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Protective Effects of Prunus Avium Extract on 10 Ghz Induced Damages in the Spleen of Swiss Albino Mice

KEYWORDS: Microwaves; spleen; antioxidants

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ABSTRACT *For the present study thirty male Swiss albino mice were selected. They were divided into three groups. Group I: Sham exposed, Group II: 10 GHz microwave exposed, Group III: PAE+MW exposed. After 30 days of treatment the animals were sacrificed to study the histological and biochemical changes in spleen. Microwave exposure resulted in significant reduction of spleen weights. Histopathological examination revealed normal architecture of spleen in sham exposed mice. Only microwave exposure resulted in the architectural disturbances of spleen with fibrosis of pulp, irregularly shaped sinusoids and giant cells. Supplementation of PAE before MW exposure resulted in less severe degree of changes. Biochemical analysis showed highly significant ($P \leq 0.01$) variations in LPO, GSH and protein levels which could be ameliorated by supplementation of PAE prior to MW exposure. Exposure to 10 GHz leads to histopathological and biochemical alterations in spleen of mice which can be ameliorated by PAE supplementation.*

INTRODUCTION

Microwave at X- band is widely used in communication systems for civil and military application devices such as aircraft, weather forecast system and various types of radars. The 10 GHz band is the easiest microwave bands to get on primarily because of its proximity to frequencies heavily used by different radars and the resulting equipment availability. This band has an advantage of giving a reasonable energy density in a waveguide of convenient size from a moderately powered microwave generator. As it happens, 10 GHz is in central region of the frequency range commonly considered hazardous, and this particular frequency penetrates tissues to a moderate depth, and an appreciable proportion of energy is absorbed (Lawrence, 1968). Its increased usage in occupational environment has caused potential threat to human health, resulting in growing public concern. This has attracted a great deal of attention (Ballardin et al. 2011, Belyaev et al. 2005, Jauchem 2008, Kumar et al. 2012, Shakhya et al. 2011). The effects of microwave radiations on biological systems are primarily identified as due to an increase in temperature i.e. thermal (Stuchley, 1988) though non thermal effects have also been identified (Paulraj and Behari, 2004). Enhancement of the presence of free radicals after electromagnetic field exposure have also be pointed (Yoshikawa et al. 2000). EMF can affect cells via different mechanisms. For instance, electromagnetic radiations effect cytoplasmic membrane which can cause a change in functional potential due to biochemical changes followed by a change in concentration of ions trafficking within the membrane (Berg, 1993). Interaction between EMF and chemical bonds could lead to formation of free radicals in the body of organisms (Rollwitz et al. 2004). Excessive production of free radicals specifically reactive oxygen species (ROS), have also been reported in wide variety of clinical disorders and environmental stress (Agarwal, 2005, Galli et al. 2005, Houten et al. 2006, Willcox et al. 2004). It is agreed that the basic mechanism for damage to body tissues involves free radicals. The interaction of MW in living systems have been reported to cause enzyme inactivation, cell damage, lipid per oxidation, DNA single stranded breaks and oxidative stress (Usikalu et al. 2010). Recently, increased interest has developed on search for potential drugs of plant origin which can quench the reactive energy of free radicals and eliminate oxygen, one of major participants in lipid per oxidation and modify radiation responses (radio protectors/sensitizers) with minimum side effects. It has been assumed that nutritional intervention to increase intake of antioxidants may reduce threat of free radicals. There are very few studies about the microwave (10 GHz) effects on hematopoietic system in the literature and all studies have been focused on the serum parameters like different enzymes. For instance Koyu et al. (2009) has demonstrated that when rats were exposed to EMF (900 MHz), activities of CAT, SOD, GSH-Px, XO were changed. On the other hand, there are data that show EMF exert their effect via formation of free radicals (Canseven et al. 2008). Earlier studies in our laboratory have shown that the fruits viz. Phalsa, Cherry having anthocyanin, carotenes, vitamin C etc. (antioxidants) possess the radio protective efficacy against gamma rays (Sisodia et al. 2009 a, b; Sharma and Sisodia, 2010; Sisodia et al. 2011). A pile of research has confirmed that non-ionizing communications radiation in the RF/microwave spectrum has the same effect on human health as ionizing gamma wave radiation from nuclear reactions. Therefore in this context the fruit of the Prunus avium (Cherry) particularly rich in anthocyanin and vitamin C has been selected to study its possible role against microwave radiations in spleen of male Swiss albino mice. Cherry commonly known as sweet cherry, wild bird cherry, mazzard cherry, gean, gilas, krusbal, which is cultivated species native to West Asia and is also found in Jammu and Kashmir, Himachal Pradesh, Uttaranchal and hills of Tamil Nadu (Gamble, 1957).

Prunus avium is rich in phenolic compounds, characterized by relatively high antioxidant activity, higher than e.g. oranges, apples or strawberries (Kayano et al. 2002). Presence of high anthocyanin content and phenolic compound with good antioxidative capacity (FRAP) of sweet cherry cultivars was reported by Vangdall and Stad (2006). According to Wang et al. (1997) anthocyanin content in sweet cherry is 350–450 mg/100 gm of fruit. According to USDA Nutrient Database for Standard Reference 1999, 100 grams of the edible portion of fruits of Prunus avium has protein 1.2 g, total lipid (fat) 0.96 g, carbohydrate 16.55 g, total dietary fiber 2.3 g,

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calcium 15 mg, Iron 0.39 mg, magnesium 11 mg, phosphorus 19 mg, potassium 224 mg, zinc 0.06 mg, copper 0.095 mg, manganese 0.092 mg, ascorbic acid 7 mg, thiamin 0.05 mg, riboflavin 0.06 mg, niacin 0.4 mg, pantothenic acid 0.127 mg, vitamin B6 0.036 mg, folate 4.2 mcg, vitamin A 214 IU, vitamin A 21 mcg RE, and vitamin E 0.130 mg ATE.

According to Pandey et al. (2008) tender stem of Prunus avium has ethnomedicinal values in heart diseases. The fruit stalks are astringent, diuretic, and tonic. A decoction is used in the treatment of cystitis, edema, bronchial complaints, looseness of the bowels, and anemia (Grieve, 1984; Rivera et al. 2005). Fruits of Prunus avium have digestive and antispasmodic uses (Rivera et al. 2005). A much more recent study reported some evidence that cherry consumption might lower levels of urate in the blood. Although no specific mention has been seen for this species, all members of the genus Prunus contain amygdalin and prunasin, substances that break down in water to form hydrocyanic acid (cyanide or prussic acid). In small amounts, this exceedingly poisonous compound stimulates respiration and improves digestion (Bown, 1995). So the objective of the present study is to investigate possible radioprotective efficacy of Prunus avium against 10 GHz induced damage in spleen of Swiss albino mice.

MATERIALS AND METHODS

Extract Preparation (Drug)

Fresh fruits of Prunus avium were washed, shade dried, and powdered after removal of seeds. Methanolic extract was then prepared by refluxing for 48 h .The extract thus obtained was vacuum evaporated so as to get it in powdered form. The extract was redissolved in double-distilled water (DDW) just before the oral administration. For the various concentrations, a known amount of PAE was suspended in DDW, and 50 µl of PAE suspension was given to each mice by oral gavage.

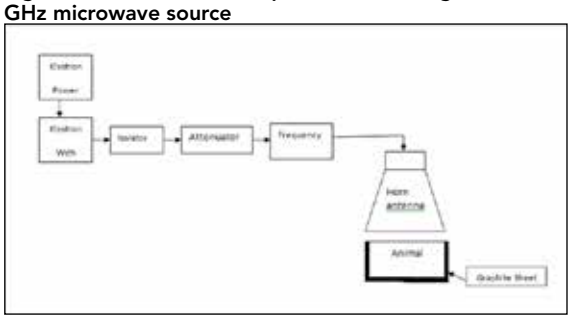
Animals

Adult male Swiss albino mice, 6-8 weeks old and weighing 25±2g, were used for the present study. These animals were maintained in the animal house as an inbred colony as per the norms established by Institutional Animal Ethical Committee (IAEC). The animals were housed in clean polypropylene cages and maintained under controlled conditions of temperature (25 ±1.5°C) and light (12L: 12D). They were maintained on standard normal diet obtained from Hindustan Lever, Delhi, India and water ad libitum.

Microwave radiation source

Mice were kept in a plexiglass cage and placed vertically below the pyramidal horn antenna aperture connected with klystron power supply. The transmitter operating in X-band (8-12 GHz) with incident power < 5 mW.

Figure 1. Sketch of the experimental arrangement of 10 GHz microwave source



Specific Absorption Rate (SAR)

The emitted power of microwaves was measured by a power meter which is a peak sensitive device (RF power sensors 6900 series and infra red (IFR) 6960 B RF power meter; made of Aeroflex Inc., Wichita, Kansas, USA). Every day the cage was placed in the same position below the horn antenna. A similar experiment was performed with sham exposed animals without energizing the system. The power density at the cage location was 0.25 mW/cm^2 and the SAR was calculated as 0.1790 W/Kg following the work of Durney et al. (1984).

Experimental design

Mice were divided into three groups, each group consisting of ten animals.

Group I: Sham exposed (Control)

Mice of this group which served as control were kept in a plexiglass cage and placed vertically below the horn antenna aperture without energizing the system for 2 hr/day for 30 consecutive days.

Group II: Microwaves exposed

Mice of this group were exposed with 10 GHz microwaves 10 GHz for 2 hrs/day for 30 consecutive days.

Group III: (PAE+MW exposed)

Mice of this group received 500mg/kg/b.wt. of Prunus avium extract (PAE) continuously once daily 1 hr before exposure to 10 GHz pulsed density (2hr/day) for 30 consecutive days. At the end of the experiment, body weights of the mice were recorded thereafter, the animals were sacrificed by cervical dislocation. For histopathological studies spleen tissue was quickly excised removed and weighed and then fixed for 12 hrs in Bouin's solution, processed for paraffin embedding method of Drury and Wallington, 1980. 5 µm sections of spleen stained with haematoxylin and eosin were observed microscopically using phase contrast microscope for histopathological changes. For biochemical studies homogenate of fresh spleen was made in saline and processed for further evaluation.

Biochemical assay in spleen

Lipid per oxidation (LPO) assay- LPO was measured by the method of Buege and Aust . (1978). A 10% tissue homogenate of the spleen (1 g) was prepared in 9 ml of 1.15% KCl. Tissue homogenate (0.8 ml) was mixed with 1.2 ml solution of TCA (15% w/v)– TBA (0.375% w/v)–HCl (0.25N) prepared in a 1:1:1 ratio. This final mixture was heated in a water bath for 30 min at 80°C and cooled. After centrifugation the absorbance was recorded at 532 nm using a UV–vis double beam spectrophotometer. A standard curve was prepared by using TMP. After comparison with a standard curve the LPO level was expressed in n mol g/tissue.

Glutathione (GSH) assay- The reduced GSH content of tissue samples was determined in spleen by the method of Moron et al. (1979). A tissue sample was homogenized in the sodium phosphate–EDTA buffer then 0.6ml DTNB was added. The optical density of the yellow colored complex developed by the reaction of GSH and DTNB was measured at 412 nm using a UV–vis spectrophotometer. The results were expressed as nmol GSH/100 mg of tissue.

Protein assay- Estimation of protein was based on the method proposed by (Bradford, 1976) 10% homogenate was prepared in NaCl and 0.1 ml of the sample was taken for the Bradford assay. Three repeats of the assay from each animal were carried out. The absorbance was read at 595 nm. The results were expressed as mg/ gm of tissue.

Statistical analysis

The values were expressed as mean ± SEM. Statistical analysis was performed using Student's 't' test.

Results

Biochemical studies

In the present study the effects of 10 GHz on spleen were investigated and possible inhibitory effects of PAE were assessed. Analysis of LPO levels by thiobarbituric acid reaction showed a highly significant ($P \leq 0.01$) increase in the MW

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exposed mice (Group II) compared to sham exposed mice (Group I). Supplementation of PAE prior to MW exposure (Group III) significantly ($P \leq 0.01$) inhibited the enhanced LPO level due to exposure. Exposure to MW resulted in significant ($P \leq 0.01$) decrease in the activities of total protein and GSH in spleen tissue compared with sham exposed group. The supplementation of PAE prior to MW exposure (Group III) resulted in significant increase ($P \leq 0.01$) in the level of total protein and GSH compared to MW exposed mice (Group II).

Table 1. Variations in the different biochemical parameters in the spleen of mice after 30 days of MW exposure in the presence or absence of Prunus avium extract (PAE). (±SEM).

Groups / Parameters	Sham N=10 (Group I)	MW exposed N=10 (Group II)	PAE+MW exposed (500 mg/kg b.wt) N=10 (Group III)
Total protein (mg/ gm)	129.8±0.01	101.43±0.02 ^{a***}	124.26±0.02^{b***}
LPO (nano mole/ gm of protein)	329.16±0.02	338.23±0.02^{a***}	334.51±0.06^{b***}
GSH (nano mole /100gm)	36.71±0.05	28.49±0.07^{a***}	30.41±0.04^{b***}

All values are expressed as mean ± SEM. a=Sham Vs MW exposed, b=MW exposed Vs PAE+MW exposed. Significance levels *= Highly significant differences at P ≤ 0.001,**
****= Significant differences at p≤0.05,**
***= Non significant**

Histopathological studies

Histopathological examination revealed normal architecture of the spleen tissue in the mice of sham exposed group. Boundaries of red pulp and white pulp were clear, trabeculae was clearly defined, and small arterioles and rich lymphocytes were noted in central white pulp of germinal center (Fig 3). In MW exposed mice congestion and changes in the architecture of spleen were noted i.e., trabeculae was not clearly defined, depletion of white pulp with shrunken germinal centers (Fig. 4). Fibrosis of pulp with enlarged, irregularly shaped sinusoids and giant cells could also be seen spleen (Fig. 6). Supplementation of PAE before MW exposure (Group III) resulted in mild degenerative changes in spleen architecture which included reduction of white pulp with shrunken germinal centers. The reduction noted was less severe than in the MW exposed group (Fig. 5). Giant cells could also be seen in spleen of PAE+ MW exposed mice but they were less in number compared to sham exposed mice. Similarly, congestion and fibrosis noted (Fig 7) were less severe compared to MW exposed mice. This indicates that PAE has radio protective effect to an extent against MW induced damage in spleen of Swiss albino mice.

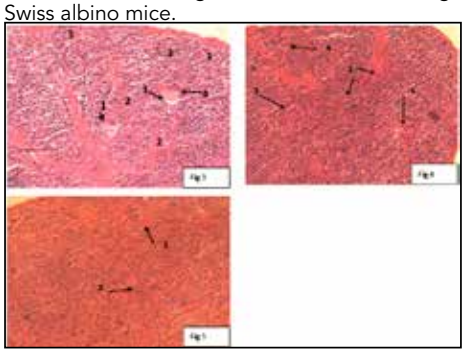
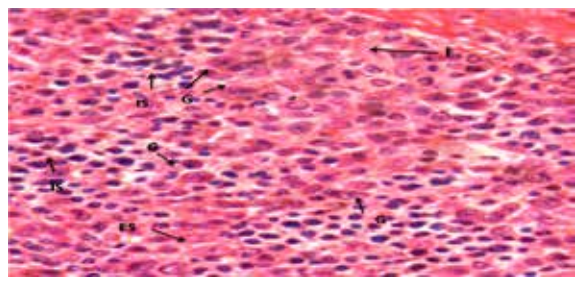


Figure 3: Photomicrograph showing a sectional view of sham exposed mice spleen showing white pulp (1), clearly defined trabeculae (2), red pulp (3) and germinal centre (4). (100 X).
Figure 4: Photomicrograph showing a sectional view of MW exposed mice spleen showing depletion of white pulp (1), less clear trabeculae (2) , congestion in red pulp (3) and shrunken germinal centre (4). (100 X).
Figure 5: Photomicrograph showing a sectional view of PAE+MW exposed mice spleen showing Shrunken germinal center (1), reduction in white pulp (2) . (100 X).



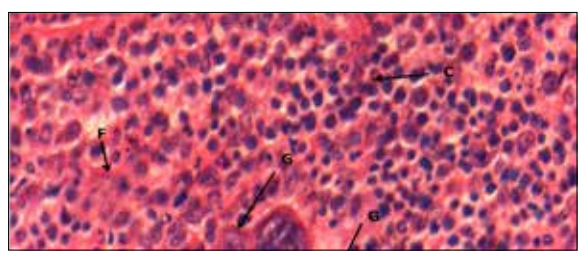


Figure 6: Photomicrograph showing a sectional view of MW exposed mice spleen showing fibrosis (F), giant cells (G), irregularly shaped sinusoid (IS), enlarged sinusoids (ES). (400X).
Figure 7: Photomicrograph showing a sectional view of PAE+MW exposed mice spleen showing mild fibrosis (F), occasional giant cells (G) , congestion in pulp (C) . (400X).

Spleen weights

The spleen weights of MW exposed and PAE+MW exposed group were found to be significantly decreased ($P \leq 0.001$) compared to sham exposed. Whereas, statistically non significant difference was noted between MW exposed group and PAE+MW exposed group.

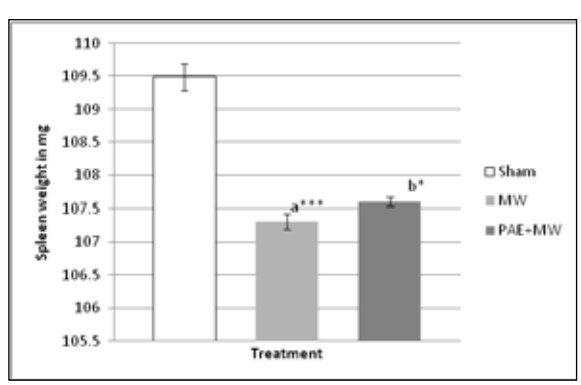


Fig 8. Variations in the spleen weights of mice after 30 days of MW exposure in the presence or absence of Prunus avium extract (PAE) (±SEM).

a=Sham Vs MW exposed, b=MW exposed Vs PAE+MW exposed.

Significance levels *** P ≤ 0.001, ** p≤0.05, *= Non significant

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Discussion

In spite of the enormous studies on the effect of EMF on living organisms studies on the effects of MW on immune system are limited on lymphatic organs. In the present study, the effect of MW (10 GHz) exposure on one of the main lymphatic organ (spleen) and radio protective role of PAE was investigated. In the present study it was observed that spleen lesions were prominent and more severe in MW exposed mice compared to PAE+ MW exposed mice. The results are in line with earlier studies of Attia and Yehia (2002) and Mevissen et al. (1998). In the present study MW exposure caused a significant elevation in lipid per oxidation in mice spleen. Lipid per oxidation not only damages cell membranes, but its products such as malondialdehyde also induce damage to other enzyme systems and DNA as well (Noda et al. 1993). Lipid peroxidation has been reported to be directly proportional to oxidative stress where the efficacy of various defense mechanisms is weakened. The defense mechanism may be strengthened by the addition of exogenous substance. In the present study, it was observed that PAE supplementation prior to MW exposure significantly altered the LPO in control mice. PAE treatment significantly lowered the radiation-induced LPO in terms of malondialdehyde. Kumar et al. (2012) also reported increase in malondialdehyde (MDA) in sperms of microwaves exposed male Wistar rats. The inhibition of LPO in biomembranes can be caused by antioxidants. Earlier studies also showed that whole-body ionizing radiations significantly increased LPO contents of mice spleen (Manda and Bhatia, 2003) and in blood serum which can be modulated by supplementation of exogenous substances (Singh et al. 2007; Sisodia et al. 2008).

The decrease in protein level of the spleen observed in MW exposed mice, may be probably due to lysis or inhibition of protein synthesis, or may be due to depression of enzymes involved in the activation of amino acid and transfer of tRNA or by the inhibition of release of synthesized polypeptides from polysomes (Samanta et al. 2004). This could be due to excessive damage to the genetic machinery. Increased protein concentration in the present study after PAE supplementation may be due to improved ribosomal activities, which enhance protein synthesis.

The depletion in GSH content after exposure to MW noted in the present study in spleen may be due to the reaction of GSH with free radicals resulting in the formation of thiol radicals that associate to produce glutathione disulfide (GSSG) (Navarro et al. 1997). Moreover, the availability of GSH can also be limited by deficiency in synthesis, enhanced efflux, or inefficient reduction of GSSG (Jones, 1985). In the normal condition, the cells are intact and healthy and GSH is restored by synthesis (Meister and Anderson, 1983) but in the MW exposed mice, normal synthesis and/or repair is disrupted due to damage to DNA and membranes. Flavonoids are known to activate glutathione-synthesizing enzyme (Myhrstad, 2002). It was also proved that oral intake of anthocyanin increases total blood GSH and DNA fragmentation in mice and rats (Weisl et al. 2006). There is evidence that some flavonoids can elevate the intracellular basal level of GSH, allowing better tolerance of free radicals (Cipaket et al. 2003; Durgo et al. 2007). The increased GSH levels suggest that protection by PAE may be mediated through the modulation of cellular antioxidant levels. GSH is a versatile protector, and executes its radio protective functions through free radical scavenging, restoration of the damaged molecules by hydrogen donation, reduction of peroxide, and maintenance of protein thiols in the reduced state (Bump et al. 1990).

Various mechanisms can explain the effect of EMF on organ function and induce cellular changes that the electromagnetic field might amplify electric currents in tissues and cells or affect these currents through resonance with local field focus (Sagan, 1992).

It was believed that EMF, by producing free radicals, could attack organic bases in the nucleotides and break hydrogen bonds between purines and pyrimidine bases that would result in chromosomal breakage, point mutation and changes in nucleotide sequences. Various studies indicated that supplementation of vitamin E and C prior and after EMF exposure has a protective effect in EMF induced damage in spleen blood and bodyweights of mice (Mohammadnejad et al. 2010; Aziz et al. 2010). Similarly Sokolovic et al. (2008) studied the protective effects of melatonin against EMF induced oxidative damage in mice brain.

The changes observed may be due to the fact that RF EMF exposure is capable of raising the temperature of a body which will in turn results in the formation of free radicals. These free radicals are capable of attacking ions in the body thereby changing their nature and breaking the protein bonds, i.e., causing cells damage. The cells degeneration observed in this experiment is similar to the findings of (Zare et al. 2007 and Usman et al. 2012) where it is shown that histopathological studies of low frequency EMF affect liver, testis and kidney of guinea pig. The findings of (Kristic et al. 2005) corroborated our results where it was reported that increased level of lipid per oxidation and protein oxidative modification as a result of exposure of mice to GSM frequency leads to significant disorders of function and structure of brain and liver cell in mice.

Flavonoids and other phenolic compounds of plant origin have been reported as scavengers and inhibitors of lipid peroxidation (Rice-Evans et al. 1996). Shimoi et al. (1996) concluded that plant flavonoids, which also show antioxidant activity in vitro, function as antioxidants in vivo, and their radio protective effects might be attributed to their radical scavenging activity. The mechanism of action of herbal drugs and their extract preparations differ in many respects from those of synthetic drugs or single substances (Wagner, 1999). The exact mechanism by which PAE protects against radiation-induced damage is not fully understood. The radio protective potential of PAE may be due to the free radical scavenging power of the antioxidants, anthocyanin, vitamins, minerals, etc present in it. Therefore, free radical scavenging seems to be one of the important radio protective mechanisms of PAE. Delgado-Vargas et al. (2000) demonstrated that anthocyanins have scavenging properties against OH and O^2, and are better agents against lipid per oxidation than α-tocopherol (up to seven times). Mechanisms of anti oxidative action of vitamin C are direct scavenging and blocking of ROS, as well as regeneration of other anti oxidative systems (Griffi and Lunec, 2001). Protective effects of vitamin C against ionizing radiation DNA damage have also been widely documented (Konopacka et al. 1998). The positive effects of anthocyanin pigments could be related to their potent antioxidant activity demonstrated in various in vitro and in vivo studies (Wang et al. 1999; Tsuda et al. 1994; Ramirez-Tortosa et al. 2001; Matsumoto et al. 2002). In the present study, we have noted that supplementation with PAE before MW exposure reduced the damages induced by MW exposure. Therefore, the modulatory action of Prunus avium against MW induced biochemical and histopathological alterations may be due to synergistic action of various antioxidants, minerals, vitamins, etc., present in the fruit.

Conclusion

From the findings of present investigation it can be concluded that MW exposure induce damage in spleen of Swiss albino mice in the form of biochemical and histopathological alterations. However, most of these damages showed signs of improvements with PAE supplementation compared to microwave exposure alone.

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