honeybee propolis, were recently found to be potent free radical scavengers and antioxidants. Mechanisms of adverse effects of EMR indicate that reactive oxygen species may play a role in the biological effects of this radiation. The present study was carried out to compare the efficacy of the protective effects of melatonin and CAPE against retinal oxidative stress due to long-term exposure to 900 MHz EMR emitting mobile phones. Melatonin and CAPE were administered daily for 60 days to the rats prior to their EMR exposure during our study. Nitric oxide (NO, an oxidant product) levels and malondialdehyde (MDA, an index of lipid peroxidation), were used as markers of retinal oxidative stress in rats following to use of EMR. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate the changes of antioxidant status in retinal tissue. Retinal levels of NO and MDA increased in EMR exposed rats while both melatonin and CAPE caused a significant reduction in

the levels of NO and MDA. Likewise, retinal SOD, GSH-Px and CAT activities decreased in EMR exposed animals while melatonin and CAPE caused a significant increase in the activities of these antioxidant enzymes. Treatment of EMR exposed rats with melatonin or CAPE increased the activities of SOD, GSH-Px and CAT to higher levels than those of control rats. In conclusion, melatonin and CAPE reduce retinal oxidative stress after long-term exposure to 900 MHz emitting mobile phone. Nevertheless, there was no statistically significant difference between the efficacies of these two antioxidants against to EMR induced oxidative stress in rat retina. The difference was in only GSH-Px activity in rat retina. Melatonin stimulated the retinal GSH-Px activity more efficiently than CAPE did.

(E) Ozgur E, Güler G, Seyhan N. Mobile phone radiation-induced free radical damage in the liver is inhibited by the antioxidants n-acetyl cysteine and epigallocatechin-gallate. Int J Radiat Biol. 86(1):935-945, 2010.

Purpose: To investigate oxidative damage and antioxidant enzyme status in the liver of guinea pigs exposed to mobile phone-like radiofrequency radiation (RFR) and the potential protective effects of N-acetyl cysteine (NAC) and epigallocatechin-gallate (EGCG) on the oxidative damage. Materials and methods: Nine groups of guinea pigs were used to study the effects of exposure to an 1800-MHz Global System for Mobile Communications (GSM)-modulated signal (average whole body Specific Absorption Rate (SAR) of 0.38 W/kg, 10 or 20 min per day for seven days) and treatment with antioxidants. Results: Significant increases in malondialdehyde (MDA) and total nitric oxide (NO(x)) levels and decreases in activities of superoxide dismutase (SOD), myeloperoxidase (MPO) and glutathione peroxidase (GSH-Px) were observed in the liver of guinea pigs after RFR exposure. Only NAC treatment induces increase in hepatic GSH-Px activities, whereas EGCG treatment alone attenuated MDA level. Extent of oxidative damage was found to be proportional to the duration of exposure ($P < 0.05$). Conclusion: Mobile phone-like radiation induces oxidative damage and changes the activities of antioxidant enzymes in the liver. The adverse effect of RFR may be related to the duration of mobile phone use. NAC and EGCG protect the liver tissue against the RFR-induced oxidative damage and enhance antioxidant enzyme activities.

(E) *Ozorak A, Nazıroğlu M, Celik O, Yüksel M, Özçelik D, Ozkaya MO, Cetin H, Kahya MC, Kose SA. Wi-Fi (2.45 GHz)- and Mobile Phone (900 and 1800 MHz)-Induced Risks on Oxidative Stress and Elements in Kidney and Testis of Rats During Pregnancy and the Development of Offspring. Biol Trace Elem Res. 2013 Oct 8. [Epub ahead of print]

The present study was designed to determine the effects of both Wi-Fi (2.45 GHz)- and mobile phone (900 and 1800 MHz)-induced electromagnetic radiation (EMR) on oxidative stress and trace element levels in the kidney and testis of growing rats from pregnancy to 6 weeks of age. Thirty-two rats and their 96 newborn offspring were equally divided into four different groups, namely, control, 2.45 GHz, 900 MHz, and 1800 MHz groups. The 2.45 GHz, 900 MHz, and 1,800 MHz groups were exposed to EMR for 60 min/day during pregnancy and growth. During the fourth, fifth, and sixth weeks of the experiment, kidney and testis samples were taken from decapitated rats. Results from the fourth week showed that the level of lipid peroxidation in the kidney and testis and the copper, zinc, reduced glutathione (GSH), glutathione peroxidase (GSH-Px), and total antioxidant status (TAS) values in the kidney decreased in the EMR groups, while iron concentrations in the kidney as well as vitamin A and vitamin E concentrations in the testis increased in the EMR groups. Results for fifth-week samples showed that iron, vitamin A, and β -carotene concentrations in the kidney increased in the EMR groups, while the GSH and TAS levels decreased. The sixth week results showed that iron concentrations in the kidney and the extent of lipid peroxidation in the kidney and testis increased in the EMR groups, while copper, TAS, and GSH concentrations decreased. There were no statistically significant differences in

kidney chromium, magnesium, and manganese concentrations among the four groups. In conclusion, Wi-Fi- and mobile phone-induced EMR caused oxidative damage by increasing the extent of lipid peroxidation and the iron level, while decreasing total antioxidant status, copper, and GSH values. Wi-Fi- and mobile phone-induced EMR may cause precocious puberty and oxidative kidney and testis injury in growing rats.

(E) Paredi P, Kharitonov SA, Hanazawa T, Barnes PJ, Local vasodilator response to mobile phones. *Laryngoscope* 111(1):159-162, 2001.

OBJECTIVES: The use of mobile phones with the resulting generation of potentially harmful electromagnetic fields (EMF) is the focus of public interest. Heat generation and the activation of the inducible form of nitric oxide (NO) synthase may be possible causes of the biological effects of EMF exposure. We investigated if a mobile telephone conversation can modify skin temperature, NO, and nasal resistance. **METHODS:** We studied the effect of an EMF (900 MHz) generated by a commercially available cellular phone during a 30-minute telephone conversation on skin temperature, nasal NO measured by chemiluminescence, and nasal minimal cross-sectional area (MCA) measured by rhinometry. Eleven normal subjects (mean age +/- standard error of mean [SEM], 32 +/- 5 y; 10 male) were studied. **RESULTS:** There was a similar and significant increase in skin temperature of the nostril and occipital area on the same side as the telephone (maximal increase 2.3 +/- 0.2 degrees C at 6 min) as well as a tendency for higher nasal NO levels (maximal increase 12.9 +/- 4.9% at 10 min), whereas the MCA was significantly reduced (maximal decrease -27 +/- 6% at 15 min). Such changes were not recorded when an earpiece was used to avoid the direct exposure to the electromagnetic field. There were no changes in the skin temperature and nasal NO measured on the opposite side to the mobile phone, whereas the MCA was significantly increased (38 +/- 10%). **CONCLUSIONS:** Exposure to EMF produced by a mobile phone produces biological effects that can be easily measured. Microwaves may increase skin temperature and therefore cause vasodilation and reduce MCA. Further studies are needed to study the long-term effects of mobile phone use and the relation among NO production, vasodilation, and temperature.

(E) Pilla AA. Electromagnetic fields instantaneously modulate nitric oxide signaling in challenged biological systems. *Biochem Biophys Res Commun.* 426(3):330-333, 2012.

This study shows that a non-thermal pulse-modulated RF signal (PRF), configured to modulate calmodulin (CaM) activation via acceleration of Ca(2+) binding kinetics, produced an immediate nearly 3-fold increase in nitric oxide (NO) from dopaminergic MN9D cultures (P<0.001). NO was measured electrochemically in real-time using a NO selective membrane electrode, which showed the PRF effect occurred within the first seconds after lipopolysaccharide (LPS) challenge. Further support that the site of action of PRF involves CaM is provided in human fibroblast cultures challenged with low serum and exposed for 15min to the identical PRF signal. In this case a CaM antagonist W-7 could be added to the culture 3h prior to PRF exposure. Those results showed the PRF signal produced nearly a two-fold increase in NO, which could be blocked by W-7 (P<0.001). To the authors' knowledge this is the first report of a real-time effect of non-thermal electromagnetic fields (EMF) on NO release from challenged cells. The results provide mechanistic support for the many reported bioeffects of EMF in which NO plays a role. Thus, in a typical clinical application for acute post operative pain, or chronic pain from, e.g., osteoarthritis, EMF therapy could be employed to modulate the dynamics of NO via Ca/CaM-dependent constitutive nitric oxide synthase (cNOS) in the target tissue. This, in turn, would modulate the dynamics of the signaling pathways the body uses in response to the various phases of healing after physical or chemical insult or injury.

(NE) Poulletier de Gannes F, Haro E, Hurtier A, Taxile M, Ruffié G, Billaudel B, Veyret B, Lagroye I. Effect of exposure to the edge signal on oxidative stress in brain cell models. Radiat Res. 175(2):225-230, 2011.

In this study we investigated the effect of the Enhanced Data rate for GSM Evolution (EDGE) signal on cells of three human brain cell lines, SH-SY5Y, U87 and CHME5, used as models of neurons, astrocytes and microglia, respectively, as well as on primary cortical neuron cultures. SXC-1800 waveguides (IT'IS-Foundation, Zürich, Switzerland) were modified for in vitro exposure to the EDGE signal radiofrequency (RF) radiation at 1800 MHz. Four exposure conditions were tested: 2 and 10 W/kg for 1 and 24 h. The production of reactive oxygen species (ROS) was measured by flow cytometry using the dichlorofluorescein diacetate (DCFH-DA) probe at the end of the 24-h exposure or 24 h after the 1-h exposure. Rotenone treatment was used as a positive control. All cells tested responded to rotenone treatment by increasing ROS production. These findings indicate that exposure to the EDGE signal does not induce oxidative stress under these test conditions, including 10 W/kg. Our results are in agreement with earlier findings that RF radiation alone does not increase ROS production.

(E) Qin F, Yuan H, Nie J, Cao Y, Tong J. [Effects of nano-selenium on cognition performance of mice exposed in 1800 MHz radiofrequency fields]. Wei Sheng Yan Jiu. 43(1):16-21, 2014. [Article in Chinese]

OBJECTIVE: To study the effects of nano-selenium (NSe) on cognition performance of mice exposed to 1800 MHz radiofrequency fields (RF). METHODS: Male mice were randomly divided into four groups, control and nano-Se low, middle and high dose groups (L, M, H). Each group was sub-divided into three groups, RF 0 min, RF 30 min and RF 120 min. Nano-se solution (2, 4 and 8 microg/ml) were administered to mice of L, M, H groups by intra-gastric injection respectively, 0.5 ml/d for 50 days, the control group were administered with distilled water. At the 21st day, the mice in RF subgroup were exposed to 208 microW/cm² 1800 MHz radiofrequency fields (0, 30 and 120 min/d respectively) for 30 days. The cognitive ability of the mice were tested with Y-maze. Further, the levels of MDA, GABA, Glu, Ach and the activities of CAT and GSH-Px in cerebra were measured. RESULTS: Significant impairments in learning and memory ($P < 0.05$) were observed in the RF 120 min group, and with reduction of the Ach level and the activities of CAT and GSH-Px and increase of the content of GABA, Glu and MDA in cerebrum. NSe enhanced cognitive performance of RF mice, decreased GABA, Glu and MDA levels, increased Ach levels, GSH-Px and CAT activities. CONCLUSION: NSe could improve cognitive impairments of mice exposed to RF, the mechanism of which might involve the increasing antioxidation, decreasing free radical content and the changes of cerebra neurotransmitters.

(E) Sharma VP, Singh HP, Kohli RK, Batish DR. Mobile phone radiation inhibits Vigna radiata (mung bean) root growth by inducing oxidative stress. Sci Total Environ. 407(21):5543-5547, 2009.

During the last couple of decades, there has been a tremendous increase in the use of cell phones. It has significantly added to the rapidly increasing EMF smog, an unprecedented type of pollution consisting of radiation in the environment, thereby prompting the scientists to study the effects on humans. However, not many studies have been conducted to explore the effects of cell **phone** EMFr on growth and biochemical changes in plants. We investigated whether EMFr from cell phones inhibit growth of Vigna radiata (mung bean) through induction of conventional stress responses. Effects of cell **phone** EMFr (power density: 8.55 microW cm⁻²); 900 MHz band width; for 1/2, 1, 2, and 4 h) were determined by measuring the generation of reactive oxygen

species (ROS) in terms of malondialdehyde and hydrogen peroxide (H₂O₂) content, root oxidizability and changes in levels of antioxidant enzymes. Our results showed that cell **phone** EMFr significantly inhibited the germination (at > or =2 h), and radicle and plumule growths (> or =1 h) in mung bean in a time-dependent manner. Further, cell **phone** EMFr enhanced MDA content (indicating lipid peroxidation), and increased H₂O₂ accumulation and root oxidizability in mung bean roots, thereby inducing oxidative stress and **cellular** damage. In response to EMFr, there was a significant upregulation in the activities of scavenging enzymes, such as superoxide dismutases, ascorbate peroxidases, guaiacol peroxidases, catalases and glutathione reductases, in mung bean roots. The study concluded that cell **phone** EMFr inhibit root growth of mung bean by inducing ROS-generated oxidative stress despite increased activities of antioxidant enzymes.

(E) Sharma VP, Singh HP, Batish DR, Kohli RK. Cell phone radiations affect early growth of Vigna radiata (mung bean) through biochemical alterations. Z Naturforsch C. 65(1-2):66-72, 2010.

The indiscriminate use of wireless technologies, particularly of cell phones, has increased the health risks among living organisms including plants. We investigated the impact of cell phone electromagnetic field (EMF) radiations (power density, 8.55 microW cm⁻²) on germination, early growth, proteins and carbohydrate contents, and activities of some enzymes in Vigna radiata. Cell phone EMF radiations significantly reduced the seedling length and dry weight of V radiata after exposure for 0.5, 1, 2, and 4 h. Furthermore, the contents of proteins and carbohydrates were reduced in EMF-exposed plants. However, the activities of proteases, alpha-amylases, beta-amylases, polyphenol oxidases, and peroxidases were enhanced in EMF-exposed radicles indicating their role in providing protection against EMF-induced stress. The study concludes that cell phone EMFs impair early growth of V radiata seedlings by inducing biochemical changes.

(E) Singh HP, Sharma VP, Batish DR, Kohli RK. Cell phone electromagnetic field radiations affect rhizogenesis through impairment of biochemical processes. Environ Monit Assess. 184(4):1813-1821, 2012.

Indiscriminate adoption and use of cell phone technology has tremendously increased the levels of electromagnetic field radiations (EMFr) in the natural environment. It has raised the concerns among the scientists regarding the possible risks of EMFr to living organisms. However, not much has been done to assess the damage caused to plants that are continuously exposed to EMFr present in the environment. The present study investigated the biochemical mechanism of interference of 900 MHz cell phone EMFr with root formation in mung bean (Vigna radiata syn. Phaseolus aureus) hypocotyls, a model system to study rhizogenesis in plants. Cell phone EMFr enhanced the activities of proteases (by 1.52 to 2.33 times), polyphenol oxidases (by 1.5 to 4.3 times), and peroxidases (by 1.5 to 2.0 times) in mung bean hypocotyls over control. Further, EMFr enhanced malondialdehyde (an indicator of lipid peroxidation), hydrogen peroxide, and proline content, indicating a reactive oxygen species-mediated oxidative damage in hypocotyls. It was confirmed by the upregulation in the activities of antioxidant enzymes (superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, catalase, and glutathione reductase) suggesting their possible role in providing protection against EMFr-induced oxidative damage. The study concluded that cell phone radiations affect the process of rhizogenesis through biochemical alterations that manifest as oxidative damage resulting in root impairment.

(E) Sokolovic D, Djindjic B, Nikolic J, Bjelakovic G, Pavlovic D, Kocic G, Krstic D, Cvetkovic T, Pavlovic V. Melatonin reduces oxidative stress induced by chronic exposure of microwave radiation from mobile phones in rat brain. J Radiat Res (Tokyo). 49(6):579-586, 2008.

PURPOSE: The aim of the study was to evaluate the intensity of oxidative stress in the brain of animals chronically exposed to mobile phones and potential protective effects of melatonin in reducing oxidative stress and brain injury. MATERIALS AND METHODS: Experiments were performed on Wistar rats exposed to microwave radiation during 20, 40 and 60 days. Four groups were formed: I group (control)- animals treated by saline, intraperitoneally (i.p.) applied daily during follow up, II group (Mel)- rats treated daily with melatonin (2 mg kg(-1) body weight i.p.), III group (MWs)- microwave exposed rats, IV group (MWs + Mel)- MWs exposed rats treated with melatonin (2 mg kg(-1) body weight i.p.). The microwave radiation was produced by a mobile test phone (SAR = 0.043-0.135 W/kg). RESULTS: A significant increase in the brain tissue malondialdehyde (MDA) and carbonyl group concentration was registered during exposure. Decreased activity of catalase (CAT) and increased activity of xanthine oxidase (XO) remained after 40 and 60 days of exposure to mobile phones. Melatonin treatment significantly prevented the increase in the MDA content and XO activity in the brain tissue after 40 days of exposure while it was unable to prevent the decrease of CAT activity and increase of carbonyl group contents. CONCLUSION: We demonstrated two important findings; that mobile phones caused oxidative damage biochemically by increasing the levels of MDA, carbonyl groups, XO activity and decreasing CAT activity; and that treatment with the melatonin significantly prevented oxidative damage in the brain.

(E) Tkalec M, Malaric K, Pevalek-Kozlina B. Influence of 400, 900, and 1900 MHz electromagnetic fields on Lemna minor growth and peroxidase activity. Bioelectromagnetics. 26(3):185-193, 2005.

Increased use of radio and microwave frequencies requires investigations of their effects on living organisms. Duckweed (*Lemna minor* L.) has been commonly used as a model plant for environmental monitoring. In the present study, duckweed growth and peroxidase activity was evaluated after exposure in a Gigahertz Transversal Electromagnetic (GTEM) cell to electric fields of frequencies 400, 900, and 1900 MHz. The growth of plants exposed for 2 h to the 23 V/m electric field of 900 MHz significantly decreased in comparison with the control, while an electric field of the same strength but at 400 MHz did not have such effect. A modulated field at 900 MHz strongly inhibited the growth, while at 400 MHz modulation did not influence the growth significantly. At both frequencies a longer exposure mostly decreased the growth and the highest electric field (390 V/m) strongly inhibited the growth. Exposure of plants to lower field strength (10 V/m) for 14 h caused significant decrease at 400 and 1900 MHz while 900 MHz did not influence the growth. Peroxidase activity in exposed plants varied, depending on the exposure characteristics. Observed changes were mostly small, except in plants exposed for 2 h to 41 V/m at 900 MHz where a significant increase (41%) was found. Our results suggest that investigated electromagnetic fields (EMFs) might influence plant growth and, to some extent, peroxidase activity. However, the effects of EMFs strongly depended on the characteristics of the field exposure.

(E) Tkalec M, Malarić K, Pevalek-Kozlina B. Exposure to radiofrequency radiation induces oxidative stress in duckweed *Lemna minor* L. Sci Total Environ. 388(1-3):78-89, 2007.

Widespread use of radiofrequency radiation emitting devices increased the exposure to electromagnetic fields (EMFs) from 300 MHz to 300 GHz. Various biological effects of exposure to these fields have been documented so far, but very little work has been carried out on plants.

The aim of the present work was to investigate the physiological responses of the plant *Lemna minor* after exposure to radiofrequency EMFs, and in particular, to clarify the possible role of oxidative stress in the observed effects. Duckweed was exposed for 2 h to EMFs of 400 and 900 MHz at field strengths of 10, 23, 41 and 120 V m⁻¹. The effect of a longer exposure time (4 h) and modulation was also investigated. After exposure, parameters of oxidative stress, such as lipid peroxidation, H₂O₂ content, activities and isoenzyme pattern of antioxidative enzymes as well as HSP70 expression were evaluated. At 400 MHz, lipid peroxidation and H₂O₂ content were significantly enhanced in duckweed exposed to EMFs of 23 and 120 V m⁻¹ while other exposure treatments did not have an effect. Compared to the controls, the activities of antioxidative enzymes showed different behaviour: catalase (CAT) activity increased after most exposure treatments while pyrogallol (PPX) and ascorbate peroxidase (APX) activities were not changed. Exceptions were reduced PPX and APX activity after longer exposure at 23 V m⁻¹ and increased PPX activity after exposures at 10 and 120 V m⁻¹. By contrast, at 900 MHz almost all exposure treatments significantly increased level of lipid peroxidation and H₂O₂ content but mostly decreased PPX activity and did not affect CAT activity. Exceptions were exposures to a modulated field and to the field of 120 V m⁻¹ which increased PPX and CAT activity. At this frequency APX activity was significantly decreased after exposure at 10 V m⁻¹ and longer exposure at 23 V m⁻¹ but it increased after a shorter exposure at 23 V m⁻¹. At both frequencies no differences in isoenzyme patterns of antioxidative enzymes or HSP70 level were found between control and exposed plants. Our results showed that non-thermal exposure to investigated radiofrequency fields induced oxidative stress in duckweed as well as unspecific stress responses, especially of antioxidative enzymes. However, the observed effects markedly depended on the field frequencies applied as well as on other exposure parameters (strength, modulation and exposure time). Enhanced lipid peroxidation and H₂O₂ content accompanied by diminished antioxidative enzymes activity caused by exposure to investigated EMFs, especially at 900 MHz, indicate that oxidative stress could partly be due to changed activities of antioxidative enzymes.

(E) *Tkalec M, Stambuk A, Srut M, Malarić K, Klobučar GI. Oxidative and genotoxic effects of 900 MHz electromagnetic fields in the earthworm *Eisenia fetida*. *Ecotoxicol Environ Saf.* 90:7-12, 2013. (WS) (LI)

Accumulating evidence suggests that exposure to radiofrequency electromagnetic field (RF-EMF) can have various biological effects. In this study the oxidative and genotoxic effects were investigated in earthworms *Eisenia fetida* exposed in vivo to RF-EMF at the mobile phone frequency (900 MHz). Earthworms were exposed to the homogeneous RF-EMF at field levels of 10, 23, 41 and 120 V m⁻¹ for a period of 2h using a Gigahertz Transversal Electromagnetic (GTEM) cell. At the field level of 23 V m⁻¹ the effect of longer exposure (4h) and field modulation (80% AM 1 kHz sinusoidal) was investigated as well. All exposure treatments induced significant genotoxic effect in earthworms coelomocytes detected by the Comet assay, demonstrating DNA damaging capacity of 900 MHz electromagnetic radiation. Field modulation additionally increased the genotoxic effect. Moreover, our results indicated the induction of antioxidant stress response in terms of enhanced catalase and glutathione reductase activity as a result of the RF-EMF exposure, and demonstrated the generation of lipid and protein oxidative damage. Antioxidant responses and the potential of RF-EMF to induce damage to lipids, proteins and DNA differed depending on the field level applied, modulation of the field and duration of *E. fetida* exposure to 900 MHz electromagnetic radiation. Nature of detected DNA lesions and oxidative stress as the mechanism of action for the induction of DNA damage are discussed.

(E) *Tök L, Nazıroğlu M, Doğan S, Kahya MC, Tök O. Effects of melatonin on Wi-Fi-induced oxidative stress in lens of rats. Indian J Ophthalmol. 62(1):12-15, 2014. doi: 10.4103/0301-4738.126166.

Introduction: Melatonin has been considered a potent antioxidant that detoxifies a variety of reactive oxygen species in many pathophysiological states of eye. The present study was designed to determine the effects of Wi-Fi exposure on the lens oxidant, antioxidant redox systems, as well as the possible protective effects of melatonin on the lens injury induced by electromagnetic radiation (EMR). Materials and Methods: Thirty-two rats were used in the current study and they were randomly divided into four equal groups as follows: First and second groups were cage-control and sham-control rats. Rats in third group were exposed to Wi-Fi (2.45 GHz) for duration of 60 min/day for 30 days. As in the third group, the fourth group was treated with melatonin. The one-hour exposure to irradiation in second, third and fourth took place at noon each day. Results: Lipid peroxidation levels in the lens were slightly higher in third (Wi-Fi) group than in cage and sham control groups although their concentrations were significantly ($P < 0.05$) decreased by melatonin supplementation. Glutathione peroxidase (GSH-Px) activity was significantly ($P < 0.05$) lower in Wi-Fi group than in cage and sham control groups although GSH-Px ($P < 0.01$) and reduced glutathione ($P < 0.05$) values were significantly higher in Wi-Fi + melatonin group than in Wi-Fi group. Conclusions: There are poor oxidative toxic effects of one hour of Wi-Fi exposure on the lens in the animals. However, melatonin supplementation in the lens seems to have protective effects on the oxidant system by modulation of GSH-Px activity.

(E) Tomruk A, Guler G, Dincel AS. The influence of 1800 MHz GSM-like signals on hepatic oxidative DNA and lipid damage in nonpregnant, pregnant, and newly born rabbits. Cell Biochem Biophys. 56(1):39-47, 2010.

The aim of our study is to evaluate the possible biological effects of whole-body 1800 MHz GSM-like radiofrequency (RF) radiation exposure on liver oxidative DNA damage and lipid peroxidation levels in nonpregnant, pregnant New Zealand White rabbits, and in their newly borns. Eighteen nonpregnant and pregnant rabbits were used and randomly divided into four groups which were composed of nine rabbits: (i) Group I (nonpregnant control), (ii) Group II (nonpregnant-RF exposed), (iii) Group III (pregnant control), (iv) Group IV (pregnant-RF exposed). Newborns of the pregnant rabbits were also divided into two groups: (v) Group V (newborns of Group III) and (vi) Group VI (newborns of Group III). 1800 MHz GSM-like RF radiation whole-body exposure (15 min/day for a week) was applied to Group II and Group IV. No significant differences were found in liver 8 OHdG/10(6) dG levels of exposure groups (Group II and Group IV) compared to controls (Group I and Group III). However, in Group II and Group IV malondialdehyde (MDA) and ferrous oxidation in xylenol orange (FOX) levels were increased compared to Group I ($P < 0.05$, Mann-Whitney). No significant differences were found in liver tissue of 8 OHdG/10(6) dG and MDA levels between Group VI and Group V ($P > 0.05$, Mann-Whitney) while liver FOX levels were found significantly increased in Group VI with respect to Group V ($P < 0.05$, Mann-Whitney). Consequently, the whole-body 1800 MHz GSM-like RF radiation exposure may lead to oxidative destruction as being indicators of subsequent reactions that occur to form oxygen toxicity in tissues.

(E) Wu W, Yao K, Wang KJ, Lu DQ, He JL, Xu LH, Sun WJ. [Blocking 1800 MHz mobile phone radiation-induced reactive oxygen species production and DNA damage in lens epithelial cells by noise magnetic fields]. [Article in Chinese] Zhejiang Da Xue Xue Bao Yi Xue Ban. 37(1):34-38, 2008.

OBJECTIVE: To investigate whether the exposure to the electromagnetic noise can block reactive oxygen species (ROS) production and DNA damage of lens epithelial cells induced by 1800 MHz mobile phone radiation. METHODS: The DCFH-DA method and comet assay were used respectively to detect the intracellular ROS and DNA damage of cultured human lens epithelial cells induced by 4 W/kg 1800 MHz mobile phone radiation or/and 2 μ T electromagnetic noise for 24 h intermittently. RESULT: 1800 MHz mobile phone radiation at 4 W/kg for 24 h increased intracellular ROS and DNA damage significantly ($P < 0.05$). However, the ROS level and DNA damage of mobile phone radiation plus noise group were not significant enhanced ($P > 0.05$) as compared to sham exposure group. CONCLUSION: Electromagnetic noise can block intracellular ROS production and DNA damage of human lens epithelial cells induced by 1800 MHz mobile phone radiation.

(E) Xu S, Zhou Z, Zhang L, Yu Z, Zhang W, Wang Y, Wang X, Li M, Chen Y, Chen C, He M, Zhang G, Zhong M. Exposure to 1800 MHz radiofrequency radiation induces oxidative damage to mitochondrial DNA in primary cultured neurons. Brain Res. 1311:189-196, 2010.

Increasing evidence indicates that oxidative stress may be involved in the adverse effects of radiofrequency (RF) radiation on the brain. Because mitochondrial DNA (mtDNA) defects are closely associated with various nervous system diseases and mtDNA is highly susceptible to oxidative stress, the purpose of this study was to determine whether radiofrequency radiation can cause oxidative damage to mtDNA. In this study, we exposed primary cultured cortical neurons to pulsed RF electromagnetic fields at a frequency of 1800 MHz modulated by 217 Hz at an average special absorption rate (SAR) of 2 W/kg. At 24h after exposure, we found that RF radiation induced a significant increase in the levels of 8-hydroxyguanine (8-OHdG), a common biomarker of DNA oxidative damage, in the mitochondria of neurons. Consistent with this finding, the copy number of mtDNA and the levels of mitochondrial RNA (mtRNA) transcripts showed an obvious reduction after RF exposure. Each of these mtDNA disturbances could be reversed by pretreatment with melatonin, which is known to be an efficient in the brain. Together, these results suggested that 1800 MHz RF radiation could cause oxidative damage to mtDNA in primary cultured neurons. Oxidative damage to mtDNA may account for the neurotoxicity of RF radiation in the brain.

(E) Yao K, Wu W, Wang K, Ni S, Ye P, Yu Y, Ye J, Sun L. Electromagnetic noise inhibits radiofrequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells. Mol Vis. 14:964-969, 2008.

PURPOSE: The goal of this study was to investigate whether superposing of electromagnetic noise could block or attenuate DNA damage and intracellular reactive oxygen species (ROS) increase of cultured human lens epithelial cells (HLECs) induced by acute exposure to 1.8 GHz radiofrequency field (RF) of the Global System for Mobile Communications (GSM). METHODS: An sXc-1800 RF exposure system was used to produce a GSM signal at 1.8 GHz (217 Hz amplitude-modulated) with the specific absorption rate (SAR) of 1, 2, 3, and 4 W/kg. After 2 h of intermittent exposure, the ROS level was assessed by the fluorescent probe, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). DNA damage to HLECs was examined by alkaline comet assay and the phosphorylated form of histone variant H2AX (gammaH2AX) foci formation assay. RESULTS: After exposure to 1.8 GHz RF for 2 h, HLECs exhibited significant intracellular ROS increase in the 2, 3, and 4 W/kg groups. RF radiation at the SAR of 3 W/kg and 4 W/kg could induce significant DNA damage, examined by

alkaline comet assay, which was used to detect mainly single strand breaks (SSBs), while no statistical difference in double strand breaks (DSBs), evaluated by gammaH2AX foci, was found between RF exposure (SAR: 3 and 4 W/kg) and sham exposure groups. When RF was superposed with 2 μ T electromagnetic noise could block RF-induced ROS increase and DNA damage. CONCLUSIONS: DNA damage induced by 1.8 GHz radiofrequency field for 2 h, which was mainly SSBs, may be associated with the increased ROS production. Electromagnetic noise could block RF-induced ROS formation and DNA damage.

(E) Yariktas M, Doner F, Ozguner F, Gokalp O, Dogru H, Delibas N. Nitric oxide level in the nasal and sinus mucosa after exposure to electromagnetic field. Otolaryngol Head Neck Surg. 132(5):713-716, 2005.

OBJECTIVE: The purpose of this study was to examine the changes in nitric oxide (NO) level in the nasal and paranasal sinus mucosa after exposure radiofrequency electromagnetic fields (EMF). STUDY DESIGN AND SETTING: Thirty male Sprague-Dawley rats were randomly grouped as follows: EMF group (group I; n, 10), EMF group in which melatonin received (group II; n, 10) and the control (sham operated) group (group III; n, 10). Groups I and II were exposed to a 900 MHz. Oral melatonin was given in group II. Control rats (group III) were also placed in the tube as the exposure groups, but without exposure to EMF. At the end of 2 weeks, the rats were sacrificed, and the nasal and paranasal sinus mucosa dissected. NO was measured in nasal and paranasal mucosa. RESULTS: The nasal and paranasal sinus mucosa NO levels of group I were significantly higher than those of the control group (group III) ($P < 0.05$). However, there was no statistically significant difference between group II and the control group (group III) regarding NO output ($P > 0.05$). CONCLUSION: Exposure to EMF released by mobile phones (900 MHz) increase NO levels in the sinus and nasal mucosa. SIGNIFICANCE: Increased NO levels may act as a defense mechanism and presumably related to tissue damage. In addition, melatonin may have beneficial effect to prevent these changes in the mucosa.

(E) Yurekli AI, Ozkan M, Kalkan T, Saybasili H, Tuncel H, Atukeren P, Gumustas K, Seker S. GSM Base Station Electromagnetic Radiation and Oxidative Stress in Rats. Electromagn Biol Med. 2006;25(3):177-188, 2006. (LI)

The ever increasing use of cellular phones and the increasing number of associated base stations are becoming a widespread source of nonionizing electromagnetic radiation. Some biological effects are likely to occur even at low-level EM fields. In this study, a gigahertz transverse electromagnetic (GTEM) cell was used as an exposure environment for plane wave conditions of far-field free space EM field propagation at the GSM base transceiver station (BTS) frequency of 945 MHz, and effects on oxidative stress in rats were investigated. When EM fields at a power density of 3.67 W/m² (specific absorption rate = 11.3 mW/kg), which is well below current exposure limits, were applied, MDA (malondialdehyde) level was found to increase and GSH (reduced glutathione) concentration was found to decrease significantly ($p < 0.0001$). Additionally, there was a less significant ($p = 0.0190$) increase in SOD (superoxide dismutase) activity under EM exposure.

(E) Zeni, O., Di Pietro, R., d'Ambrosio, G., Massa, R., Capri, M., Naarala, J., Juutilainen, J. and Scarfi, M. R. Formation of Reactive Oxygen Species in L929 Cells after Exposure to 900 MHz RF Radiation with and without Co-exposure to 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone. Radiat. Res. 167, 306-311, 2007.

The aim of this study was to investigate the induction of reactive oxygen species in murine L929 fibrosarcoma cells exposed to radiofrequency (RF) radiation at 900 MHz, with or without co-exposure to 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a potent environmental carcinogen produced during chlorination of drinking water. Both continuous-wave and GSM mobile phone signals were applied for 10 or 30 min at specific absorption rates of 0.3 and 1 W/kg. Simultaneous sham exposures were performed for each exposure condition. MX treatment was performed at a subtoxic level of 500 µM, and the RF-field exposure was carried out during the first 10 or 30 min of the chemical treatment. The formation of reactive oxygen species was followed soon after the exposure and at different harvesting times until 1 h after RF-field treatment. The studied provided no indication that 900 MHz RF-field exposure, either alone or in combination with MX, induced formation of reactive oxygen species under any of the experimental conditions investigated. In contrast, exposure to MX resulted in a statistically significant increase in the formation of reactive oxygen species for all the treatment durations investigated, confirming that MX is an inductor of oxidative stress in L929 cells.

(E) Zmyslony M, Politanski P, Rajkowska E, Szymczak W, Jajte J. Acute exposure to 930 MHz CW electromagnetic radiation in vitro affects reactive oxygen species level in rat lymphocytes treated by iron ions. Bioelectromagnetics. 25(5):324-328, 2004.

The aim of this study was to test the hypothesis that the 930 MHz continuous wave (CW) electromagnetic field, which is the carrier of signals emitted by cellular phones, affects the reactive oxygen species (ROS) level in living cells. Rat lymphocytes were used in the experiments. A portion of the lymphocytes was treated with iron ions to induce oxidative processes. Exposures to electromagnetic radiation (power density 5 W/m², theoretical calculated SAR = 1.5 W/kg) were performed within a GTEM cell. Intracellular ROS were measured by the fluorescent probe dichlorofluorescein diacetate (DCF-DA). The results show that acute (5 and 15 min) exposure does not affect the number of produced ROS. If, however, FeCl₂ with final concentration 10 µg/ml was added to the lymphocyte suspensions to stimulate ROS production, after both durations of exposure, the magnitude of fluorescence (ROS level during the experiment) was significantly greater in the exposed lymphocytes. The character of the changes in the number of free radicals observed in our experiments was qualitatively compatible with the theoretical prediction from the model of electromagnetic radiation effect on radical pairs.