

# The non-thermal biological effects and mechanisms of microwave exposure

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## ABSTRACT

### ► Review article

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Revised: April 2020

Accepted: June 2020

Int. J. Radiat. Res., July 2021;  
19(3): 483-494

DOI: 10.29252/ijrr.19.2.483

The purpose of this article was to present a brief review of pertinent information regarding the effects of microwave radiation on biological systems. Researchers have been intrigued by the interaction of electromagnetic fields (EMFs) and various life processes since the 18th century. Microwaves refer to the oscillation of an EMF with a wavelength of 1 mm to 1 m, which penetrates matter to varying degrees. With the widespread and ever-increasing use of microwaves, such as cellular telephones and other wireless technologies, great attention and research has been paid to the potential adverse biological effects. It is well recognized that microwaves affect the biological functions of living organisms at both the cellular and molecular levels, and can lead to the appearance of toxicity, genotoxicity and transformation. However, until now no satisfactory mechanism has been proposed to explain the biological effects of these fields. Therefore, increasing attention should be focused on the biological effects of microwaves in the future, especially since microwaves have extensive applications in various fields.

**Keywords:** Microwaves, biological effects, mechanism, thermal, non-thermal.

## INTRODUCTION

Microwave radiation is considered a type of non-ionizing electromagnetic field (EMF) and it is present in the environment due to its ever-increasing applications, such as in microwave ovens, radio/television (TV) transmission, cellular phones and in occupational use (e.g. radar operators), as well as through medical exposure via diathermy. Much has been published supporting the notion that microwaves have both thermal and non-thermal effects, which can be identified in terms of their manifestations on cell physiology. Many studies have shown that microwaves cause different biological effects depending upon the field strength, frequency, wave form, modulation and duration of exposure <sup>(1)</sup>. These effects have mainly been attributed to microwave heating, but the non-thermal effects of microwaves on cells or animals have been

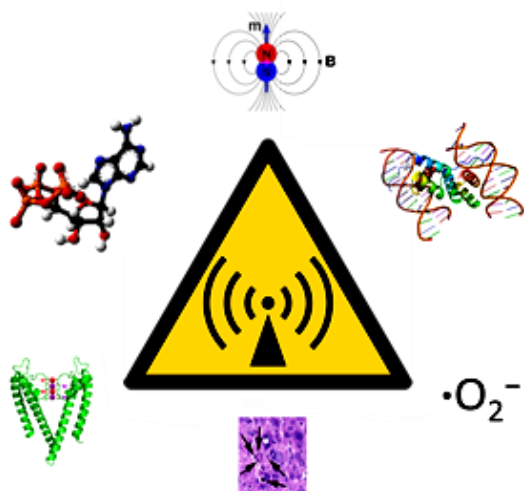
documented over the last three decades <sup>(2)</sup>. Although the exact mechanism underlying the biological effects of microwaves has not been specified, there is a hypothesis that microwaves either cause ions to accelerate and collide with other molecules or cause dipoles to rotate and line up rapidly with an alternating electric field <sup>(3)</sup>.

In previous investigations it was found that microwave exposure was able to induce several changes at the cellular and molecular levels of cells, including increasing the permeability of the blood-brain barrier, causing the death of single-cell organisms, inhibiting cell proliferation, increasing DNA single and double strand breaks, altering gene expression in different cell types, inducing micronuclei, altering protein conformation and inducing oxidative stress <sup>(4)</sup>. However, identifying and evaluating the biological effects of microwaves is complex and controversial because the

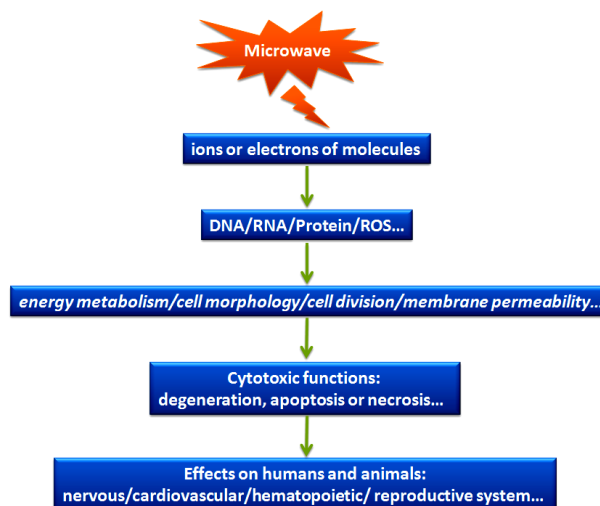
mechanisms by which microwaves exert their biological effects remain poorly characterized. Consequently, there is still a great need for elucidation of the real targets and mechanisms of action of pulsed microwaves. Thus, in order to familiarize the reader with pertinent information regarding the non-thermal effects of microwave irradiation, this systematic review had covered the original articles in the biological effects and mechanisms of microwave exposure, such as the ions or electrons of molecules, the characteristics of ion channels, energy metabolism, reactive oxygen species (ROS) and response of biological cells. Moreover, the possible protective measure against the biological effects of microwave radiation were also discussed in this review.

### The mechanism of biological effects

The mechanism of the biological effects of microwave radiation is still unknown because of inadequate equipment and techniques, which have left most data open to question. However, many hypotheses have developed, which will now be discussed (figure 1). The biological effects of organisms caused by microwave radiation are complex, possible mechanism of action of biological systems were included in this review (figure 2).



**Figure 1.** Possible adverse biological effects of microwave radiation.



**Figure 2.** Possible mechanism of action of microwave on biological systems.

### Microwaves can affect the ions or electrons of molecules

Microwaves can cause ions or electrons to become misplaced, vibrate or collide with other molecules, and it can cause a change in the direction of a polar molecule's dipole moment, which may explain the mechanism of action with regards to the physiological reactions in cells at the molecular level, whereby biochemical consequences, changes in metabolism and alteration of the proliferation of living cells have been noted <sup>(3)</sup>.

### Microwaves can affect the characteristics of ion channels

Microwaves can affect the characteristics of many ion channels, such as the  $\text{Ca}^{2+}$  channel, and the transfer of  $\text{Na}^{+}$  and  $\text{K}^{+}$  across the plasma membrane. Pall <sup>(4)</sup> found that the EMF activation of voltage-gated calcium channels (VGCCs) led to rapid elevation of intracellular  $\text{Ca}^{2+}$ , nitric oxide and, in some cases at least, peroxynitrite, resulting in pathophysiological effects mediated through the  $\text{Ca}^{2+}$ /nitric oxide/peroxynitrite pathway.

### Microwaves can affect reactive oxygen species

In biological systems affected by electromagnetic radiation (EMR), the

mechanisms of tissue damage are thought to involve ROS. Excessive ROS can damage membrane structures and large cellular molecules, such as lipids, proteins and nucleic acids; they may also account for failure of the nuclear DNA chain structure following EMR exposure. Moustafa *et al.* <sup>(6)</sup> found that microwave exposure could increase the levels of free radicals and detrimentally impact antioxidant mechanisms in the body.

### **Microwaves can impact energy metabolism**

Microwave radiation can result in disordered cell energy metabolism, which can affect the function of cells. Srivastava *et al.* <sup>(7)</sup> found that microwave irradiation decreased adenosine triphosphate (ATP) and increased free  $Mg^{2+}$  ions, impacting ATP hydrolysis and the release of  $Mg^{2+}$  ions into intracellular spaces. Following exposure of the brain to microwaves, the equilibrium constants of certain metabolic reactions were grossly distorted, affecting determination of the cytosolic phosphorylation potential.

### **Response of biological cells to microwave irradiation**

#### **Effect of microwaves on cells**

Microwave radiation may alter cell morphology, disrupt cell division and change cell membrane permeability <sup>(8)</sup> resulting in degeneration, apoptosis or necrosis in cells at different stages and the details of these results were listed in table 1. One current theory suggests that these effects may be mainly attributed to microwave heating, but other reports have shown or suggested that non-thermal microwaves are able to produce molecular transformations and alterations <sup>(3)</sup>. For example, it has been reported that the cell cycle is altered in Chinese hamster ovary (CHO) cells by 27 MHz microwave exposure <sup>(9)</sup>. Furthermore, human amnion cells exposed to 960 MHz show a decreased cell growth rate with increased exposure time. Wu *et al.* <sup>(10)</sup> found that cytokines produced by microwave-radiated Sertoli cells induced apoptosis and lipid peroxidation in the membranes of germ cells. In addition, germ cell apoptosis was associated

with the up-regulation of Bcl-2 Associated X/B-cell lymphoma-2 (Bax/Bcl-2) and caspase-3. Song *et al.* <sup>(11)</sup> observed that the rate of early stage apoptosis in A549 human lung carcinoma cells reached 6.10–17.98% and the rate of advanced stage apoptosis and the necrosis rate reached 8.04–44.06% after 6 hours of continuous microwave radiation. Furthermore, the down-regulation of Bcl-2 expression reached its lowest level and the up-regulation of tumor protein 53 (P53) expression peaked at about 3 hours. This study showed that cell damage, apoptosis and protein expression differed depending on the radiation intensity and duration. Recently, Zhao *et al.* <sup>(12)</sup> also showed that microwave dose-dependently induced cellular apoptosis, cell cycle arrest and decreased cytotoxic activity in Natural killer cells 92 (NK-92), possibly through extracellular regulated protein kinases (ERK)-mediated regulation of apoptosis and perforin expression. Additionally, Zhang *et al.* <sup>(13)</sup> demonstrated that continuous microwave radiation once a day for up to 7 days efficiently induced Lewis lung carcinoma (LLC) tumor cell apoptosis in mice even at the low intensity of 10 mW/cm<sup>2</sup> for 5 min each time, and revealed that non-thermal effect played a critical role in the induction of tumor cell apoptosis *in-vivo*.

Many studies have shown that biological systems exposed to extremely high frequency microwaves may suffer significant effects via non-thermal mechanisms primarily involving the interaction of microwaves with phospholipid membrane structures. Biological membranes are likely to be highly sensitive to frequencies in the 1–80 GHz range <sup>(14)</sup>. Zhadobov *et al.* <sup>(15)</sup> found that membrane system exposure at 60 GHz significantly increased the lateral pressure of phospholipid monolayer films at power densities as low as 9  $\mu$ W/cm<sup>2</sup>, although there was no statistically significant phospholipid microdomain reorganization. Furthermore, the ionic transmembrane current has been found to be involved. For example, Geletyuk *et al.* <sup>(16)</sup> observed significant alterations in the transport of  $Ca^{2+}$  ions across a single  $Ca^{2+}$ -activated  $K^{+}$  channel in kidney cells when exposed to 42.25 GHz radiation. In addition, Rougier *et al.* <sup>(17)</sup> and

Zeng *et al.* <sup>(18)</sup> respectively observed that 2.45 GHz microwave exposure induced membrane modifications to increase cellular permeability

to ions and molecules in *Escherichia coli* and Yeast.

**Table 1.** Effect of microwaves on cells.

| Time | Author                                 | Microwave Parameter               | Experimental Cell               | Detection Index                                                       | Result                                                                                |
|------|----------------------------------------|-----------------------------------|---------------------------------|-----------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| 1995 | Cao <i>et al.</i> <sup>(9)</sup>       | 27 MHz                            | CHO cells                       | Cell cycle                                                            | changed                                                                               |
| 2012 | Wu <i>et al.</i> <sup>(10)</sup>       | 50 Hz                             | human mesenchymal stem cells    | cell apoptosis                                                        | the up-regulation of Bax/ Bcl-2 and caspase-3                                         |
| 2011 | Song <i>et al.</i> <sup>(11)</sup>     | 200-600 mW/cm <sup>2</sup>        | A549 cells                      | cell apoptosis                                                        | the rate of early stage: 6.10–17.98%<br>the rate of advanced stage: 8.04–44.06%       |
| 2017 | Zhao <i>et al.</i> <sup>(12)</sup>     | 10, 30, and 50 mW/cm <sup>2</sup> | NK-92 cells                     | Ultrastructural changes, cellular apoptosis and cell cycle regulation | Changed possibly through ERK-mediated regulation of apoptosis and perforin expression |
| 2017 | Zhang <i>et al.</i> <sup>(13)</sup>    | 10 mW/cm <sup>2</sup>             | LLC cells                       | cell apoptosis                                                        | changed                                                                               |
| 2006 | Zhadobov <i>et al.</i> <sup>(15)</sup> | 60 GHz                            | Artificial Biological Membranes | lateral pressure                                                      | increased                                                                             |
| 1995 | Geletyuk <i>et al.</i> <sup>(16)</sup> | 42.25 GHz                         | kidney cells                    | K <sup>+</sup> channel                                                | changed                                                                               |
| 2014 | Rougier <i>et al.</i> <sup>(17)</sup>  | 2.45 GHz                          | <i>Escherichia coli</i>         | cellular permeability                                                 | increased                                                                             |
| 2014 | Zeng <i>et al.</i> <sup>(18)</sup>     | 2.45 GHz                          | Yeast                           | cellular permeability                                                 | increased                                                                             |

### Effect of microwaves on free radicals

It is already known that aging, several diseases and exposure to various toxic substances and radiation increase the amount of ROS. The increase of ROS results in the alteration of macromolecules, such as polyunsaturated fatty acids in membrane lipids, vital proteins and DNA. The resulting oxidative stress causes an increase of malondialdehyde (MDA), which causes damage to membrane lipids and a decreased glutathione (GSH) concentration, which plays a central role in defense against different diseases and cell insults. GSH and MDA levels have been evaluated to determine whether microwaves can induce oxidative damage. However, contradictory results regarding the effect of microwaves on the parameters of oxidative stress have been reported and the details of these results were listed in table 2.

Several research groups have found that radiofrequency (RF) radiation has no effect on ROS production at special absorption rate (SAR) values <2.0 W/kg. Ferreira *et al.* <sup>(19)</sup> found that

microwave exposure did not increase the MDA concentration in the brains of rats. In our previous study, we found that electromagnetic pulse (EMP) prevents free radical generation by activating antioxidant enzyme activity and reducing oxygen consumption *in-vitro* <sup>(20)</sup>. Additionally, our findings indicate that the biological effect of microwave at a SAR of 4 W/kg resulted in instantaneous and recoverability changes *in-vivo* <sup>(21)</sup>. However, there is evidence that microwaves may stimulate the formation of ROS in exposed cells *in-vivo* and *in-vitro* <sup>(22)</sup>.

Yurekli *et al.* <sup>(23)</sup> investigated 945 MHz frequency EMFs and the oxidative stress effects on rats. They used a gigahertz transverse electromagnetic (GTEM) cell as the exposure environment. When the EMF was at a power density of 3.67 W/m<sup>2</sup> (SAR of 11.3 mW/kg), which is well below current exposure limits, the MDA level increased and the GSH concentration decreased significantly. Additionally, there was a less significant increase in superoxide dismutase (SOD) activity, demonstrating that oxidative stress resulted from electromagnetic exposure.

Esmekaya *et al.* <sup>(24)</sup> found that MDA levels were increased significantly and GSH levels were significantly lowered in the liver, lung, testis and heart tissues of an exposed group compared to sham and control groups after rats were exposed to 900 MHz pulse-modulated RF radiation at a SAR level of 1.20 W/kg for 20 min/day for 3 weeks. Thus, pulse-modulated RF radiation causes oxidative injury in tissues mediated by lipid peroxidation.

Recently, Narayanan *et al.* <sup>(25)</sup> reported that exposure to microwave radiation with frequencies ranging from 890-915 MHz at SAR 1.58 W/Kg, for 28 days resulted in oxidative stress as indicated by increased level of

thiobarbituric acid-reactive substances (TBARS) in brain. Furthermore, in the study reported by Xing *et al.* <sup>(26)</sup>, it has been shown that 1800MHz microwave activates caspase-3 signaling pathway that leads to cell apoptosis through enhancing ROS production and DNA damage, which is consistent with another study <sup>(27)</sup> in that microwave induced non-small cell lung cancer (NSCLC) cells apoptosis by increasing the production of ROS *in-vivo*. In addition, Kavindra *et al.* <sup>(28)</sup> also revealed that exposure of 2.45 GHz microwave frequency adversely affects the whole brain and cause oxidative damage by increased levels of lipid peroxide (LPO) and ROS.

**Table 2.** Effect of microwaves on free radicals.

| Time | Author                                  | Microwave Parameter                | Experimental Animal                           | Detection Index | Result                                   |
|------|-----------------------------------------|------------------------------------|-----------------------------------------------|-----------------|------------------------------------------|
| 2006 | Ferreira <i>et al.</i> <sup>(19)</sup>  | 800–1800 MHz                       | brain of rats                                 | MDA             | unchanged                                |
| 2015 | Wang <i>et al.</i> <sup>(21)</sup>      | 2.856 GHz<br>4 W/kg                | PC12 cells                                    | ROS             | instantaneous and recoverability changes |
| 2008 | Yao <i>et al.</i> <sup>(22)</sup>       | 1.8 GHz<br>2, 3, and 4 W/kg        | human lens epithelial cells                   | ROS             | increased                                |
| 2006 | Yurekli <i>et al.</i> <sup>(23)</sup>   | 945 MHz<br>11.3 mW/kg              | GTEM cells                                    | MDA and GSH     | MDA :increased<br>GSH: decreased         |
| 2011 | Esmekaya <i>et al.</i> <sup>(24)</sup>  | 900 MHz<br>1.20 W/kg               | liver, lung, testis and heart tissues of rats | MDA and GSH     | MDA :increased<br>GSH: decreased         |
| 2014 | Narayanan <i>et al.</i> <sup>(25)</sup> | 890-915 MHz<br>1.58 W/Kg           | discrete brain regions of rats                | TBARS           | increased                                |
| 2016 | Xing <i>et al.</i> <sup>(26)</sup>      | 1800MHz<br>1209 mW/m <sup>2</sup>  | Mouse NIH/3T3 and human U-87 MG cells         | ROS             | increased                                |
| 2018 | Zhao <i>et al.</i> <sup>(27)</sup>      | 433 MHz                            | NSCLC cells                                   | ROS             | increased                                |
| 2017 | Kavindra <i>et al.</i> <sup>(28)</sup>  | 2.45 GHz<br>0.2 mW/cm <sup>2</sup> | brain of rats                                 | LPO and ROS     | increased                                |

### Genetic effects of microwaves

Numerous experimental reports have shown that RF radiation does not induce genetic effects <sup>(29)</sup>. However, recent work has pointed to the chromosomes and DNA as targets for resonance interactions between living cells and microwaves and exposure to microwave radiation can damage DNA and gene structures <sup>(30)</sup> and the details of these results were listed in table 3. Cleary *et al.* <sup>(31)</sup> found that DNA synthesis was increased in glioma cells and suppressed in lymphocytes after exposure to

microwave radiation. In addition, cytogenetic damage has been reported in human lymphocytes from blood samples exposed to a 945 MHz field <sup>(32)</sup>. Cytogenetic studies of microwave radiation were conducted *in-vitro* and *in-vivo*, and have yielded contradictory and often intriguing experimental results. Garaj-Vrhovac *et al.* <sup>(33)</sup> found that workers occupationally exposed to pulsed marine radar frequencies (3, 5.5 and 9.4 GHz) have statistically significant differences between the mean tail intensities (0.67 vs. 1.22) and moments (0.08 vs.

0.16) assessed via comet assays and micronucleus tests, showing the effects of microwaves on micronuclei, nucleoplasmic bridges and nuclear buds. Additionally, Zhao *et al.* <sup>(34)</sup> have reported that microwave irradiation at a frequency of 2.45 GHz induced an inactivation of DNA-based hybrid catalyst even at low temperatures (such as 5°C), and a substantial amount of DNA molecules degraded and lost their characteristic structures. Furthermore, Deshmukh *et al.* <sup>(35)</sup> have also shown that low level subchronic microwave radiation at frequencies 900, 1800, and 2450 MHz for 2h a day, 5d a week for 90 days might lead to decline in cognitive function on rat brain as evidenced by DNA damage and increased heat shock protein 70 (HSP70) level in hippocampus tissues which could induce hazardous effects in rats. The results suggested that microwaves may cause genetic and cellular alterations.

Trosic *et al.* <sup>(36)</sup> researched Wistar rats exposed to a power density of 2.4 W/m<sup>2</sup> and a carrier frequency of 915 MHz with Global System Mobile (GSM) signal modulation. They found that repeated 915 MHz radiation could cause DNA breaks in renal and liver cells, but it did not affect the cell genome to a high extent compared to the basal damage. Further investigation is needed on the genotoxic effects

of RF radiation, especially to evaluate dependence on the SAR level and to specify possible mechanisms leading to alterations in DNA structure.

Some studies have found that oxidative stress may be one possible mechanism of DNA damage. Luukkonen *et al.* <sup>(37)</sup> found that human SH-SY5Y neuroblastoma cells exposed to 872 MHz RF radiation at 5 W/kg for 1 h exhibited increased DNA breakage. Furthermore, Kesari *et al.* <sup>(38)</sup> had 45-day-old male Wistar rats exposed for 2 h a day for 60 d to a mobile phone and found that the ROS content showed a positive linear correlation with DNA damage. Megha *et al.* <sup>(39)</sup> have also reported that prolonged exposure to low intensity microwave radiation at frequencies 900, 1800 and 2450 MHz might lead to oxidative stress and inflammatory imbalances which subsequently contribute to DNA damage in the rat brain, and revealed that increase in frequency could enhance the severity of effect. The results suggested that RF radiation might enhance chemically induced ROS production and thus cause secondary DNA damage. Any exposure, including RF and microwave exposure, which results in increased free radical production may be considered a plausible biological mechanism for carcinogenesis.

**Table 3.** Genetic effects of microwaves

| Time | Author                                      | Microwave Parameter             | Experimental Animal             | Detection Index                                     | Result                                    |
|------|---------------------------------------------|---------------------------------|---------------------------------|-----------------------------------------------------|-------------------------------------------|
| 1992 | Cleary <i>et al.</i> <sup>(31)</sup>        | 27 MHz<br>25 W/kg               | glioma cells and lymphocytes    | DNA synthesis                                       | increased                                 |
| 1996 | Maes <i>et al.</i> <sup>(32)</sup>          | 945 MHz<br>1.5 W/kg             | human lymphocytes               | cytogenetic                                         | cytogenetic damage                        |
| 2011 | Garaj-Vrhovac <i>et al.</i> <sup>(33)</sup> | 3, 5.5 and 9.4 GHz              | workers                         | micronuclei, nucleoplasmic bridges and nuclear buds | changed                                   |
| 2016 | Zhao <i>et al.</i> <sup>(35)</sup>          | 2.45 GHz                        | salmon testes                   | DNA                                                 | inactivation of DNA-based hybrid catalyst |
| 2016 | Deshmukh <i>et al.</i> <sup>(35)</sup>      | 900, 1800, and 2450 MHz         | hippocampus tissues of rats     | DNA                                                 | DNA damage                                |
| 2011 | Trosic <i>et al.</i> <sup>(36)</sup>        | 915 MHz<br>2.4 W/m <sup>2</sup> | brain, liver and kidney of rats | DNA                                                 | DNA breaks                                |
| 2009 | Luukkonen <i>et al.</i> <sup>(37)</sup>     | 872 MHz<br>5 W/kg               | SH-SY5Y cells                   | ROS and DNA                                         | ROS production and DNA damage             |
| 2014 | Kesari <i>et al.</i> <sup>(38)</sup>        | 3G mobile phone                 | brain of rats                   | ROS and DNA                                         | ROS production and DNA damage             |
| 2015 | Megha <i>et al.</i> <sup>(39)</sup>         | 900, 1800 and 2450 MHz          | brain of rats                   | ROS and DNA                                         | ROS production and DNA damage             |

### Effect of microwaves on gene and protein expression

If microwave radiation produces any biological effects in animals, this result must imply changes in cell behavior, and gene and protein expression. Microwave radiation can result in thermal damage in association with high rates of energy absorption; it has been shown that the effect of microwaves on gene and protein expression relies on non-thermal effects induced by lower intensities of exposure and the details of these results were listed in table 4. For example, Zhao *et al.* <sup>(40)</sup> demonstrated that following mice whole body exposure to low level microwaves (2100 MHz), 41 genes (0.45 W/kg group), 29 genes (1.8 W/kg group), and 219 genes (3.6 W/kg group) were differentially expressed, primarily involved in learning and memory processes. Furthermore, after exposure at an average power density of 30 mW/cm<sup>2</sup> for 10 min daily for 3 consecutive days, Zhao *et al.* <sup>(41)</sup> found that microwave induced differentially expressed miRNAs profiles in rats' hippocampus. Genes known to be stress responsive (heat shock and immediate early genes) have been investigated most frequently since the first publication regarding heat shock gene expression in *Caenorhabditis elegans* after exposure to microwaves at intensities too low to elicit any measurable temperature change <sup>(42)</sup>. However, a number of studies have failed to show a consistent effect on the expression of either genes or proteins following microwave exposure. Calabrò *et al.* <sup>(43)</sup> investigated SH-SY5Y human neuroblastoma cells exposed to microwaves for 2 and 4 h at 1800 MHz frequency bands. Under their experimental conditions, neither cell viability, heat shock protein 27 (Hsp27) expression nor caspase-3 activity was significantly changed.

That said, special attention has been given to the investigation of the effects of low power microwave radiation (<10 mW/cm<sup>2</sup>) on gene and protein expression. In human lymphocytes, 450 MHz fields at around 1.0 mW/cm<sup>2</sup> affected cyclic adenosine monophosphate- (cAMP-) independent protein kinase activity <sup>(44)</sup>.

Furthermore, Yao *et al.* <sup>(45)</sup> studied cultured rabbit lens epithelial cells exposed to continuous microwave radiation at a frequency of 2450 MHz, and power densities of 0.10, 0.25, 0.50, 1.00 and 2.00 mW/cm<sup>2</sup> for 8 h. They found that the protein expression of P27<sup>Kip1</sup> (a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals) that was available for interaction with cyclin-dependent kinases (CDKs) was markedly increased after microwave radiation. However, the mRNA levels were unchanged. In addition, microwave could result in abnormalities in the N-methyl-D-aspartate receptor-postsynaptic density-95-calcium/calmodulin- dependent protein kinase II (NMDAR-PSD95-CaMKII) pathway; Wang *et al.* <sup>(46)</sup> have demonstrated that the expression and phosphorylation of synapsin I, which could serve as a link between synaptic transmission and the NMDAR-PSD95-CaMKII pathway, was inhibited and altered in pheochromocytoma-12 (PC12) Cells.

Synapses are important structures for information transmission, and neurotransmitters are released as messengers mainly by Ca<sup>2+</sup>-dependent synaptic vesicle exocytosis. Qiao *et al.* <sup>(47)</sup> investigated hippocampal synaptosomes in Wistar rats and differentiated (neuronal) PC12 cells exposed to 2.856 GHz microwave radiation for 5 min at a mean power density of 30 mW/cm<sup>2</sup>. They found that the expression of phosphorylated synapsin I (p-Syn I) (Ser-553) and gamma-aminobutyric acid (GABA) release were both attenuated after exposure, suggesting that the abnormal release of neurotransmitters after microwave exposure may cause learning and memory deficits. Additionally, Wang *et al.* <sup>(48)</sup> have shown that although a stable C-to-T variant at nucleotide position -217 of the N-methyl-D-aspartate receptor-2B (NR2B) promoter sequences, a central synaptic receptor subunit, was not induced by exposure to microwave radiation (30 mW/cm<sup>2</sup>) for 5 min, NR2B mRNA and protein expression was decreased in the TT genotype rats but not the CC and CT genotype rats, and caused memory impairment.

**Table 4.** Effect of microwaves on gene and protein expression.

| Time | Author                          | Microwave Parameter                                         | Experimental Animal           | Detection Index                                  | Result                                             |
|------|---------------------------------|-------------------------------------------------------------|-------------------------------|--------------------------------------------------|----------------------------------------------------|
| 2015 | Zhao et al. <sup>(40)</sup>     | 2100 MHz 0.45, 1.8 and 3.6 W/kg                             | mice                          | gene expression in learning and memory processes | changed                                            |
| 2014 | Zhao et al. <sup>(41)</sup>     | 30 mW/cm <sup>2</sup>                                       | hippocampus of rats           | miRNAs                                           | differentially expressed miRNAs profiles           |
| 1998 | Daniells et al. <sup>(42)</sup> | 750 MHz                                                     | <i>Caenorhabditis elegans</i> | heat shock gene expression                       | changed                                            |
| 2012 | Calabrò et al. <sup>(43)</sup>  | 1800 MHz                                                    | SH-SY5Y cells                 | heat shock gene expression                       | unchanged                                          |
| 1984 | Byus et al. <sup>(44)</sup>     | 450 MHz<br>1.0 mW/cm <sup>2</sup>                           | human lymphocytes             | cAMP-independent protein expression              | changed                                            |
| 2004 | Yao et al. <sup>(45)</sup>      | 2450 MHz 0.10, 0.25, 0.50, 1.00 and 2.00 mW/cm <sup>2</sup> | rabbit lens epithelial cells  | mRNA and P27 <sup>Kip1</sup> expression          | mRNA: unchanged<br>P27 <sup>Kip1</sup> : increased |
| 2015 | Wang et al. <sup>(46)</sup>     | 30 mW/cm <sup>2</sup>                                       | PC12 cells.                   | NMDAR-PSD95-CaMKII pathway                       | inhibited                                          |
| 2014 | Qiao et al. <sup>(47)</sup>     | 2.856 GHz<br>30 mW/cm <sup>2</sup>                          | PC12 cells                    | p-Syn I expression                               | decreased                                          |
| 2016 | Wang et al. <sup>(48)</sup>     | 30 mW/cm <sup>2</sup>                                       | PC12 cells                    | NR2B mRNA and protein expression                 | decreased                                          |

### Microwave irradiation studies in humans and animals

Exposure to high density microwaves may cause detrimental effects on the testis and eye, and induce significant biological changes involving the central nervous system <sup>(49)</sup>, the cardiovascular system <sup>(50)</sup>, the hematopoietic system <sup>(51)</sup> and the endocrine system <sup>(52)</sup>. In addition, it can affect uteroplacental function,

and development and behavior of the fetus through its thermal action <sup>(53)</sup>. Guo et al. <sup>(54)</sup> found that various blood cell parameters of Wistar rats exposed to microwave irradiation changed and the bone marrow smear showed obvious destruction after exposure to 200 mW/cm<sup>2</sup> microwave radiation (The details of each article we referenced in this review were listed in table 5).

**Table 5.** Microwave irradiation studies in humans and animals.

| Time | Author                            | Microwave Parameter                              | Experimental Animal                     | Detection Index                                                     | Result              |
|------|-----------------------------------|--------------------------------------------------|-----------------------------------------|---------------------------------------------------------------------|---------------------|
| 2010 | Wang et al. <sup>(49)</sup>       | 30 mW/cm <sup>2</sup>                            | cerebral cortex and hippocampus of rats | central nervous system                                              | changed             |
| 2015 | Zhu et al. <sup>(50)</sup>        | 100, 150 and 200 mW/cm <sup>2</sup>              | heart tissue of rabbits                 | cardiovascular system                                               | changed             |
| 2010 | Cao et al. <sup>(51)</sup>        | 900-MHz                                          | hippocampus of mice                     | hematopoietic system                                                | changed             |
| 2012 | Wang et al. <sup>(52)</sup>       | 2.1 W/kg                                         | urine of rats                           | endocrine system                                                    | changed             |
| 2015 | Zhao et al. <sup>(53)</sup>       | 9.417-GHz<br>2.0 W/kg                            | fetal of mice                           | uteroplacental function                                             | changed             |
| 2011 | Guo et al. <sup>(54)</sup>        | 200 mW/cm <sup>2</sup>                           | hematopoietic system of rats            | bone marrow                                                         | changed             |
| 1985 | Nawrot et al. <sup>(55)</sup>     | 2.45-GHz<br>30 mW/cm <sup>2</sup>                | Pregnant CD-1 mice                      | fetal toxicity and teratogenicity                                   | teratogenic effects |
| 2010 | Kesari and Behari <sup>(56)</sup> | 50 GHz                                           | male rats                               | sperm glutathione peroxidase (GPx), SOD activity and histone kinase | decreased           |
| 2014 | Shahin et al. <sup>(57)</sup>     | 2.45-GHz<br>0.018 W/Kg                           | male mice                               | sperm count and sperm viability                                     | decreased           |
| 2018 | Wu et al. <sup>(58)</sup>         | 30 mW/cm <sup>2</sup> and 100 mW/cm <sup>2</sup> | testis of rats                          | TLRs signalling                                                     | activated           |

However, there is no epidemiologic evidence showing that occupational or daily life exposure to microwaves harm human reproductive processes, although experimental animal studies have suggested that microwaves can produce intrauterine effects, including teratogenic effects<sup>(55)</sup>. Kesari and Behari<sup>(56)</sup> investigated the effects of 50 GHz microwave frequency EMFs on the reproductive systems of male Wistar rats. Their results showed a significant decrease in the levels of sperm glutathione peroxidase (GPx), SOD activity and histone kinase after exposure; furthermore, the results also indicated a decrease in the G<sub>2</sub>/M transition phase of the cell cycle in the exposed group. Shahin *et al.*<sup>(57)</sup> have also shown a decrease in sperm count and sperm viability at a non-thermal low-level 2.45-GHz microwave radiation (CW for 2 h/day for 30 days, power density 0.029812 mW/cm<sup>2</sup> and SAR 0.018 W/Kg) in male mice. Furthermore, Wu *et al.*<sup>(58)</sup> have demonstrated that Toll-like receptors (TLRs) signaling can be activated by microwave radiation in testis, which may provide novel clues into the preventive and therapeutic strategies for the impairment of spermatogenesis and male fertility by microwave radiation. The authors concluded that radiation may have a significant effect on the reproductive system of male rats, which may relate to male infertility.

## CONCLUSION

During recent years there has been increasing public concern regarding the biological effects of microwave radiation emissions from wireless communications. The number of reports on the effects induced by microwave radiation in various cellular and molecular systems continues to increase, attracting public attention toward microwave radiation protection. However, until now no satisfactory mechanism has been proposed to explain the biological effects of these fields. The theories and hypotheses that explain some of the local physiological phenomena have limitations, making it difficult to formulate a specific diagnostic criteria and specific methods

of prevention and treatment.

Currently, physical protection is the main measure of EMR protection, including protective clothing with metal wires. In addition, medical interventions through the use of antioxidants are also beneficial for protection against EMR, though specific drugs are lacking. Possible medications include melatonin<sup>(28)</sup>, carnitine, caffeic acid phenethyl ester (CAPE)<sup>(59)</sup>, vitamin C<sup>(60)</sup> and different kinds of Chinese medicines<sup>(61)</sup>.

Whether mobile phones or other wireless technologies are harmful to humans is still controversial, but research increasingly suggests that they are unlikely to cause brain cancers in adults<sup>(62)</sup> or affect learning and memory processes<sup>(63,64)</sup>. In addition, some studies have even found that microwaves emitted from mobile phones can induce adaptive responses that make animals more resistant to ionizing radiation<sup>(65)</sup>. That said, efforts should be made to minimize microwave radiation exposure by remaining remote from TV towers, radar stations and other strong radiation areas. Furthermore, while mobile phone use has not been linked to brain cancer, long-term use may be harmful to the eyes<sup>(66)</sup> and brain<sup>(67)</sup>. Thus, mobile devices should be kept at a safe distance from the head and, if possible, earphones should be used.

Microwave radiation may also be emitted from microwave ovens, which are widely used for food preparation in daily life. A commonly used frequency in such ovens is 2450 MHz<sup>(68)</sup>. In general, microwave ovens may be considered a safe form of cooking<sup>(68)</sup>; however, improper use can damage the eyes or even result in scald injuries and burns<sup>(69)</sup>. As a safety precaution, a safe distance should be maintained from microwave ovens when in use. Thus, some studies suggest that although current literature lacks definitive evidence associating male infertility with cell phone exposure, limitation of exposure to the possible harmful effects of cell phone, laptop, and microwave ovens is recommended<sup>(70)</sup>.

In conclusion, although the biological effects of microwaves are controversial, future studies regarding the non-thermal effects and

mechanisms of microwave radiation are likely to progress our understanding, as well as our comprehension of temporary biological effects that may have long-term impacts on microbial cells, animals and humans. An increased number of studies are paying attention to the biological effects of microwaves and they are expected to increase in number in the future since microwaves have extensive applications in various fields.

**Conflicts of interest:** Declared none.

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