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MODULATION OF 10 GHZ MICROWAVES INDUCED BIOCHEMICAL CHANGES IN DIFFERENT ORGANS OF SWISS ALBINO MICE BY *PRUNUS DOMESTICA* FRUIT EXTRACT

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ABSTRACT: For the present study thirty male Swiss albino mice were selected from an inbred colony. They were divided into three groups consisting of ten mice in each group. Group I: Sham exposed, Group II: 10 GHz MW (microwave) exposed, Group III: PDE (*Prunus domestica* extract) +MW exposed. After 30 days of treatment the animals were sacrificed to study alterations in body weights and biochemical parameters in different organs of Swiss albino mice viz. intestine, liver, testis and spleen. MW exposure did not resulted in significant reduction in body weights compared to sham exposed group of mice. PDE supplementation prior MW exposure did not result in any significant weight gain compared to MW exposed group. Biochemical analysis showed highly significant ($p<0.001$) variations in Lipid Per Oxidation (LPO), Glutathione (GSH) and protein levels which could be ameliorated by supplementation of PDE prior to MW exposure. Exposure to 10 GHz leads to biochemical alterations in different organs studied in mice which can be ameliorated by PDE supplementation. It can be concluded from the current study that exposure to microwave radiation caused significant alterations in the LPO, GSH and protein content of different organs of Swiss albino mice and supplementation of PDE prior microwave exposure repaired the damage to an extent.

INTRODUCTION: Many forms of "radiation" are encountered in the natural environment and are produced by modern technology. Most of them have the potential for both beneficial and harmful effects. Based on new studies, there is growing evidence among scientists and the public about possible health risks associated with these technologies.

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Use of wireless communicating devices is increasing at an exponential rate in present time and is raising serious concerns about possible adverse effects of microwave (MW) radiation emitted from these devices on human health.

Microwaves are a specific category of radio waves that can be defined as radiofrequency radiation where frequencies range upward from several hundred megahertz (MHz) to several gigahertz (GHz). The Microwave X-band lies in frequency ranging from 8-10 GHz and 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. Its increased usage in occupational environment has caused potential threat to human health, resulting in growing public concern [1]. This has attracted a great deal of attention.

The effect of microwave radiations on biological systems is primarily identified as due to an increase in temperature i.e. thermal though non thermal effects have also been identified [2]. There are several reports indicating that electromagnetic fields, such as those originating from power distribution systems, house hold electrical wiring, medical devices, cellular phones and wireless communication produce a variety of biological effects [3]. These studies have generally used rodents. Experiments have pointed enhancement of the presence of free radicals after electromagnetic field exposure [4]. Excessive production of free radicals specifically reactive oxygen species (ROS), have also been reported in wide variety of clinical disorders and environmental stress [5]. The balance between production and neutralization of ROS levels can increase dramatically, which may cause damage to cell structures leading to behavioral, histopathological, biochemical alterations.

What is a person to do about these symptoms? Right now it looks like the best defense against radiation poisoning is the same as the best defense against all. This defense begins with diet and supplements. Eating a diet high in apples, citrus fruits, cruciferous vegetables, drinking red wine, and using fresh rosemary have been scientifically shown to be effective. 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 [6]. Supplementation of Vitamin E and C known antioxidants can reduce the effects of damage produced by microwaves [7].

Lot of research has confirmed that non-ionizing communications radiation in the radio frequency (RF)/microwave spectrum has the same effect on human health as ionizing gamma wave radiation from nuclear reactions. In this context, Plums (Family Rosaceae) commonly known as Alu bukhara, used as a traditional medicinal food in humans to enhance immunity against infectious agents, has been used for exploring its anti radiation effects. They are fruit rich in phenolic compounds, characterized by relatively high antioxidant activity, higher than e.g. oranges, apples or strawberries [8]. Pre and post treatment with PDE significantly ameliorated the endogenous protein in brain and improved spatial learning [9]. Therefore, in the present study effects of 10 GHz and possible ameliorative role by PDE were assessed through biochemical estimations of protein, lipid per oxidation (LPO) and glutathione (GSH) in selected organs viz. testes, intestine and liver and spleen of Swiss albino mice.

MATERIALS AND METHODS:

Experimental animals: Adult male Swiss albino mice, 6-8 weeks old and weighing 25±2 grams were used for the present study. Initially the mice were procured from Central Drug Research Institute (CDRI), Lucknow, India and 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 ±1.5ºC) (12 hours Light: 12 hours Dark). They were maintained on standard normal diet obtained from Hindustan Lever, Delhi, India and water *ad libitum*.

10 GHz exposure system, exposure conditions and dosimetry: Mice were divided into three groups consisting of ten mice in each group. Two mice were housed at a time in a rectangular partitioned cage made of plexi glass which was well ventilated with holes of 1 centimeter (cm) diameter. The dimensions of the cage (4.5×9×9cm) were such that animals were comfortably placed, though they could not move. The horn antenna was kept in H (Magnetic field) plane configuration. Therefore electric field was perpendicular to the ground surface. Field was almost uniform because the dimension of the cage is of the order of wavelength. At near field distance from the horn antenna, it was found that the power density measured was 0.25 mW/cm^2 (milliwatt per centimeter square) which was maximum. Every day, the cage with mice was placed in the same position facing the horn antenna. The mice were

exposed with 10 GHz MW radiation source through the antenna for 2 hours/ day for 30 days as shown in **Fig.1**. The whole microwave exposure system was procured from Wavetech, Faridabad, Haryana, India.

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 in front of horn antenna. A similar experiment was performed with sham exposed animals without energizing the system. The power density at the cage location was 0.25mW/cm^2 and the SAR was calculated as 0.1790 W/Kg (watt per kilogram) following the work of Durney [10].

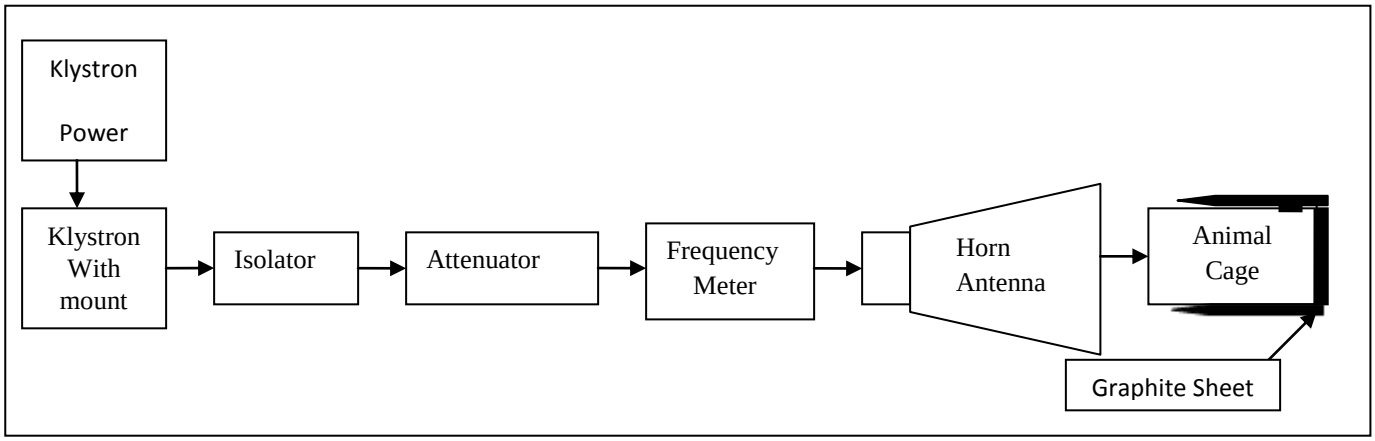

FIG.1: DIAGRAMMATIC VIEW OF 10 GHZ MICROWAVE EXPOSURE SETUP

Plant material and extraction procedure:
Fresh fruits of *Prunus domestica* were washed, shade dried, and powdered after removal of seeds. Methanolic extract was then prepared by refluxing for 48 hours (4 x 12) at 40°C. 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 *Prunus domestica* extract (PDE) was dissolved in DDW. The mice were given 500 mg/kg body weight of PDE by oral gavage.

Selection of optimum dose of *Prunus domestica* extract against 10 GHz pulsed density microwaves: Dose selection of *Prunus domestica* was carried out on the basis of a drug tolerance study. Various doses of *Prunus domestica* 100, 200, 400, 500, 700, 1000, and 1200 mg/kg b.wt. were tested against 10 GHz MW radiation . Thereafter, 500 mg/kg/day was selected as the optimum dose based on LPO and GSH estimation in liver of mice. This dose was used for 30 days for therapeutic experimentation with 10 GHz MW exposure. Optimum dose of *Prunus domestica* extract was calculated as 500 mg/kg b. wt. from the data interpreted from (**Fig.2**) which leads to maximum possible decrease in LPO levels along with maximum possible increase in GSH levels in liver of Swiss albino mice exposed to 10 GHz microwave.

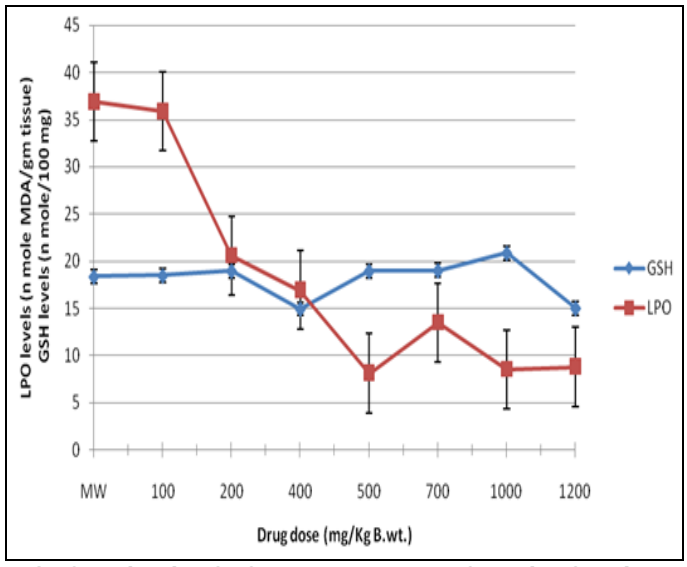

FIG. 2: VARIATIONS IN LIPID PER OXIDATION AND GLUTATHIONE LEVELS IN LIVER OF SWISS ALBINO MICE EXPOSED TO MICROWAVES ADMINISTERED WITH DIFFERENT CONCENTRATIONS OF *PRUNUS DOMESTICA* EXTRACT

Experimental design:
Mice were dived into three groups:

Group I: Sham exposed (Control): Mice of this group which served as control were kept in a plexiglass cage and placed symmetrically along the pyramidal horn antenna aperture connected with klystron power supply without energizing the system for 2 hours/day for 30 consecutive days.

Group II: Microwaves exposed: Mice of this group were exposed with microwaves 10 GHz for 2 hours/day for 30 consecutive days.

Group III: PDE treated +MW exposed: Mice of this group received 500mg/kg/b.wt. of *Prunus domestica* extract (PDE) once daily 1 hr before exposure to 10 GHz pulsed density (2hr/day) for 30 consecutive days.

At the end of the experiment, the animals were weighed for observing changes in body weights if any and sacrificed by cervical dislocation. For biochemical studies liver, intestine, testes and spleen tissues were quickly excised and removed; homogenate of fresh tissues was made in saline and processed for further evaluation.

Biochemical assay:
LPO assay: MDA (Malondialdehyde) levels were estimated by the double heating method of Draper and Hadley [11]. The principle of the method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA. For this purpose, 2.5 (mililitre) mL of 100 g L-1 trichloroacetic acid (Sigma-aldrich, St. Louis, MO, USA) solution was added to 0.5 millli liter (ml) supernatant in each centrifuge tube and the tubes were placed in a boiling water bath for 15 minutes. After cooling in tap water, the tubes were centrifuged at 1000 g for 10 minutes and 2 ml of the supernatant was added to 1 ml of 6.7 g L-1 TBA solution in a test tube and the tube was placed in a boiling water bath for 15 minutes.

The solution was then cooled in tap water and its absorbance was measured using a spectro photometer (Double Beam Spectrophotometer 2203, Systronics, India) at 532 nano meter (nm). The concentration of MDA was calculated by the absorbance coefficient of the MDA–TBA complex (absorbance co-efficient = 1.56 × 105 cm-1 M-1) and is expressed as nano moles per gram units (nM g-1) wet tissue.

GSH assay: The reduced GSH content of tissue samples was determined by the method of Moron et al [12]. Tissue samples from intestine, liver, testes and spleen were homogenized in the sodium phosphate– ethylenediaminetetraacetic acid (EDTA) buffer then 0.6ml Beta dystrobrevin (DTNB) (Sigma-aldrich, St. Louis, MO, USA) 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 ultra violet (UV)–visible spectrophotometer. The results were expressed as n mol GSH/100 mg of tissue.

Protein assay:
Estimation of protein was based on the method proposed by Bradford [13]. The procedure is based on interaction of dye, coomassie brilliant blue, with proteins. The unbound dye has an absorbance maximum at 465 nm. However on interaction with protein the dye turns blue and its absorbance maxima is displaced to 595 nm. Thus, from the absorbance at 595 nm the amount of protein in a sample solution was estimated in mg/gm (milligram/ gram) tissue. 10% homogenate of each excised tissue was prepared in 0.85 molar (M) NaCl (Himedia, Mumbai, Maharashtra, India) solution and 0.1 ml of the sample was taken for the Bradford assay.

The volume in the test tube was adjusted to 1 ml with phosphate buffer (pH 7.4) (Sigma-aldrich, St. Louis, MO, USA). Five milliliters of Bradford reagent (Sigma-aldrich, St. Louis, MO, USA) was added to the test tube and the contents were mixed by vortexing. The absorbance at 595 nm was measured after 2 minutes in 3 ml cuvettes against a reagent blank prepared from 0.1 ml of the phosphate buffer (pH 7.4) and 5 ml of protein reagent by using spectrophotometer. Three repeats of the assay from each animal were carried out. The weight of protein was plotted against the corresponding absorbance resulting in a standard curve used to determine the protein in unknown samples.

Statistical Analysis: Data were analyzed using one-way ANOVA (Analysis of variance) with Bonferroni's multiple comparison tests.

RESULTS:

Body weights: The body weights were recorded at the beginning and at the end of the experiment in all the three groups. The average body weight of experimental animals was 25±2 grams at the start of experiment. It is obvious that animals showed a progressive increase in body weight with the lapse of time. The animals in the experimental group exposed to microwave radiations (Group II) showed less (0.07%) weight gain compared to sham exposed group (4.05%) (**Group I**). PDE supplementation prior MW exposure (Group III) however, did not result in any significant weight gain (0.42%) compared to MW exposed (**Table 1**).

TABLE 1: VARIATIONS IN THE BODY WEIGHTS OF MICE AFTER 30 DAYS OF 10 GHz MW EXPOSURE IN THE PRESENCE OR ABSENCE OF *PRUNUS DOMESTICA* FRUIT EXTRACT

Groups	Body weights (grams)	
	At the beginning of the experiment	After completion of the experiment
Sham (Group I)	25.18±0.09	26.2±0.01 (4.05 %)
MW (Group II)	25.83±0.07	25.85±0.01 (0.07 %)
PDE+MW (Group III)	25.71±0.15	25.82±0.02 (0.42 %)

Values in the parentheses indicate percent increase in body weights of mice after the end of experiment compared to the body weights of mice at the beginning of experiment.

LPO: Results showed that LPO levels increased significantly ($p<0.001$) after MW exposure (group III) in all the tissues viz intestine, liver, testes and spleen compared to sham exposed (group I). PDE administration prior to microwave exposure could significantly ($p<0.001$) reduce LPO in all the tissues studied (**Table 2**).

TABLE 2: VARIATIONS IN THE LPO CONTENT (NANO MOLE MDA/ml OF PROTEIN) IN THE INTESTINE, LIVER, TESTIS AND SPLEEN OF MICE AFTER 30 DAYS OF MW EXPOSURE IN THE PRESENCE OR ABSENCE OF *PRUNUS DOMESTICA* FRUIT EXTRACT

Groups	Intestine	Liver	Testis	Spleen
Sham (Group I)	27.8±0.01	345.8±0.01	97.81±0.01	329.16±0.02
MW (Group II)	55.43±0.02**	384.43±0.02**	171.43±0.02**	338.23±0.02**
PDE+MW (Group III)	44.6±0.08**	368.6±0.08**	156.6±0.08**	334.51±0.06**

Each value represents Mean ± SEM (n=10)
Statistical comparison: Sham Vs MW, MW Vs PDE +MW, **- $p<0.001$- highly significant

GSH: MW exposure resulted in highly significant depletion ($p<0.001$) of GSH levels in intestine, liver testis and spleen of mice compared to sham exposed (group I). PDE supplementation prior MW exposure (group III) augmented the levels of GSH up to a large extent in liver, intestine, testes and spleen respectively (**Table 3**).

TABLE 3: VARIATIONS IN THE GSH CONTENT (NANO MOLE/ 100gm) IN THE INTESTINE, LIVER, TESTIS AND SPLEEN OF MICE AFTER 30 DAYS OF MW EXPOSURE IN THE PRESENCE OR ABSENCE OF *PRUNUS DOMESTICA* FRUIT EXTRACT

Groups	Intestine	Liver	Testis	Spleen
Sham (Group I)	22.8±0.01	55.8±0.05	18.76±0.03	36.71±0.05
MW (Group II)	13.6±0.09**	41.43±0.02**	14.38±0.02**	28.49±0.07**
PDE+MW(Group III)	17.43±0.02**	44.9±0.16**	17.8±0.08**	30.41±0.01**

Each value represents Mean ± SEM (n=10)
Statistical comparison: Sham Vs MW, MW Vs PDE +MW, **- $p<0.001$- highly significant

Protein: Microwave exposure resulted in significant depletion ($p<0.001$) of protein content in liver, intestine and testis and spleen compared to sham exposed (group I). Significant increase ($p<0.001$) in the levels of protein was observed in all the tissues studied with PDE supplementation. Elevated levels of protein were observed in mice of PDE supplemented group (group III) (**Table 4**).

TABLE 4: VARIATIONS IN THE PROTEIN CONTENT (mg/gm) IN THE INTESTINE, LIVER, TESTIS AND SPLEEN OF MICE AFTER 30 DAYS OF MW EXPOSURE IN THE PRESENCE OR ABSENCE OF *PRUNUS DOMESTICA* FRUIT EXTRACT

Groups	Intestine	Liver	Testis	Spleen
Sham (Group I)	88.51±0.01	142.51±0.15	106.51±0.10	129.8±0.01
MW (Group II)	76.43±0.02**	107.43±0.02**	82.43±0.02**	101.43±0.02**
PDE+MW (Group III)	81.6±0.08**	118.6±0.08**	87.6±0.08**	124.26±0.02**

Each value represents Mean ± SEM (n=10)
Statistical comparison: Sham Vs MW, MW Vs PDE +MW, **- p<0.001- highly significant

DISCUSSION: The potential of EMF adversely affecting the health of the human population is an issue which continues to receive a great deal of attention in both public and scientific forums. There is growing evidence that the effects of microwave irradiation are mediated by the formation of ROS and free radicals, which are highly reactive, removing hydrogen atoms from fatty acids, causing lipid per oxidation and consequently cell death[14].

In our experiment, the non significant change in the body weights (**Table 1**) noted following microwave exposure is in agreement with other studies. Rats exposed to EMF at an intensity of 6.25 T for 8 hrs/day, body weights of experimental and control groups showed no difference after three months [15]. Adult male rats were treated by 50 Hz sinusoidal magnetic field (250 mg) for 18 consecutive weeks no significant changes were recorded on the absolute body weight of the exposed rats [16]. 60 Hz EMF exposure did not decrease the body of mice [17]. However, there are studies which report change in the body weights after microwave exposure [18]. Reported body weight changes when rats were exposed for long term to electromagnetic field with a well defined frequency [19].

Exposed mice to electromagnetic field by placing 40 cm away from the cathode ray technology based video display unit (computer monitor) for 10-12hrs/day for four weeks. Exposed mice had less gain in body weight compared to control mice after three weeks of exposure [20]. Reported decrease in the body weight by second week of treatment with SMF. Various studies have 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 body weights of mice [21, 22]. Significant decrease was noted in body weights of mice exposed to 50 Hz compared to control [23].

The LPO is a good biomarker of damage occurring due to radiation and inhibition of LPO is suggestive of radio protective action. Significant acceleration in the oxidation of lipids associated with depletion in antioxidant enzymes levels due to 10 GHz microwave exposure were noticed in our study (**Table 2**). Lipid per oxidation not only damages cell membranes, but its products such as MDA also induce damage to other enzyme systems and DNA as well [24]. Lipid per oxidation 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. PDE treatment significantly lowered the radiation-induced LPO in terms of MDA [25]. Also reported increase in MDA in sperms of microwaves exposed male wistar rats.

The inhibition of LPO in bio membranes can be caused by antioxidants. Earlier studies also showed that exposure to 10 GHz MW significantly increased LPO contents of mice spleen [26] and in blood serum which can be modulated by supplementation of exogenous substances [27]. Increase in LPO after MW exposure in the present study gets support from the findings of [28]. Glutathione is an important non-enzymatic antioxidant which plays a critical role in cellular defense system against toxic chemicals of exogenous and endogenous origin. Depletion of cellular GSH increases cell vulnerability to oxidative stress [29].

In the present study the decrease in the activities of antioxidant enzyme GSH in liver intestine and testis following microwave exposure (**Table 3)** noticed may be due to the damaging effect of free radicals produced following radiation exposure or alternatively could be a direct effect of formaldehyde formed from oxidation of free radicals, on these enzymes. GSH acts as a free radical scavenger and regenerator of alpha

tocopherol and plays a significant role in sustaining protein sulfhydryl groups [30].

Decreased hepatic GSH contents result in increased susceptibility to hepatic injury via induction of lipid per oxidation [31]. GSH is the main antioxidant found in liver cells and plays a protective role in the metabolism of a large number of toxic agents, including oxidative stress. Enhanced radiation toxicity has been associated with decreased hepatic/intestinal GSH, which may reflect the depletion of GSH by the overproduction of ROS and subsequent oxidative stress caused by radiation. ROS is kept at physiologically low levels by intracellular free radical scavenger. Our results showed that PDE supplementation prior to MW exposure significantly inhibited the radiation induced depletion of GSH. The decrease in GSH activity correlates with the increase in lipid per oxidation. This may account for the increased levels of oxidized lipids in the serum lipoproteins of irradiated mice following consumption of a diet rich in oxidized lipids [32], since the intestinal/hepatic GSH detoxifies dietary lipids before they enter the circulation [33, 34].

Reported that exposure to 2450 MHz, 0.25mW/cm^2 continuous waves MW induces significant decrease in antioxidant enzymes activities of GSH-Px, superoxide dismutase (SOD), catalase (CAT) compared to control group [35]. Also reported decreased LPO and GSH content in testis and epididymis of rats exposed to 0.9/1.8 GHz [36]. Also reported decreased GSH content in Liver, heart, kidney and plasma of rats exposed to extremely low frequency (ELF)-EMF (60 Hertz). An enhanced production of ROS after combined exposure to RF radiation (930 MHz, SAR 1.5 Wkg^{-1}) and iron ions was also reported in an experimental model of rat lymphocytes and induced lipid per oxidation, accompanied by decreased activity of superoxide dismutase (SOD), myelo per oxidase (MPO) and glutathione peroxidase (GSH-Px) by RF exposure has been reported in various organs, such as rat kidney and guinea pigs liver. Moreover, in the latter animal model, treatment with epigallocatechin-gallate, the main active component of green tea, and N-acetyl cysteine, a glutathione (GSH) precursor, provided protection against oxidative stress-induced liver injury caused by RF-EMFs [37].

The decrease in protein level observed in MW exposed mice (**Table 4**), 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 [38].

Increased protein concentration in the present study after PDE supplementation may be due to improved ribosomal activities, which enhance protein synthesis. Decrease in the protein content after exposure to radiation has been reported by [39] which might be due to either decline in the rate of protein synthesis or an increase in the protein consumption. The reduction in the protein biosynthesis could be attributed to any of the following factors: (a) activation of RNAase (b) depletion of mRNA or effect on the formation and/or maturation of RNAase. Radiation may also include local defects in the microstructure of protein molecules, which becomes the centre of thermal denaturation and cross linkage, thus causing tissue damage [40]. Reduction in rate of protein synthesis may be due to unfavorable conditions like unavailability of one or more essential enzymes and/or reduction in the sites of protein synthesis. Some studies have indicated that oxidative stress diminishes and the levels of some proteins vary during the progression of Alzheimer's disease [41, 42]. Reported a decrease in the content of the cytoskeletal protein tubulin in endothelial cells exposed to multiple pulses of high power microwaves (HPM). Our results are in line with [43] who also reported decrease in protein content of brain after 10 GHz MW exposure.

CONCLUSION: In conclusion, this study demonstrates that oral pre-supplementation with PDE (500 mg/kg b. wt) reduces the effect of biochemical alterations induced by 10 GHz MW exposure at a statistical significant level. This study suggests that the natural antioxidants offer protection against MW induced hepatic/ intestinal/reproductive damages in albino mice.

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