

ORIGINAL ARTICLE

Protective Effect of Date Palm Fruits (*Phoenix Dactylifera L.*) versus Vitamin C Against Mobile Phone Radiation-induced Pituitary Gland Damage in RatsRasha M.S.M. Mohamed^{1*}, Maha M. Ahmed Abdul Rahman², Sahar Kamal Younes Ali¹, Heba S Ahmed¹¹ Clinical Pharmacology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt² Human Anatomy and Embryology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt***Corresponding author:**Rasha Mohamed Sabry
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University, Zagazig, Egypt**E-mail address:**rmsabry@medicine.zu.edu.eg**Submit Date** 2021-09-28
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Accept Date 2022-01-09**ABSTRACT****Background:** Date palm fruit is one of the most precious nutraceutical compounds. Now, it is well established that mobile phone radiation causes deleterious health hazards. The pituitary gland is the master gland in the human body and is highly affected by mobile phone radiation as it's the nearest part of the body to mobile phones during calls. This study was designed to investigate the cytoprotective effect of date palm fruit against the mobile phone radiation-induced pituitary gland damage compared to vitamin C.**Methods:** Male Wister albino rats were exposed to mobile phone electromagnetic radiations (EMR) for 1 hour per day for 4 weeks. Rats were divided into 4 groups: control normal, EMR, EMR+ vitamin C (100 mg/kg), and EMR+ date palm fruit groups. Pituitary oxidative stress markers (malondialdehyde, MDA, and superoxide dismutase, SOD), DNA damage (8-hydroxy-2'-deoxyguanosine, 8-OHdG), and apoptosis (caspase 3) were assessed.**Results:** Mobile phone radiation significantly increased pituitary gland oxidative stress, DNA damage, and apoptosis while both vitamin C and date palm fruit significantly alleviated these detrimental effects with superior cytoprotective effect for date palm fruit over vitamin C. **Conclusions:** These results can introduce date palm fruit as an affordable nutraceutical agent owing to its antioxidant and antiapoptotic properties.**Keywords:** Date Palm Fruits; mobile (cell) Phone Radiation; Pituitary gland; Caspase; Oxidative stress**INTRODUCTION**

Date palm fruit (*Phoenix dactylifera L.*) is a traditional fruit in the Middle East and Africa, especially in Arab regions. It acquires particular importance owing to their religious background, however, its significance in western countries has not been explored yet due to different cultures and eating habits. It is commonly used in traditional medicine. Date palm fruit is likely to provide a valuable source of low-cost food. Interestingly, date palm fruit is rich in vitamins (vitamin B1, B2, B3, B6, and vitamin C). Date palm fruit also consists of fibers, proteins, carbohydrates, fats, and minerals (Cu, Na, Ca, Mg, P, K, Zn, and Fe) [1]. Moreover, the Egyptian date palm fruit exhibited significant antioxidant and antimicrobial activities.

The presence of mobile phones has become indispensable in our daily activities. According to the International Telecommunication Union (2017), mobile phone users accounted for about ~7.68 billion [2]. Mobile phones emit electromagnetic radiations (EMR). The magnetic field is hazardous due to its ability to penetrate the living tissues. The electromagnetic radiations (EMR) consist of radiofrequency (RF), microwaves, infrared, visible light, ultraviolet light, x-rays, and gamma rays [3]. Radiofrequency radiation is the type of EMR that ranges from 30 kHz–300 GHz. The distance of the source of radiofrequency EMR from the body is one of the most important factors that determine the damaging effect of cell phones on the body tissues [4].

Mobile phones emit radiofrequency EMR signals. This escalating increase in the use of mobile phones draws serious attention surrounding its health hazards which are either thermal or non-thermal [5]. Thermal effects are due to the increase in tissue temperature which can cause temporary facial nerve dysfunction [6]. Moreover, there are reports on the effects of EMR on the apoptotic pathway of the mitochondria, cell differentiation, free radical metabolism, heat shock proteins, DNA damage, and the plasma membrane [7]. Also, prolonged ipsilateral use of mobile phones reduced individuals' cognitive functions [8] especially in children [9] but its carcinogenic effect is still controversial [4]

Oxidative stress is the imbalance between the synthesis and degradation of reactive oxygen species (ROS) in the cell and the ability of the body to get rid of it. Reactive oxygen species are generated during normal metabolism and in response to stressors (i.e., radiations) [10]. Mobile phone radiations were proved to induce oxidative stress and DNA damage in rat brain tissue [11]. Moreover, ROS formation was proved to cause oxidative degeneration in the reproductive tissue [12], heart, lungs, and liver [13]. Furthermore, chronic inflammation is linked to the continuous production of ROS which causes tissue damage including lipid peroxidation, proteins degradation, and DNA damage with the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) [14][15].

The pituitary gland is the master gland of the endocrine system. Mobile phones, with a frequency of 900 MHz, were proved to have high penetration power of the nearest tissues especially the brain and the pituitary gland. There is great controversy regarding the increased incidence of intracranial tumors, including pituitary tumors, with the long-term use of mobile phones [16]. According to Shahabi et al 2018, brain histological structure and pituitary hormones level were significantly changed after prolonged exposure to mobile phone EMR [17].

Date palm fruit has shown promising ameliorative effects in various diseases. It has antioxidant and anti-inflammatory characteristics. It has protective effects on

diabetes mellitus, liver, renal, and reproductive system diseases [18],[19], [20] Moreover, date palm fruit has been found to be beneficial in the treatment of pancreatic cancer due to its potent antifibrotic effect [21]

This study aimed to investigate the cytoprotective effect of date palm fruit compared to vitamin C against mobile phone-induced pituitary damage in adult male wistar albino rats.

METHODS

Animal and ethical statement:

Sixteen Adult male Wistar rats were brought from the Faculty of Veterinary Medicine, Zagazig University, Egypt. Each rat weighed about 150 g and rats were distributed as three per cage and kept for about 1 week for acclimatization. Rats had food and water ad libitum. Temperature ($23^{\circ}\text{C} \pm 2^{\circ}\text{C}$), humidity ($60\% \pm 10\%$), and light/dark cycles (12/12 h) were kept constant. All animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals Act. All animal handling procedures were authorized by the Ethical Committee for Animal Handling at Zagazig University, Egypt, with approval no. ZU-IACUC/3/F/91/2021.

Exposure to mobile phone radiation:

Exposure to mobile phone radiation was carried out by using Darago D5 (2G, GSM: 900 mHz) mobile phone. The mobile phone was in silent switched-on mode all the time of the experiment and was installed close to the rat cages. The radiation groups were exposed to missed calls for one hour per day for 4 weeks.

Drugs and experimental design:

Rats were divided into four groups: Control normal group (n = 4) and Wister albino rats who were exposed to mobile phone radiation (1h/day for 4 weeks) [22] and randomly divided into 3 groups: Electromagnetic radiation group (EMR) (n = 4), EMR + vitamin C group (n = 4) in which rats received 100 mg/kg body weight daily dissolved in drinking water (vitacid-c effervescent 1g, chemical industries development, Giza, ARE) [34][34][34][34], and EMR + dates group (n = 4) in which Egyptian date palm fruit was

purchased from the market in the form of paste and One-third of rat food was made of date palm fruit paste mixed with the standard chow diet.

Collection of pituitary samples for laboratory analysis:

At the end of the experiment, rats were anaesthetized by urethane (1.3 g/kg, ip, Sigma-Aldrich, USA). The pituitary gland was dissected, washed with ice-cold saline, kept on ice then stored at -20°C .

Enzyme-linked immunosorbent assays (ELISA):

Pituitary levels of malondialdehyde (MDA, biodiagnostic, Giza, Egypt), superoxide dismutase (SOD, biodiagnostic, Giza, Egypt), caspase 3 (CASP3) (Cloud-Clone Corporation, Fernhurst Dr., Katy, USA), and 8-hydroxy-2-deoxyguanosine (8-OHdG) (MyBioSource San Diego, USA) were assayed using rat ELISA kits. All procedures were done according to the manufacturers' instructions.

Ultra-performance liquid chromatography-electrospray tandem mass spectrometry (UPLC-ESI-MS/MS) separation:

Using Ultra-performance liquid chromatography-electrospray tandem mass spectrometry (UPLC-ESI-MS/MS) UPLC-ESI-MS/MS technique revealed the presence of sucrose, L-ascorbic acid, (iso)citric acid, caffeoyl shikimic acid hexoside, ferulic acid, cyanidin-3-O-hexoside, (+)-catechin, (-)-epicatechin, quercetin, luteolin, and apigenin as major metabolites.

Statistics:

Data are presented as mean \pm SEM. Statistical analysis was done using GraphPad Prism version 5 (GraphPad Software, Inc., CA, USA). All groups were analyzed by one-way ANOVA and post-hoc Tukey test. P-values <0.05 were considered significant.

RESULTS

In Figure 1, Oxidative stress was evaluated by assessing pituitary levels of MDA, as an oxidative stress marker, and SOD, as a marker of antioxidant activity. Data revealed that rats exposed to EMR showed a significant increase

in MDA (Fig. 1A) with a significant decrease in SOD levels (Fig. 1B) in the pituitary tissues compared to the control group ($P <0.001$) reflecting increased oxidative damage under the effect of EMR.

Rats treated with vitamin C or date palm fruits presented significant protection against mobile phone-EMR-induced oxidative stress as demonstrated by the significant decrease in MDA (Fig. 1A) and a significant increase in SOD levels (Fig. 1B) in the pituitary tissues compared to the EMR group ($P <0.001$). Interestingly, there was a significantly higher pituitary SOD level in rats receiving date palm fruits compared to the vitamin C group ($P <0.05$) indicating higher tissue antioxidant activity, and subsequent tissue protection.

In Figure 2A, DNA damage was assessed by measuring pituitary levels of 8-OHdG, a marker of oxidative damage to DNA (2'-deoxyguanosine). Data revealed that rats exposed to EMR showed a significant increase in 8-OHdG levels in pituitary tissues compared to the control group ($P <0.001$) reflecting increased DNA damage.

Rats treated with vitamin C or date palm fruits presented significant protection against EMR-induced DNA damage as demonstrated by the significant decrease in 8-OHdG levels in the pituitary tissue ($P <0.001$) compared to the EMR group. Date palm fruit-treated rats exhibited higher protection against DNA damage compared to the vitamin C group ($P <0.001$).

In Figure 2B, Cell apoptosis was assessed by measuring pituitary levels of caspase 3. Data revealed that rats exposed to EMR exhibited a significant increase in caspase-3 levels in pituitary tissues compared to the control group ($P <0.001$) reflecting increased cell death under the effect of mobile phone-EMR.

Rats treated with vitamin C or date palm fruits exhibited significant protection against mobile phone-EMR-induced pituitary apoptosis ($P <0.01$ respectively) compared to the EMR group.

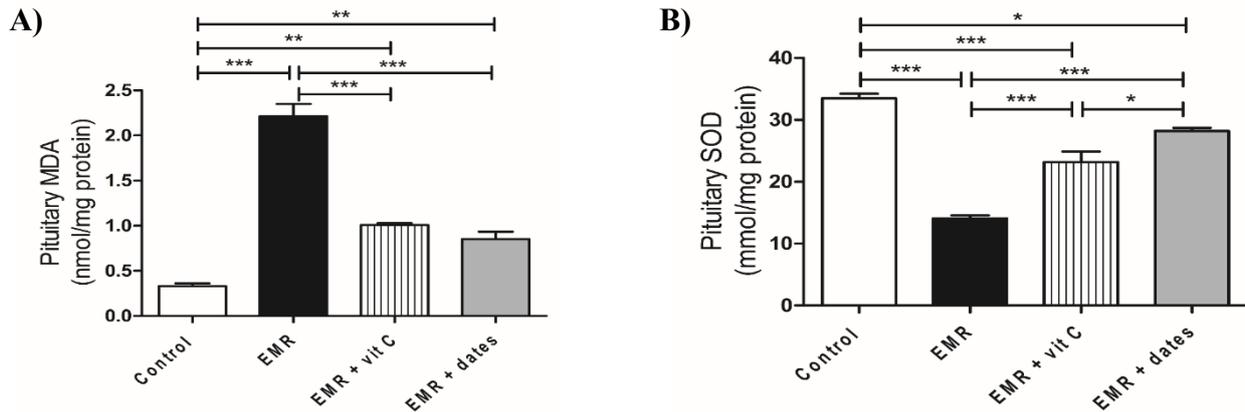


Figure 1. Effect of vitamin C and date palm fruits on mobile phone EMR-induced oxidative stress in the pituitary gland. Graphical presentation of malondialdehyde (MDA) (A), and superoxide dismutase (SOD) (B). Rats were exposed to mobile phone electromagnetic radiations (EMR) for 1 hour per day for 4 weeks. Rats were divided into 4 groups: control normal, EMR, EMR+ vitamin C (100 mg/kg orally), and EMR+ date palm fruit groups. Groups were analyzed using two-way ANOVA and post-hoc Tukey test. All values were shown as mean \pm SE. (n= 3). * P < 0.05, ** P < 0.01, and *** P < 0.001.

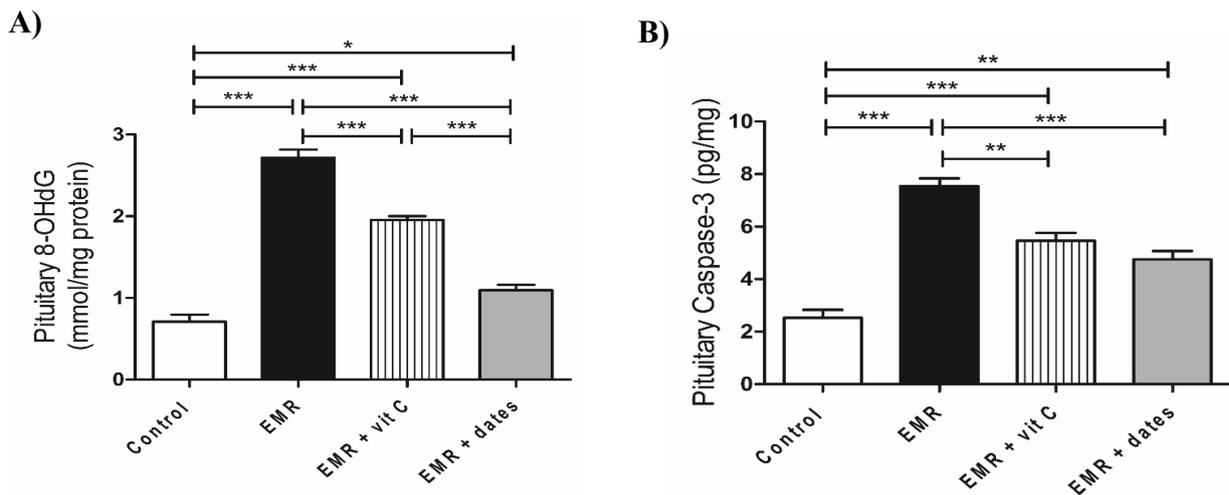


Figure 2. Effect of vitamin C and date palm fruits on mobile phone EMR-induced DNA damage and apoptosis in the pituitary gland. Graphical presentation of 8-hydroxy-2’deoxyguanosine (8-OHdG) (A), caspase 3 (B). Rats were exposed to mobile phone electromagnetic radiations (EMR) for 1 hour per day for 4 weeks. Rats were divided into 4 groups: control normal, EMR, EMR+ vitamin C (100 mg/kg orally), and EMR+ date palm fruit groups. Groups were analyzed using two-way ANOVA and post-hoc Tukey test. All values were shown as mean \pm SE. (n= 3). * P < 0.05, ** P < 0.01, and *** P < 0.001.

DISCUSSION

Date palm fruit is a valuable and affordable source of vitamins, minerals, fibers, and sugars. It was used for a long time in traditional medicine in Arab countries with Islamic religious backgrounds [1]. Now, date palm fruit is introduced as a nutraceutical agent with both protective and therapeutic properties. In this study, we investigated its cytoprotective

role in the case of mobile phone radiation-induced pituitary damage compared to vitamin C as a standard antioxidant. Our results showed that the oxidative stress markers, MDA and 8-OHdG, significantly increased, while the antioxidant enzyme, SOD, significantly decreased under the effect of EMR compared to the control group. Moreover, the caspase 3 enzyme, the hole

mark of both intrinsic and extrinsic apoptotic pathways, significantly increased in the EMR group compared to the control group.

Consistent with our results, Shahabi et al proved that prolonged exposure to mobile phones can induce both brain and adrenal tissue pathological changes [17]. Moreover, there was a significant increase in MDA and a reduction in SOD levels in the brain, kidneys, liver, and myocardial tissues after exposure to mobile phone radiation [23]. On the contrary, rats exposed to mobile phone radiation for only 10 minutes per day did not exhibit a significant change in oxidative stress, supporting the critical role of duration of exposure to the source of radiation in determining the extent of tissue damage [24]. Similarly, it has been proved that mobile phone EMR-induced adverse effects on cognitive function of healthy volunteers depend on both distances from the device generating EMR and the duration of exposure [8].

8-Hydroxydeoxyguanosine (8-OHdG) is a sensitive marker of DNA oxidation which increases in oxidative stress conditions. Reactive oxygen species (ROS) stimulate 8-OHdG synthesis which, in turn, activates the apoptotic pathways leading to cell death [26]. This is in accordance with our results that showed a significant, and simultaneous increase in both DNA damage and cell apoptosis. In the same context, Alkis and colleagues proved that mobile phone radiation can induce DNA damage and increase brain oxidative stress in rats [11].

Results of the current study showed that vitamin C significantly reduced pituitary oxidative stress and apoptosis as reflected by decreased levels of MDA, 8-OHdG, and caspase-3 and increased levels of SOD compared to rats exposed to mobile phone EMR. Vitamin C is a powerful natural antioxidant that protects cells from the damaging effect of ROS by scavenging free radicals [27]. In addition, previous reports showed that vitamin C can prevent EMR-induced oxidative stress damage and apoptosis of endometrium and brain cells [27] [28].

Date palm fruit is a naturally occurring and affordable nutraceutical agent. It has a

powerful anticancer property in the case of pancreatic cancer due to its antifibrotic effect [21]. In addition, date palm fruit has a cytoprotective effect on kidneys [19] and liver [18]. In the present study, date palm fruit significantly reduced pituitary oxidative stress and apoptosis as reflected by decreased levels of MDA, 8-OHdG, and caspase-3 and increased levels of SOD compared to rats exposed to mobile phone EMR. Noteworthy, the improvement in oxidative stress and apoptosis markers in rats treated with date palm fruit was significantly higher than in those treated with vitamin C. In accordance with our results, a previous study showed a cardioprotective effect of Ajwa date extract due to its antioxidant, anti-inflammatory, and antiapoptotic properties. The antiapoptotic effect has been proved to be due to the downregulation of the apoptotic pathway (caspase and BAX) and upregulation of the antiapoptotic protein (BCL2) [29]. In the same context, the anti-inflammatory property of date palm fruit has been attributed to its inhibitory effect on the cyclooxygenase enzyme [30].

The aqueous methanolic extract of date palm fruit is rich in phenolic components such as caffeic, ferulic, luteolin, rutin, and quercetin. These compounds can inhibit key enzymes associated with the inflammatory process [31], and inhibit the production of pro-inflammatory cytokines such as TNF- α [32] [33]. Moreover, phenolic compounds can increase the levels of plasma antioxidants such as vitamin C, E, A, β - carotene, and decrease lipid peroxides levels [34]. Previous studies showed that phenolic compounds can target reactive oxygen species and prostaglandins and inhibit the immune cells' filtration to the inflammation site [35].

CONCLUSION

The present study showed promising cytoprotective effects of date palm fruit against mobile phone EMR-induced pituitary gland damage, possibly by mitigation of oxidative stress and apoptosis.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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