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ORIGINAL ARTICLE

Treatment Effects of Ocimum Basilicum on Kidney Cells Apoptosis Produced by Exposure to Electromagnetic Field (EMF) in Rats

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ABSTRACT

Background: Anelectromagnetic field (alsoEMForEM field) is a physical field produced byelectrically charged objects. It affects the behavior of charged objects in the vicinity of the field. Objective:Medicinal use of ocimum basilicum dates back to ancient Iran,China and India. It has been used since ancient time as medicinal and food origin as an antioxidant's ocimum basilicum has a protective effect on kidney tissue injury and apoptosis to causality of emf harmful effects. Materials and Methods:Wistar male rat (n=40) were allocated into four groups, control (n=10) and test groups (n=30), that subdivided into groups of 3, the extract group were received of ocimum basilicum extract (1.5g/kg body),second extract group were received of ocimum basilicum extract (1.5g/kg body) and emf group that exposed to50 *Hz* for 40 consequence day. Animals were kept in standard conditions. In end of study the kidney tissue of Rats in whole groups were removed and and prepared for pathology analysis.Results:Apoptotic cells significantly decreased in groups that have received ocimum basilicum extract ($P<0.05$) in comparison to experimental groups ($P<0.05$).Conclusion*:* Results revealed that administration of 1.5g/kg body of ocimum basilicum extract significantly decreased cell injury and apoptosis and preventive effect in kidney tissue damages.

Key words: Apoptosis, Emf, Ocimum basilicum, Kidney, Rat.

Introduction

The antioxidant capacity of phenolic compounds, flavonoids and food containing them has been repeatedly shown in various in vitro and in vivo systems [1]. Ocimum basilicum (Basil) is an annual herb of the Lamiacae family widely cultivated in Asia as a nourishing food and herbal medicine. It is widely used in folk medicine to treat wide range of diseases. For example the aerial part of O. basilicum is traditionally used as antispasmodic, aromatic, digestive, carminative, stomachic and tonic agents. They are also used externally for treatment acne, insect stings, snake bites and skin infections [20].

Anelectromagnetic field(alsoEMForEM field) is a physicalfieldproduced byelectrically charged objects. It affects the behavior of charged objects in the vicinity of the field. The electromagnetic field extends indefinitely throughout space and describes theelectromagnetic interaction [18,4]. It is one of the four fundamentalof nature (the others aregravitation, theweak interaction, and thestrong interaction).The field can be viewed as the combination of anelectric fieldand amagnetic field.

With the increased use of power lines and modern electrical devices concern about the public health hazards of chronic exposure to EMF has gained more attention.

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It has been shown that exposure to EMF adversely affects spermatogenic, Sertoli and Leydig cells [14]. Magnetic fields of 50 Hz may induce cytotoxic and cytostatic changes in the differentiating spermatogonia of mice [12]. Little is known about the effect of EMF on the cytoarchitecture of the boundary tissue of the seminiferous tubules that perform a number of crucial functions, including the mechanical support and transport of nutrients for the spermatozoa [17] and sperm discharge by maintaining pressure on the tubules [8]. Other studies have been made of the transitory effects of EMF on the testes and no study has revealed, to date, the possibility of recovery from the potentially harmful effects of EMF exposure after an exposure-free time. Furthermore, kidney cells necrosis an apoptosis in order to effect of EMF has been studied [19]. The present study was aimed to investigate possible beneficial effects of Ocimum basilicum as a source of natural antioxidant on kidney apoptosis on rats which exposed to the 50Hz EMF (non-ionising radiation).

Material and methods

Preparation of Extract:

Aerial parts of O.basilicum were purchased from local store. The explants was authenticated by one of us (F.F.). Fresh aerial parts of the plant were extracted by maceration with EtOH-Water (80:20) to produce a total extract (Hydroalcoholic extract, HAE), which were included total phenols and flavonoids of the plant.

Experimental Animals:

Animals and maintenance total of 40 male wistar rats were used for the study. Rats of the same sex were housed together (10per cage) .Rats were fed on compact food in the form of granules and water. This food consisted of all the essential ingredients, including vitamins and minerals. The environmental conditions (temperature and humidity) in all the animal holding areas were continuously monitored. Temperature was maintained in the range of 23^c and humidity was monitored at 35–60%. Light was provided on a 12 h light/dark cycle and kept turned on from 7 a.m. till 7 p.m.

EMF-producing System:

The equipment was based on the Helmholtz coil, which works following Fleming's right hand rule. This produced an alternate current of 50 Hz, creating an EMF of 80 G. The intensity of the EMF could be controlled by a transformer. The equipment had two main parts. In the first there were two copper coils placed one above the other and separated by a distance of 50 cm.

Between the coils (the exposure area) there was a cylindrical wooden vessel, the interior of which had a chamber for holding the cages of the experimental animals. The second part was the transformer, which checked the input and output voltage with a voltmeter and the current with an ampere meter. To prevent increases in temperature inside the chamber a fan was utilised as necessary. Five cages at a time were placed within the chamber with seven or eight rats per cage [12].

Surgical Procedure:

In day 40, the Pentobarbital sodium (40 mg/kg) was administered intra peritoneal for anesthesia, and the peritoneal cavity was opened through a lower transverse abdominal incision. Thereafter testis in control and experimental groups were immediately removed. The weights of testis in each group were registered. The animals were decapitated between 10:00 AM and 12:00 AM, and blood samples were obtained. Blood samples were centrifuged at 4°C for 10 min at 250Xg and the serum obtained was stored at −20°C until assayed.

TUNEL Analysis of Apoptosis:

The *in-situ* DNA fragmentation was visualized by TUNEL method (16). Briefly, dewaxed tissue sections were predigested with 20 mg/ml proteinase K for 20 min and incubated in phosphate buffered saline solution (PBS) containing 3 % H_2O_2 for 10 min to block the endogenous peroxidase activity. The sections were incubated with the TUNEL reaction mixture, fluorescein-dUTP (*in situ* Cell Death Detection, POD kit, Roche, Germany), for 60 min at 37°C. The slides were then rinsed three times with PBS and incubated with secondary antifluorescein-POD-conjugate for 30 min. After washing three times in PBS, diaminobenzidine-H_2O_2 (DAB, Roche, Germany) chromogenic reaction was added on sections and counterstained with hematoxylin. As a control for method specificity, the step using the TUNEL reaction mixture was omitted in negative control serial sections, and nucleotide mixture in reaction buffer was used instead. Apoptotic dark brown cells were quantified by counting the number of TUNEL stained nuclei per kidney tissues cross sections. Cross sections of 100 kidney tissues per specimen were assessed and the mean number of TUNEL positive apoptotic cells per cross- section was calculated [12].

Measurement of Serum Total Antioxidant Capacity (TAS):

TAS was measured in serum by means of a commercial kit (Randox Co-England).

The assay is based on the incubation of 2, 2'-azino-di-3-ethylbenzthiazoline sulphonate (ABTS) with a peroxidase (methmyoglobin) and hydrogen peroxide to produce the radical cation ABTS+, which has a relatively stable blue-green color, measured at 600 nm. The suppression of the color is compared with that of the Trolox, which is widely used as a traditional standard for TAS measurement assays, and the assay results are expressed as Trolox equivalent (mmol/L) [11].

Measurement of Serum MDA:

Tissue MDA levels were determined by the thiobarbituric acid)TBA(method and expressed as nmol MDA formed/mL .Plasma MDA concentrations were determined with spectrophotometer. A calibration curve was prepared by using 1,1',3,3'-tetramethoxypropane as the standard [11].

Statistical Analysis:

Statistical comparisons were made using the ANOVA test for comparison of data in the control group and the experimental groups. The results were expressed as mean ± S.E.M (standard error of means). Significant difference is written in parentheses.

Results:

Results of Serum Albumin:

Serum albumin, in control, EMF, extract and EMF plus extract groups respectively was (3.23 ± 0.11), (4.63 ± 0.11)* (2.93 ± 0.11) and (3.93 ± 0.11). These changes was significant as p value less than 0.05 (P>0.05) in compared with the control group, (Table I).

Table I: The effect of the 1.5g/kg body ocimum basilicum extract on Albumin, serum total antioxidant capacity (TAC) , Malondialdehyde (MDA) ,apoptotic cells of control and experimental groups in the rats.

Groups	Control (n=10)	HAE** (1.5g/kg body) (n=10)	EMF+HAE (1.5g/kg body) (n=10)	EMF
Serum Albumin	3.23 ± 0.11	2.93 ± 0.11	3.93 ± 0.11	4.63 ± 0.11
Total Antioxidant capacity (TAC)	0.53±0.77	2.13±0.11	0.43±0.66	0.11±0.190
Malondialdehyde (MDA)	2.64±0.193	1.03±0.11	4.14±0.180	,) 6.64±0.190
Percentage of apoptotic cells	6.25±1.14	1.05±0.41	8.10±7.17	12.15±8.17

Data are presented as mean ± SE.

*Significant different at p> 0.05 level, (compared with the control group).

**HAE, Hydroalcoholic extract.

Results of TUNEL Positive Cells:

Number of apoptotic cells colored brown, in control, EMF,extract and EMF plus extract groups respectively was (6.25±1.14), (12.15±8.17)*, (1.05±0.41) and (8.10±7.17). These changes was significant as p value less than 0.05 (P>0.05) in compared with the control group, (Table I).

Results of total antioxidant capacity (TAC) and Malondialdehyde (MDA) concentrationmeasurement in Serum.

The mean concentration of Malondialdehyde (MDA) level in control, EMF,extract and EMF plus extract groups respectively was (2.64±0.193), (6.64±0.190)*, (1.03±0.11) and (4.14±0.180)*. These changes was significant as p value less than 0.05 (P>0.05) in compared with the control group, (Table I). Total antioxidant capacity (TAC) was (0.53±0.77), (0.11±0.190)*, (2.13±0.11) * and (0.43±0.66).

These changes was significant as p value less than 0.05 (P>0.05) in compared with the control group, (Table I).

Discussion:

Previous study showed two hours of 60?Hz EMF exposure might immediately alter the metabolism of free radicals, decreasing SOD activity in plasma and GSH content in heart and kidney, but does not induce immediate lipid peroxidation [13]. Another results of EMF effect on canine kidney histopathology was revealed that EMF are able to modify the distribution of TJ and AJ structural proteins, tending to stabilize these cell contacts. The quantitative changes of beta-catenin suggest a causative relationship between EMF effects on the cell junctional complex and the protein signaling pathway [19]. Exposure of biological systems to electromagnetic radiation as an ionizing radiation results in the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These reactive species inflict damage to the various bio-macromolecules like DNA, lipids and proteins present in the cell [16]. These damages lead to early signs (e.g., cataract induction, haemologic deficiencies, damages to skin and fertility impairment) or late sickness (e.g., cancer appearing several year after exposure (of radiation injury) [18]. The harmful effects of EMF ionizing radiations)e.g. X-rays and gamma rays(have previously been demonstrated on gonadal tissues [9]. Since plants and natural products are extensively used in several traditional systems of medicine, screening of radio protective compounds from them has several advantages because usually they are considered non-toxic and are widely accepted by humans.

Many natural antioxidants, whether consumed before or after radiation exposure, are able to confer some level of radioprotection. In addition to achieving beneficial effects from established antioxidants such as vitamins C and E and their derivatives, vitamin A, beta carotene, curcumin, caffeine, chlorogenic acid, ellagic acid and bixin, protection is also conferred by several novel molecules, such as flavonoids, eppigallocatechin and other polyphenols [16]. Basil (Ocimum basilicum L. Family Lamiaceae) is used as a kitchen herb and as an ornamental plant in house garden [7]. Our results confirmed that EMF cause to increasing free radical and reactive oxygen spices)ROS(and this make cell injury and increase ability of kidney cell membrane and apoptosis and this findings are same with other reports [20,14,4] serum albumin mean in EMF group were significantly increased and this is agree with other researchers results [6]. But hydroalcoholic extract of ocimum basilicum increase antioxidant capacity and have beneficial effect to neutralize free radicals in those EMF groups which receiving it and cause to decrease level of total apoptotic cells and serum albumin measure ,and this results confirmed Previous chemical studies on Ocimum basilicum have shown the presence of flavonoids, phenylpropanoids and rosmarinic acid in aerial parts of the plant [2,15]. These reports have also indicated the antioxidant and radical scavenging activity of O. basilicum [5,3]. In conclusion Many herbal such as O. basilicum well-known flavonoid and a strong antioxidant and long-term treatment of many health disorders and it has been shown to reduce oxidative stress.

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