Ameliorating Role of Grape Seeds Extraction Compensating Unbalanced Apoptotic to Anti-apoptotic Proteins (P53/Bcl2) Ratio Induced by Microwave Irradiation

Bassem M. Raafat, Saad S. Al-Zahrani and El-Morsy A. El-Morsy

Faculty of Applied Medical Sciences, Taif University, KSA. Genetic Engineering and Biotechnology Division, National Research Centre, Cairo, Egypt.

Abstract: Purpose: This study aimed to determine the oxidative stress of microwave as well as overcoming these effects by administrating grape seeds extraction as a natural antioxidant to return back the unbalancing in apoptotic to anti-apoptotic related proteins ratio as a results of microwave irradiation. Procedure: experiments were performed on adult sixty Wistar rats weighing 175 g (± 10 g). Animals were subdivided into control in which animals neither irradiated with 2450 MHz of continuous microwave (MW) that is the cell phone carrier band nor received grape seeds extraction (GSE). The second group represents animals irradiated with MW with no antioxidant administration. The third group represents animals received 200 mg/kg b wt GSE only. The Fourth group represents animals received the same dose of GSE as antioxidant before MW irradiation sessions. Super Oxide Dismutase (SOD, glutathione peroxidase, P53, Bcl2 as well as hemoglobin auto-oxidation spectrum were studied. Results showed that apoptotic to anti-apoptotic related protein ratio is significantly (p < 0.05) returned back to its normal ratio when GSE was administered prior to MW irradiation. Antioxidant activities returned to their normal levels which are clearly proved by the auto-oxidation pattern. Conclusion: GSE extraction may be used as an ameliorating agent of MW irradiating side effects.

Key words: Microwave (MW) - Grape Seeds Extraction (GSE), apoptosis, oxidative stress.

INTRODUCTION

Microwaves, a subset of radio waves, have wavelengths ranging from as long as one meter to as short as one millimeter, or equivalently, with frequencies between 300 MHz (0.3 GHz) and 300 GHz (David, 1993). This broad definition includes both UHF and EHF (millimeter waves), and various sources use different boundaries. In all cases, microwave includes the entire SHF band (3 to 30 GHz, or 10 to 1 cm) at minimum, with RF engineering often putting the lower boundary at 1 GHz (30 cm), and the upper around 100 GHz (Goldsmith, 1997). Apparatus and techniques may be described qualitatively as "microwave" when the wavelengths of signals are roughly the same as the dimensions of the equipment, so that lumped-element circuit theory is inaccurate (Merrill, 2001). As a consequence, practical microwave technique tends to move away from the discrete resistors, capacitors, and inductors used with lower-frequency radio waves. Instead, distributed circuit elements and transmission-line theory are more useful methods for design and analysis. Microwave and coaxial transmission lines give way to waveguides and stripline, and lumped-element tuned circuits are replaced by cavity resonators or resonant lines (Breckenkamp et al., 2003). Effects of reflection, polarization, scattering, diffraction, and atmospheric absorption usually associated with visible light are of practical significance in the study of microwave propagation. The same equations of electromagnetic theory apply at all frequencies (Jauchem, 2008).

The prefix "micro-" in "microwave" is not meant to suggest a wavelength in the micrometer range. It indicates that microwaves are "small" compared to waves used in typical radio broadcasting, in that they have shorter wavelengths. The boundaries between far infrared light, terahertz radiation, microwaves, and ultra-high-frequency radio waves are fairly arbitrary and are used variously between different fields of study (Masoodi and Nicolaou, 2006). Vacuum tube devices operate on the ballistic motion of electrons in a vacuum under the influence of controlling electric or magnetic fields, and include the magnetron, klystron, traveling-wave tube (TWT), and gyrotron (Rokach et al., 2004). These devices work in the density modulated mode, rather than the current modulated mode. This means that they work on the basis of clumps of electrons flying ballistically through them, rather than using a continuous stream (Simon et al., 2005).

A maser is a device similar to a laser, which amplifies light energy by stimulating photons. The maser, rather than amplifying light energy, amplifies the lower frequency, longer wavelength microwaves and radio frequency emissions (Wolf et al., 2008). Wireless LAN protocols, such as Bluetooth and the IEEE 802.11 specifications, also use microwaves in the 2.4 GHz ISM band, although 802.11a uses ISM band and U-NII frequencies in the 5 GHz range. Licensed long-range (up to about 25 km) Wireless Internet Access services
have been used for almost a decade in many countries in the 3.5-4.0 GHz range (Ivanek, 1989). The FCC recently carved out spectrum for carriers that wish to offer services in this range in the U.S. — with emphasis on 3.65 GHz. Dozens of service providers across the country are securing or have already received licenses from the FCC to operate in this band. The WIMAX service offerings that can be carried on the 3.65 GHz band will give business customers another option for connectivity (Pratt and Bostian, 1986).

Metropolitan area network (MAN) protocols, such as WiMAX (Worldwide Interoperability for Microwave Access) are based on standards such as IEEE 802.16, designed to operate between 2 to 11 GHz. Commercial implementations are in the 2.3 GHz, 2.5 GHz, 3.5 GHz and 5.8 GHz ranges (Bailey, 1985).

Mobile Broadband Wireless Access (MBWA) protocols based on standards specifications such as IEEE 802.20 or ATIS/ANSI HC-SDMA (such as iBurst) operate between 1.6 and 2.3 GHz to give mobility and indoor building penetration characteristics similar to mobile phones but with vastly greater spectral efficiency (Mustafa Ergen, 2009). Some mobile phone networks, like GSM, use the low-microwave/high-UHF frequencies around 1.8 and 1.9 GHz in the Americas and elsewhere, respectively. DVB-SH and S-DMB use 1.452 to 1.492 GHz, while proprietary/incompatible satellite radio in the U.S. uses around 2.3 GHz for DARS (Heeks, 2008).

Microwave radio is used in broadcasting and telecommunication transmissions because, due to their short wavelength, highly directional antennas are smaller and therefore more practical than they would be at longer wavelengths (lower frequencies). There is also more bandwidth in the microwave spectrum than in the rest of the radio spectrum; the usable bandwidth below 300 MHz is less than 300 MHz while many GHz can be used above 300 MHz. Typically, microwaves are used in television news to transmit a signal from a remote location to a television station from a specially equipped van. See broadcast auxiliary service (BAS), remote pickup unit (RPU) and studio/transmitter link (STL) (Mingtao, 2007).

Most satellite communications systems operate in the C, X, Ka, or Ku bands of the microwave spectrum. These frequencies allow large bandwidth while avoiding the crowded UHF frequencies and staying below the atmospheric absorption of EHF frequencies. Satellite TV either operates in the C band for the traditional large dish fixed satellite service or the Ku band for direct-broadcast satellite. Military communications run primarily over X or Ka-band links, with the Ku band being used for Milstar (Reardon, 2008).

It is well known that low frequency microwaves include the more harmful frequencies. Yet the design of new high frequency machines has also increased the harmful fractions. Reports of hypertension, headaches, memory failure, brain damage, dementia, abortion, and breast cancer have been related to exposure to strong microwaves (Radovanobic et al., 1994). High frequency microwaves increase the temperature of human tissue, which is especially harmful to reproduction and fertilization organs and to blood forming cells (Trosic, 2002, Trosic et al., 2004). Further, microwaves generate free radicals that accelerate the aging process in human tissue, and promote adult chronic diseases and cancer. Waters et al. (2007) reported that microwaves generate harmful oxygen radicals that lead to DNA damage (Bavincoba, 1993). Moustafa et al. (2001) also observed that 2.45 GHz microwaves produce an increase in lipid peroxide. With the introduction of cellular telephones, even more public attention has been drawn to the possible bioeffects of low-level exposure to radiofrequency and microwave radiation (RF/MW). Many epidemiological studies have addressed the possible links between exposure to RF/MW fields and excess risk for cancer (Tice et al., 2002 and Vijayaalaxmi, 2004).

A part of a larger investigation designed to determine biological indicators of microwave radiation after whole-body exposure of rats were the studies of (Trosic et al., 2002 and Garag-Vrhovac et al., 2009) which described the incidence of cytogenetic damage as assessed by the micronucleus assay, and incidence of DNA damage as assessed by comet assay.

Grape seed extract (GSE) contains a number of polyphenols including proanthocyanidins and procyanidins (Bagchi et al., 2003 and Thomas et al., 2009). Recent studies reported that Proanthocyanidins exhibited potent antioxidant properties in both lipid peroxidation (Ariga, 2004 and Fing et al., 2005) and oxidative cell death (Li et al., 2004). Also grape seed proanthocyanidins extract (GSPE) has multiple health benefits due to antioxidant/antiradical activity due to a high degree of oxygen free radical scavenging potential (Devi et al., 2006). In addition to antioxidant/antiradical of (GSPE) activity, were shown to possess many biological properties including the inhibition of DNA damage (Balu et al., 2006), prevent chromosomal damage in human lymphoblastoid cells (Sugisawa et al., 2004), prevent oxidative injury by modulating the expression of antioxidant enzyme systems (Ariga, 2004), modulation of lipid metabolism and inhibition of low-density lipoprotein oxidation (Devi et al., 2006 and Balu et al., 2006).

Apoptosis is the process of programmed cell death (PCD) that may occur in multicellular organisms (Green, 2011). Biochemical events lead to characteristic cell changes (morphology) and death. These changes include blebbing, loss of cell membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation (Alberts et al., 2008). The apoptotic mode of cell death is an active and defined process which plays an important role in the development of multicellular organisms and in the regulation and maintenance of the cell populations in tissues upon physiological and pathological conditions. It should be stressed that apoptosis is a well-defined and possibly the most frequent form of
programmed cell death, but that other, non-apoptotic types of cell death also might be of biological significance (Leist and Jaattela, 2001).

The rate of apoptosis is controlled by the balance between proteins that activate processes resulting in such death and other proteins that act to inhibit these processes. Thus, for example, the Bcl-2 proto-oncogene was originally identified on the basis of its activation by chromosomal translocation in non-Hodgkin B cell lymphomas (Tsujimoto et al., 1985) and was subsequently shown to protect a wide variety of different cell types from programmed cell death or apoptosis (Reed, 1994). Conversely, the p53 anti-oncogene protein, as well as inhibiting cellular proliferation, can also stimulate programmed cell death. It is clear therefore that Bcl-2 and p53 represent proteins with opposite effects on the rate of apoptosis (Agrawal et al., 2006). Moreover, it appears that Bcl-2 can specifically inhibit p53-dependent apoptosis. Thus, although over expression of p53 can induce apoptosis in different cell types, this is prevented by over expression of Bcl-2 (Chiou et al., 1994 and Marcellus et al., 1996). In this regard, it is of interest that high levels of p53 are associated with low levels of Bcl-2 and vice versa, both during normal rat development (Ibrahim et al., 1997) and in different types of tumors (Harn et al., 1996 and Lee et al., 1996).

The aim of this work was to determine the oxidative stress of microwave as well as overcoming these effects by administrating grape seeds extraction as a natural antioxidant to return back the unbalancing in apoptotic to anti-apoptotic related proteins ratio as a results of microwave irradiation.

MATERIALS AND METHODS

The experiments were performed on adult sixty Wistar rats weighing 175 g (± 10 g). All through the experiment duration, animals were housed in separate cages, fed standard laboratory food and allowed free access to water in room lightening with a 12 hour light-dark cycle in animal house of the faculty of medicine, Cairo university. They were under responsibility of veterinary in compliance with the Guide for the Care and Use of Laboratory Animals for Scientific Purposes. Animal design and other experimental procedure were approved by the National Research Center ethical committee before starting the study procedures.

Animals were subdivided into four groups; fifteen rats in each. The first group represented control animals those neither irradiated with microwave (MW) nor received grape seeds extraction (GSE). The second group represents animals irradiated with 2450 MHz of continuous microwave (MW) that is the cell phone carrier with no antioxidant administration. The third group represents animals received 200 mg/kg b wt GSE only to find out any effects that may result from GSE administration itself. The Fourth group represents animals received the same dose of GSE as antioxidant half hour before MW irradiation sessions.

The experimental groups were exposed to 2450 MHz continuous waves MW for 1h daily for 30 days and every day at the same hour. Animals were placed in individual Plexiglas cages and exposed to the MW oven (550 Watt, 2450 MHz Imperial V- 850ST Model). The device was modified to supply no thermal conditions by water–cooled coils. Thus, the experiments carried out at 37°C (±1 °C). The power density of the field within the individual cages was measured with an EM Radiation Monitor, (Wandel & Golterman GmbH & Co. Germany) at ‘average mode’ option. Mean total-body specific absorption rates were estimated according to standard procedures (ICNIRP, 1998) (2 W/kg) and average power density (0.251 mW/cm2).

The grape seed extract (GSE) was composed of 89.3% proanthocyanidins, 2.24% moisture, 1.06% protein, 0.8% ash and 7.0% gallate ester (Agli et al., 2004). The obtained GSE was dissolved in water. During sample preparation, care was paid to avoid contamination, therefore, every item from the moment of sampling until analysis was regarded as potential source of contamination and was checked not to contain or leach detectable amount of any contaminant.

Determination of molecular markers: apoptosis-related proteins P53 and Bcl2 in animals liver, was determined by assay designs following the manufacturer instruction.

Measurement of auto-oxidation rate was carried out spectrophotometrically as described by Wallace et al. (1952) and Guilllochon et al. (1986) in air saturated 0.1 M phosphate buffer of pH 7.05 with 2 mg/ml HbO2. The pH was checked before and after each experiment and spectrum of hemoglobin was recorded in order to confirm the absence of hemichrome during auto-oxidation rate measurements.

Determination of superoxide dismutase (SOD) activity was carried out by a RANDOX kit package, cat. No. SD125 (McCord and Fridovich, 1969).

Determination of glutathione peroxidase activity was carried out by a RANDOX kit package, cat. No. RS504 according to (Wendel, 1981).

Observed data were statistically analyzed by using of SPSS program version 16. All tests were two-tailed, and a value of P < 0.05 was considered statistically significant. According to the data distribution and types, one way ANOVA was the statistical analysis test.
**Results:**

Table (1) and Fig. (1) demonstrate apoptotic and anti-apoptotic related proteins in rats liver irradiated with microwave as compared to those received GSE before irradiation sessions as well as those represented controls. P53 activity in liver of animals irradiated with MW showed the maximum significant increase (10.85 ± 0.828). Increase in the Bcl2 of the same animals was recorded but with lesser value. Attention should be drawn to the ratio between both proteins activity; it is very clear that this ratio is highly elevated (2.10) in animals irradiated with MW with no prophylactic administration. Apoptotic to anti-apoptotic proteins ratio returned back to normal value when grape seeds extraction was administered before irradiation.

**Table 1:** Apoptotic protein P53 and anti-apoptotic protein Bcl2 liver concentration in rats irradiated with microwave as compared to those treated with grape seeds extraction as well as those neither irradiated with microwave nor treated with GSE (mean ± SD).

<table>
<thead>
<tr>
<th>Group (n=15)</th>
<th>P53 (pg/ml)</th>
<th>Bcl2 (pg/ml)</th>
<th>P53 : Bcl2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.12 ± 0.458</td>
<td>4.38 ± 0.352</td>
<td>1.39</td>
</tr>
<tr>
<td>MW</td>
<td>10.85 ± 0.828</td>
<td>5.15 ± 0.681</td>
<td>2.10</td>
</tr>
<tr>
<td>GSE</td>
<td>4.82 ± 0.508</td>
<td>3.71 ± 0.278</td>
<td>1.29</td>
</tr>
<tr>
<td>GSE + MW</td>
<td>6.78 ± 0.478</td>
<td>4.82 ± 0.311</td>
<td>1.40</td>
</tr>
</tbody>
</table>

a: Mean ± SD; p < 0.05; b: Significant difference compared to control; c: Significant difference as compared to second group.

**Fig. 1:** Protein P53 and anti-apoptotic protein Bcl2 liver concentration in rats irradiated with microwave as compared to those treated with grape seeds extraction as well as those neither irradiated with microwave nor treated with GSE (mean ± SD).

Fig. (2) represents hemoglobin spectrum between 450 to 650 nm to show the spectrum value at 630 nm which is characteristic to met-hemoglobin that is the oxidized form of hemoglobin. Blue line represents the highest value of absorbance at 630 nm for animals hemoglobin irradiated with microwave. When GSE is administered before MW irradiation hemoglobin spectrum (green line) is nearly become similar to that of control (red line).

**Fig. 2:** Hemoglobin auto-oxidation spectrum of animals irradiated with microwave as compared to those treated with GSE before MW irradiation as well as control.
Table (2) shows superoxide dismutase and glutathione peroxidase activities in rat’s blood irradiated with microwave as compared to those received prophylactic agent (GSE) before MW irradiation as well as control. Very highly significant increase in both antioxidants was recorded with MW irradiation (172.11 ± 14.08 and 9903.48 ± 825.14 respectively). Attention should be drawn to the dramatic reduction of both antioxidants activities when GSE administered before microwave irradiation (96.08 ± 7.41 and 5148.71 ± 412.5 respectively).

Table 2: Super Oxide Dismutase (SOD) and Glutathione peroxidase (GPx) in rats irradiated with microwave as compared to those treated with grape seeds extraction as well as those neither irradiated with microwave nor treated with GSE (mean ± SD).

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (U/ml)</th>
<th>GPx (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=15)</td>
<td>82.68 ± 6.85</td>
<td>4985.25 ± 375.15</td>
</tr>
<tr>
<td>MW (n=15)</td>
<td>172.11 ± 14.08</td>
<td>9903.48 ± 825.14</td>
</tr>
<tr>
<td>GSE (n=15)</td>
<td>87.25 ± 5.88</td>
<td>4752.45 ± 385.15</td>
</tr>
<tr>
<td>GSE + MW (n=15)</td>
<td>96.08 ± 7.41</td>
<td>5148.71 ± 412.5</td>
</tr>
</tbody>
</table>

a: Mean ± SD; p < 0.05; b: Significant difference compared to control; c: Significant difference as compared to second group.

Discussion:

The descriptive term microwaves is used to describe electromagnetic waves with wavelengths ranging from 1 cm to 1 m. The corresponding frequency range is 300 MHz up to 30 GHz for 1-cm-wavelength waves. Electromagnetic waves with wavelengths ranging from 1 to 10 mm are called millimeter waves (Kinsler, 2011). In recent years, microwave frequencies have also come into widespread use in communication links, generally referred to as microwave links. Since the propagation of microwaves is effectively along line-of-sight paths, these links employ high towers with reflector or lens-type antennas as repeater stations spaced along the communication path. Such links are a familiar sight to the motorist traveling across the country because of their frequent use by highway authorities, utility companies, and television networks (Weinberg, 1995). A further interesting means of communication by microwaves is the use of satellites as microwave relay stations. The first of these, the Telstar, launched in July 1962, provided the first transmission of live television programs from the United States to Europe (Paul and Monk, 2004).

Since that time a large number of satellites have been deployed for communication purposes, as well as for surveillance and collecting data on atmospheric and weather conditions. For direct television broadcasting the most heavily used band is the C band. The up-link frequency used is in the 5.9- to 6.4-GHz band and the receive or down-link frequency band is between 3.7 and 4.2 GHz (Kinsler, 2011). For home reception an 8-foo diameter parabolic antenna is commonly used. A second frequency band has also been allocated for direct television broadcasting. For this second band the up-link frequency is in the 14- to 14.5-GHz range and the down-link frequencies are between 10.95 and 11.2 GHz and 11.45 and 11.7 GHz. In this band a receiving parabolic antenna with a 3-ft diameter is adequate. At the present time this frequency band is not being used to any great extent in the United States. It is more widely used in Europe and Japan (Kinsler, 2011). Terrestrial microwave links have been used for many years. The TD-2 system was put into service in 1948 as part of the Bell Network. It operated in the 3.7- to 4.2-GHz band and had 480 voice circuits, each occupying a 3.1-kHz bandwidth. In 1974, the TN-1 system operating in the 10.7- to 11.7-GHz band was put into operation (Paul and Monk, 2004). This system had a capacity of 1,800 voice circuits or one video channel with a 4.5-MHz bandwidth. Since that time the use of terrestrial microwave links has grown rapidly (Weinberg, 1995).

At the present time most communication systems are shifting to the use of digital transmission i.e., analog signals are digitized before transmission. Microwave digital communication system development is progressing rapidly. In the early systems simple modulation schemes were used and resulted in inefficient use of the available frequency spectrum. The development of 64-state quadrature amplitude modulation (64-QAM) has made it possible to transmit 2,016 voice channels within a single 30-MHz RF channel. This is competitive with the available frequency spectrum. The development of 64-state quadrature amplitude modulation (64-QAM) has made it possible to transmit 2,016 voice channels within a single 30-MHz RF channel. This is competitive with the available frequency spectrum.

During World War II, it was observed that individuals in the radiation path of radar installations experienced clicks and buzzing sounds in response to microwave radiation. This microwave auditory effect was thought to be caused by the microwaves inducing an electric current in the hearing centers of the brain (Allen and Hagness, 2004). Research by NASA in the 1970s has shown this to be caused by thermal expansion in parts of the inner ear (Guay, 2004).
When injury from exposure to microwaves occurs, it usually results from dielectric heating induced in the body. Exposure to microwave radiation can produce cataracts by this mechanism, because the microwave heating denatures proteins in the crystalline lens of the eye (in the same way that heat turns egg whites white and opaque) faster than the lens can be cooled by surrounding structures (Galli and Racagni, 1982). The lens and cornea of the eye are especially vulnerable because they contain no blood vessels that can carry away heat. Exposure to heavy doses of microwave radiation (as from an oven that has been tampered with to allow operation even with the door open) can produce heat damage in other tissues as well, up to and including serious burns that may not be immediately evident because of the tendency for microwaves to heat deeper tissues with higher moisture content (Rafnsson et al., 2005).

A paradox in metabolism is that while the vast majority of complex life on Earth requires oxygen for its existence, oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species (Davies, 1995). Consequently, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids (Sies, 1997 and Vertuani et al., 2004). In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell (Sies, 1997 and Davies, 1995). However, since reactive oxygen species do have useful functions in cells, such as redox signaling, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level (Rhee, 2006).

The reactive oxygen species produced in cells include hydrogen peroxide (H₂O₂), hypochlorous acid (HClO) and free radicals such as the hydroxyl radical (OH·) and the superoxide anion (O₂⁻) (Valko et al., 2007). The hydroxyl radical is particularly unstable and will react rapidly and non-specifically with most biological molecules. This species is produced from hydrogen peroxide in metal-catalyzed redox reactions such as the Fenton reaction (Stohs, 1995). These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins. Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms (Nakabeppu et al., 2006), while damage to proteins causes enzyme inhibition, denaturation and protein degradation (Stadtman, 1992).

The use of oxygen as part of the process for generating metabolic energy produces reactive oxygen species (Raha and Robinson, 2000). In this process, the superoxide anion is produced as a by-product of several steps in the electron transport chain (Lenaz, 2001). Particularly important is the reduction of coenzyme Q in complex III, since a highly reactive free radical is formed as an intermediate (Q⁻). This unstable intermediate can lead to electron "leakage", when electrons jump directly to oxygen and form the superoxide anion, instead of moving through the normal series of well-controlled reactions of the electron transport chain (Finkel and Holbrook, 2000). Peroxide is also produced from the oxidation of reduced flavoproteins, such as complex I (Hirst et al., 2008). However, although these enzymes can produce oxidants, the relative importance of the electron transfer chain to other processes that generate peroxide is unclear (Seaver and Imlay, 2004). In plants, algae, and cyanobacteria, reactive oxygen species are also produced during photosynthesis (Demmig-Adams and Adams, 2002) particularly under conditions of high light intensity (Krieger, 2004). This effect is partly offset by the involvement of carotenoids in photoinhibition, which involves these antioxidants reacting with over-reduced forms of the photosynthetic reaction centres to prevent the production of reactive oxygen species (Szabó et al., 2005).

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals (Halliwell, 2008). In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell (Duarte Lunec 2005). When the chain reaction occurs in a purified monomer, it produces a polymer resin, such as a plastic, a synthetic fiber, or an oil paint film. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Schneider, 2005).

Glutathione is a cysteine-containing peptide found in most forms of aerobic life (Meister and Anderson, 1983). It is not required in the diet and is instead synthesized in cells from its constituent amino acids (Meister, 1988). Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione-ascorbate cycle, glutathione peroxidases and glutaredoxins, as well as reacting directly with oxidants (Meister, 1994). Due to its high concentration and its central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants (Meister and Anderson, 1983). In some organisms glutathione is replaced by other thiols, such as by mycothiol in the Actinomycetes, bacillithiol in some Gram-positive bacteria, or by trypanothione in the Kinetoplastids (Gabella et al., 2010).

Superoxide dismutases (SODs) are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide (Zelko, 2002). SOD enzymes are present in almost all
aerobic cells and in extracellular fluids (Johnson and Giulivi, 2005). Superoxide dismutase enzymes contain metal ion cofactors that, depending on the isozyme, can be copper, zinc, manganese or iron. In humans, the copper/zinc SOD is present in the cytosol, while manganese SOD is present in the mitochondrion (Zelko, 2002). There also exists a third form of SOD in extracellular fluids, which contains copper and zinc in its active sites (Nozik et al., 2005) The mitochondrial isozyme seems to be the most biologically important of these three, since mice lacking this enzyme die soon after birth (Melov et al., 1998). In contrast, the mice lacking copper/zinc SOD (Sod1) are viable but have numerous pathologies and a reduced lifespan (see article on superoxide), while mice without the extracellular SOD have minimal defects (sensitive to hyperoxia) (Reaume et al., 1996). In plants, SOD isozymes are present in the cytosol and mitochondria, with an iron SOD found in chloroplasts that is absent from vertebrates and yeast (Van Camp et al., 1997).

Grape seed extracts are industrial derivatives from whole grape seeds that have a great concentration of vitamin E, flavonoids, linoleic acid, and OPCs. The typical commercial opportunity of extracting grape seed constituents has been for chemicals known as polyphenols, including oligomeric proanthocyanidins recognized as antioxidants (Bagchi et al., 2003). Human case reports and results from laboratory and animal studies show that grape seed extract may be useful to treat heart diseases such as high blood pressure and high cholesterol (Bagchi et al., 2003). By limiting lipid oxidation, phenolics in grape seeds may reduce risk of heart disease, such as by inhibiting platelet aggregation and reducing inflammation. While such studies are promising, more research including long-term studies in humans is needed to confirm initial findings (Vitseva et al., 2005).

A polyphenol contained in grape seeds is resveratrol, which may interfere with cancer cell growth and proliferation, as well as induce apoptosis, among a variety of potential chemopreventive effects (Kundu and Surh, 2008). Grape seed components may also be active against HIV by inhibiting virus expression and replication (Nair et al., 2002). Recently, meta-analysis of randomized controlled trials, concluded that “grape seed extract appears to significantly lower systolic blood pressure and heart rate, with no effect on lipid or C-reactive protein levels (Feringa et al., 2011). Oral grape seed extract is typically used as capsules or tablets usually containing 50 mg or 100 mg, or as a liquid to add drops to water and/or other drinks. Insufficient scientific information is known, however, about how long-term use of grape seed extract might affect health or any disease (Feringa et al., 2011). In a 12-month study, the safety of dietary intake of grape seed proanthocyanidins in a dose of 100 mg per kg per day was demonstrated in rodents (Ray et al., 2001). The US National Center for Complementary and Alternative Medicine (NCCAM) reports that oral administration of grape seed extract was well tolerated in people over 8 weeks of a clinical trial (Shamuganayagam et al., 2002).

Present work can be considered as a complete of the above mentioned benefits of grapes seed extraction. The main core of the work is presented in table 1 which shows the liver apoptotic and anti-apoptotic proteins in rats irradiated with microwaves as compared to those treated with GSE before irradiation sessions as well as controls neither received GSE nor irradiated with microwave. Attention should be drawn to the balanced ratio between both proteins P53 and Bcl2 that is in normal controls was 1.39 and was highly unbalanced in liver of animals irradiated with microwave to reach 2.10 or the balanced ratio is increased with 51%.

The results within hand are in full agreed with Agli (2004) who stated that ach cell is under constant surveillance to maintain the integrity of its genome. Genomic lesions in a cell must be repaired before the onset of DNA replication and cell division. In the scenario that the genomic lesion is not repairable, the damaged cells are disposed in an orderly manner known as programmed cell death or apoptosis. Apoptosis and cell cycle progression are two intimately linked phenomena. Uncontrollable cell proliferation perturbs the cellular homeostasis and this can lead to malignancies, as well as organ dysfunction and developmental abnormalities. The biological pathway controlling cell fate is sequentially organized at the molecular level. Recent studies have made important contributions in advancing our knowledge of the mechanisms of cell cycle control and apoptosis regulation. A oncogene derived protein (Bcl2), confers negative control in the pathways of cellular suicide machinery. A Bcl2- homogous protein, Bcl2-Baz, promotes cell death by competing with Bc2. While Bax-Bax homodimers act as apoptosis inducer, Bcl2-Bax heterodimer formation evokes a survival signal for the cells. Both Bcl2 and Bax are transcriptional targets for tumor suppressor (P53), which induces cell cycle arrest or apoptosis in response to DNA damage. In all coordinate performance of these molecules is crucial for controlling life and death of a cell.

So, the key role of both molecules is mainly depends on their gene expression or in other words it may depends on their concentration. So the balance between their presences in and around the cell may act on the apoptosis process starting and rating. Grapes seed extraction administration to animals before microwave irradiation returned back the balance between the two molecules concentration (P53 : Bcl2) after it had been elevated with the microwave application.

This prophylactic role of GSE may be due to its antioxidants role, measurement of antioxidants activity in all groups came to verify this postulate. Increasing of the antioxidants activity is a reflect of higher free radical formation rates, what we found in animals irradiated with MW with no antioxidant application. Antioxidant potency of GSE is obviously expressed in animals received GSE before microwave irradiation sessions. Over
view of these results may be considered as an evidence of returning back the balanced (P53: Bcl2) ratio by means of exclusion of excess free radicals formed due to MW irradiation.

Auto-oxidation rate of hemoglobin can be another evidence of the antioxidant potency of the grapes seeds extraction. A characteristic peak at 630 nm is very specific to the formation of non-functional hemoglobin form that is called Met-hemoglobin. Regarding the hemoglobin spectrum shown in figure 2 it is very clear in the line representing the hemoglobin spectrum of animals irradiated with microwave. Formation of Met-hemoglobin is a direct evidence of excess free radicals production (Hanada et al., 1995) after irradiation. It also returned to the normal pattern by administrating of GSE before MW irradiation.

In conclusion, it may be stated that, grapes seed extraction controlled the excess production of free radicals produced by microwave irradiation which play a role in maintaining a balanced ratio of apoptotic to anti-apoptotic related proteins that in role maintain normal cell proliferation rate.

Declarations of Interest:

The author reports no conflicts of interest. The author alone is responsible for the content and writing of this paper.

REFERENCES


