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Protective Effect of Vitamin D against Harmful Effect of Cell Phone Radiation Exposure on Albino Rat Testis

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ABSTRACT

OBJECTIVE: The aim of this study was to investigate the possible effects of electromagnetic radiation from cell phone use on the oxidant and antioxidant status in testicular tissue and determine the possible protective role of vitamins D in modulating effect of chronic exposure to cell phone radiation on the testicular functions of male albino rats and its underlying mechanism
MATERIALS AND METHODS: control group A (vehicle treated) andthe rats were exposed to the mobile phone while it was switched off. In Group B mobile radiation exposed (call mode) + vehicle treated and Group C: mobile radiation exposed (call mode) + oral administration of vitamin D by gavage (500 IU/Kg/day). Group B and C were exposed to the same electromagnetic frequency for 60 min daily for 6 weeks.
RESULTS: Mobile radiation exposure in male rats induced significant decrease in serum FSH, LH, testosterone levels, epididymal sperm count, sperm motility with insignificant changes in either body weight gain or testicular weight, significant increase in testicular malondialdehyde (MDA) with significant decrease in testicular antioxidant SOD and GPx activity in comparison to control group and showed structural changes in the form of rupture of the basement membrane, dropped off spermatogenic cells, inter tubular fibrosis and leydig cells hyperplasia. However, administration of vitamin D resulted in a significant recovery of all the above-mentioned parameters in the mobile radiation exposed rats' group.
CONCLUSION:we confirmed the harmful effect of mobile phone radiations on testicular function in male albino rats, in addition, we confirmed the protective effect of Vitamin D on the testicular functions via restoring steroidogenesis and spermatogenesis in exposed rats.

KEYWORDS: Electromagnetic Radiation; Testes; Vitamin D, Rats.

1. Introduction

Radiation can be characterized into ionizing and non-ionizing radiations, of which the latter is differentiated in two forms: extremely low frequency (ELF) or power line (60 Hz) electromagnetic fields (EMFs), and radio frequency (RF) EMFs which are produced by wireless radio waves/microwaves products **(1).** The possible health effects of radio-frequency modulated electromagnetic fields (RF-EMF) emitted by cell phones, cordless phones, base stations, and Wi-Fi routers are a matter of growing public concern around the world today.

Reactive Oxygen Species (ROS) production is associated with enhanced exposure to EMR **(2)** and together with free radicals affect the reproductive system in both human and animals **(3).** The extensive worry over EMR exposure has grown along with the decreasing fertility in the human population **(4).**

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Besides decreased sperm production, disorders in sperm structure and motility, abnormal sperm function or blockage in sperm delivery are often correlated with testicular oxidative stress caused by RFR exposure **(5-7).**

Vitamin D (VD) has been considered an interesting subject of study due to its role, including autocrine, paracrine and endocrine function on several target organs and systems **(8).**

The expression of VD receptors (VDR) and VD metabolizing enzymes in the male reproductive system has been widely analysed in animals and human studies. VDR protein was found in prostate, seminal vesicles, epididymis, as well as in germ cells **(9).** In the same context, testicular testosterone synthesis enzymes appeared down-regulated in VD deficient diet-fed mice **(10).**

2. Materials and Methods

2.1 Drugs and chemicals

Oral vitamin D3 (vidrop) (cholecalciferol) (Medical union pharmaceuticals, Abu-Sultan, Ismailia, Egypt).

2.2 Experimental animals

A total number of 30 (local strain) adult male albino rats weighing 160- 180 gm, were obtained from the animal house of Faculty of Veterinary Medicine Zagazig University. The rats were accommodated to animal house conditions for three weeks before the experiments going on **(43)** The experimental protocol was approved by Physiology Department and by Zagazig University institutional animal care unit committee (ZU-IACUC; Sharkia; Egypt) with approval number:" ZU-IACUC/3/F/117/2019

The animals were kept in plastic cages (30 inches in length,18 inches in width and 24 inches in height). Each 10 rats were housed per cage in the animal house of Physiology Department, Faculty of Medicine, Zagazig University under hygienic conditions.

2.3 Experimental protocol

The study was conducted on 30 healthy adult male albino rats (10-12 weeks) After acclimatization for 3 weeks, the rats were divided into 3 equal groups (n=10 rats). GroupA: control group shame exposed to closed mobile for 6 weeks. Group B: were exposed to mobile radiation 1 hour daily for 6 weeks. Group C: were exposed to mobile radiation + daily oral administration Vit.D for 6 weeks.
The experiment lasts for 6 weeks Handsets of mobile phones of the same brand and model were used, the properties of mobile phones were as follows: - The highest SAR value was 0.96 W/kg and each of them had an 890–915 MHz carrier frequency band 217 Hz modulation frequency, 250 mW maximum average power and 2 W maximum peak power **(11)**. A call was made from one set of GSM (Global System for Mobile Communications) mobile phones to another set of mobile phones after ensuring that each mobile phone was powered-on and with the call accepting mode (answering mode) switched on. Then, the cell phones were placed inside plastic experimental boxes devoid of any metallic fitting, mobile phones were placed in special sections in the middle of the experimental cages in order to prevent damage that could be caused by rats, the cell phone was placed in the center of the cage to provide almost equal electromagnetic radiation to the whole body of the animals **(12)**. Animals were free to move about in the cage during the exposure period. The electromagnetic field radiation generated by mobile phones was approximately equal the average of downlink frequency of Orange network

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in Egypt (935– 945) MHz electromagnetic field = 935 + 945 = 1880/2 = 940 MHz In Group A: control group. (Sham group): the rats were exposed to the mobile phone while it was switched off in a similarly sized cage for the same period in a separate, similar room for 6 weeks **(7).**Vitamin D3 supplementation: Vitamin D3 (cholecalciferol) was diluted in corn oil and then given orally by gavage daily (500 IU/Kg/day) **(13)** for a total duration of 6 weeks. At the same time, rats in groups A and B were given oral corn oil by gavage.

2.4. Blood sampling and biochemical analysis

After 24 h of the final drug treatment, all rats were sacrificed by intraperitoneal injection of Ketamine (90 mg/kg body weight) Blood samples were immediately obtained directly from cardiac puncture, then centrifuged at approximately 3000 rpm for 10 minutes and serum was collected.

2.5. Gonadal extraction and testicular tissue preparation

By making vertical midline lower abdominal incision, testes and epididymis were extracted and dissected cautiously. The weights of the testes were measured using an electronic balance.

After removing the adherent connective tissues, the testicular tissue was washed with ice cold saline and one testis of each animal was fixed in 10% formalin for histopathological and immunohistochemical evaluation. The contralateral testes were immersed immediately in liquid nitrogen and stored at -80 C till use in biochemical enzymatic assay and western blot analysis.

2.6. Sperm analysis

The right epididymis of each rat was dissected, removed and minced in 2 ml of Hank's buffer salt solution (HBSS) at 37 °C **(14)**. After 5 min incubation at 37°C, the cauda epididymis sperm was determined using the standard hemocytometric method. The epididymal fluid was drawn up to the 0.5 mark of WBC pipette (White Blood Cell pipette) and the semen diluting fluid (sodium bicarbonate 5 g, formalin 1 ml, distilled water 99.0 ml) was drawn up to '11' mark, and subsequently mixed well. One drop was added to the haemocytometer chamber and allowed the sperms to settle by keeping haemocytometer in humid place (wet chamber) for 1 h. After incubation the number of spermatozoa in the appropriate large squares of the haemocytometer was counted under the light microscope. The sperm concentration refers to the number of spermatozoa / ml fluid, and calculated using the following formula. Sperm count =No. of spermatozoa counted x dilution factor x volume factor/ No. of areas counted **(15)**. The percentage of sperm motility was calculated using the number of live sperm cells over the total number of sperm cells, both motile and nonmotile. The sperm cells that were not moving at all were considered to be nonmotile, while the rest, which displayed some movements were considered to be motile **(44).**

2.7. Evaluation of serum testosterone, FSH and LH levels.

Free testosterone levels were measured using testosterone enzyme immunoassay test kit (Catalog Number: BC1115, BioCheck, Inc 323 Vintage Park Dr. Foster City, CA 94404). FSH using rat enzyme immunoassay test kit (Catalog Number: BC-1029, BioCheck, Inc 323 Vintage Park Dr. Foster City, CA 94404. LH using rat enzyme immunoassay test kit (Catalog Number: BC-1031, BioCheck, Inc 323 Vintage Park Dr. Foster City, CA 94404): according to the manufacturers protocol

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2.8. Evaluation of testicular tissue oxidative stress markers.

The testicular tissues were homogenized in 50 mM potassium phosphate (pH 7.4). The homogenates were used to assess the oxidative stress parameters; SOD activity was measured using rat ELISA kit (Egyptian Company for Biotechnology (SAE), Obour city, Cairo, Egypt.), GPx activity using rat ELISA kit (Egyptian Company for Biotechnology (SAE), Obour city, Cairo, Egypt.). and MDA content using Rat ELISA kit (Egyptian Company for Biotechnology (SAE), Obour city, Cairo, Egypt.) according to the manufacturers protocol.

2.9. Histopathological examination.

The testes were immersed in Bouin's fluid then paraffin, sectioned at 5 μm thicknesses and stained in hematoxylin & eosin. The sections were examined under a light microscope and the general histological appearance was assessed. The histopathological analysis was carried out blindly by an expert pathologist.

2.10. Statistical Analysis.

The data obtained in the present study were expressed as mean ±SD for quantitative variables and statistically analyzed by using SPSS program (SPSS Inc. Chicago, IL, USA). ANOVA with [Post hoc (LSD)] test was used to compare means among all studied groups, P value < 0.05 was considered statistically significant.

3. Results

3. 1. Effect of mobile radiation exposure and Vit D treatment on body weight gain and testicular weights

The present study showed that mobile radiation exposure didn't significantly affect the body weight gain nor the testicular weight. Treatment with vit-D showed non-significant change in body weight gain or the testicular weight compared to untreated mobile radiation exposed (table 1).

3.2. Effect of mobile radiation exposure and Vit D treatment on sperm characteristics

Mobile radiation exposure showed significant decrease in sperm count and motility compared to control rats. Treatment with vit-D induced significant increase in sperm count and motility compared to untreated mobile radiation exposed(table 1).

3.3. Effect of mobile radiation exposure and Vit D treatment on serum testosterone, FSH and LH levels.

Mobile radiation exposure showed significant decrease in serum testosterone level in addition to decrease in serum FSH and LH levels compared to control rats. Interestingly, treatment with vit-D significantly increased the serum levels of testosterone, FSH and LH levels compared to untreated mobile radiation exposed group(table 1).

3.4. Effect of mobile radiation exposure and Vit D treatment on testicular tissue oxidative stress and lipid peroxidation parameter.

The testicular MDA level as a marker of lipid peroxidation was elevated significantly in non-treated mobile radiation exposed rats, in association with significant reductions of testicular GPx and SOD activity compared to control rats. Meanwhile these parameters were significantly reversed in vit-D treated groups when compared with untreated mobile radiation exposed group

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(table 2).

3.4. Histopathologic results

Figure (1): light photo microscopic picture of H & E stain x 400 magnification. Group A showing: Normal testis formed of uniform seminiferous tubules lined by normal layers of spermatogenic cells up to mature sperm formation, basement membrane of normal thickness and normal Leydig cells. Group B showing rupture of the basement membrane, dropped off spermatogenic cells, inter tubular fibrosis and Leydig cells hyperplasia Fig (1B). Group C showing seminiferous tubules lined by nearly normal layers of spermatogenic cells with mature sperms, the basement membrane of nearly normal thickness, no Leydig cells hyperplasia and slight focal drop of spermatogenic cells Fig (1C).

Table (1) Effect of mobile radiation exposure and Vit D treatment on body wt. gain, testicular weights, sperm count, sperm motility, serum testosterone, FSH and LH level in all studied groups.

Parameters		Group A	Group B	Group C
Body weight gain (gm)	Mean ± SD	69.5 ± 14.8	67.4 ±10.6	71.1 ±7.7
	P value of LSD		P > 0.05[a]	P > 0.05[b]
Right testicular wt (gm)	Mean ± SD	1.68 ±0.081	1.69±0.088	1.70±0.101
	P value of LSD		P > 0.05 [a]	P > 0.05 [b]
Left testicular wt (gm)	Mean ± SD	1.67 ±0.083	1.7 ± 0.09	1.71 ±0.091
	P value of LSD		P > 0.05 [a]	P > 0.05 [b]
Sperm count ($x10^6$ spermatozoa /ml)	Mean ± SD	73.5± 5.87	38.9 ± 5.15	69.6± 6.09
	P value of LSD		P < 0.001[a]	p> 0.05[a] P < 0.001[b]
Sperm motility rate (%)	Mean ± SD	69.5 ± 5.52	61.1 ± 4.72	68.8± 5.03
	P value of LSD		P < 0.01[a]	p> 0.05[a] P < 0.01[b]
serum Testosterone (ng/ml)	Mean ± SD	6.27 ± 1.18	4.37 ± 0.8	6.56 ± 1.34
	P value of LSD		P <0.01[a]	p> 0.05[a] P < 0.001[b]
: Serum FSH levels (mIU/mL)	Mean ± SD	3 ± 0.61	2.12 ± 0.55	3.4 ± 0.76
	P value of LSD		P < 0.01[a]	p> 0.05[a] P < 0.001[b]
: Serum LH levels (mIU/mL)	Mean ± SD	4.13 ± 0.65	3.18 ±0.45	4.37 ±0.82
	P value of LSD		P < 0.01[a]	p> 0.05[a] P < 0.001[b]

a Significant compared to group A. b Significant compared to group B.

Table (2) Effect of mobile radiation exposure and Vit D treatment on the testicular levels of MDA, SOD activity and GPx activity in all studied groups.

Parameters	Group A	Group B	Group C

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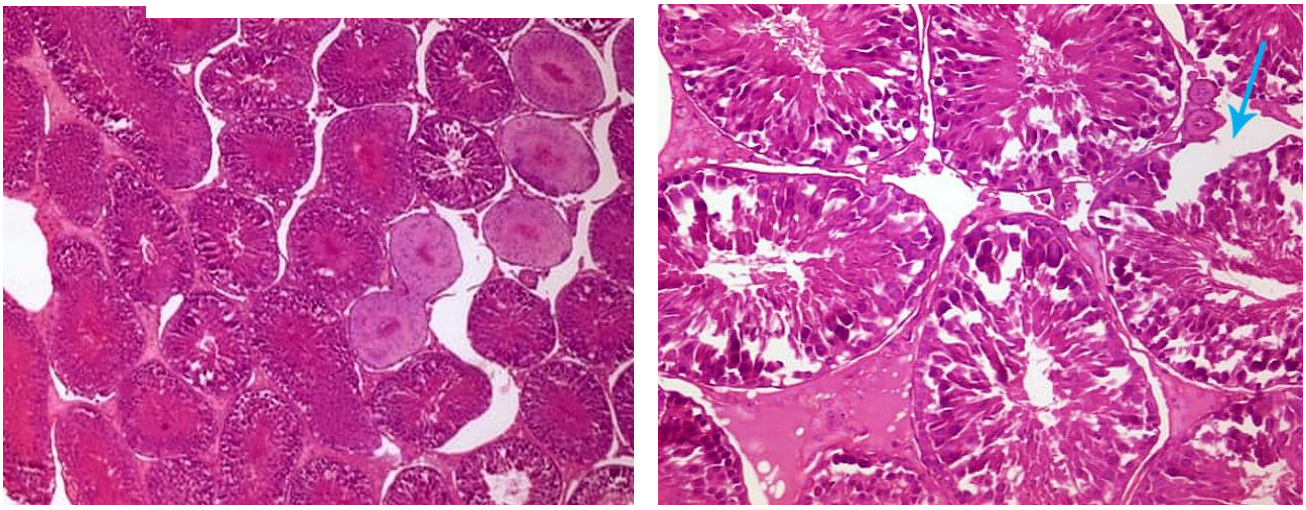
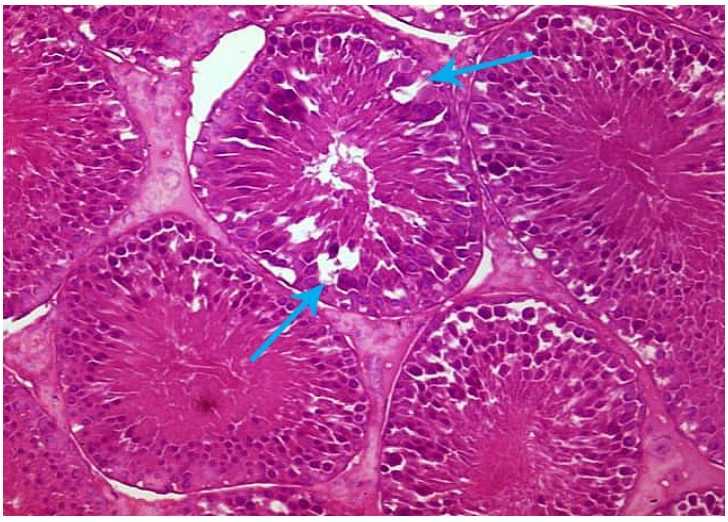
MDA (nmol/gm protein)	Mean ± SD	119.6 ± 7.5	231.7 ± 8.38 7.5	141 ± 11.88
	P value of LSD		P < 0.001a	p>0.001a P< 0.001b
SOD activity (U/gm protein)	Mean ± SD	80 ± 8.42	59.9 ± 11.35	74.7 ± 7.72
	P value of LSD		P < 0.001a	p> 0.05a P< 0. 01b
GPX activity (U/gm) protein)	Mean ± SD	30.4 ±5.85	22.7 ± 4.22	29.2 ± 4.13
	P value of LSD		P < 0.001a	p> 0.05a P< 0.01b

a Significant compared to group A b Significant compared to group B.

A



B



C

Figure (1): light photo microscopic picture of H & E stain x 400 magnification (A) (group A) showing: Normal testis formed of uniform seminiferous tubules lined by normal layers of

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spermatogenic cells up to mature sperm formation, basement membrane of normal thickness and normalLeydig cells. (B) (group B) showing: rupture of the basement membrane, dropped off spermatogenic cells, inter tubular fibrosis and Leydig cells hyperplasia (group C) showing seminiferous tubules lined by nearly normal layers of spermatogenic cells with mature sperms, the basement membrane of nearly normal thickness, no Leydig cells hyperplasia and slight focal drop of spermatogenic cells.

4.Discussion

The rapid growth of mobile phone use has been accompanied by a parallel increase in the Electromagnetic field (EMF) density **(16, 17)**. The direct biological effects of an EMF are either thermal effects via the EMF energy absorption by the induced electric current or athermic action by the long-term exposure **(18)**.
The present study revealed that exposure to mobile radiation in male rats induced significant decrease in serum FSH, LH, testosterone levels, epididymal sperm count and sperm motility and with insignificant changes in either body weight gain or testicular weight in comparison to control group.

Kumar et al. **(6)** have reported a decline in the level of testosterone in male rats after 10 GHz of microwave exposure. On the other hand, Hajioun**(19)** and Salama et al. **(20)** found that mobile phones (800 MHz) did not change the serum testosterone levels in rabbits while Nisbet et al. **(21)** and Kim et al. **(22)** reported that EMF (2.45 GHz) increased testosterone levels in rats but did not alter FSH/LH levels.
Our results are in line with Meo et al. **(23)** who reported that EMFzzs with 84 MHz frequency did not significantly affect body weight, testis
In reports on rabbits, a 50 Hz of super low-frequency (SLF)-EMFs caused reduction in sperm's motility and their viability **(24)**. On the other hand, SLF-EMFs had no impact on the viability and morphology of boars 'sperm**(25)**. However, Kesari et al. **(26)** indicated a significantly diminished total sperm count after wireless exposure (2 h/day for 35 days).
In the present study, light photo microscopic picture of testicular tissue isolated from mobile radiation exposed rats showed structural changes in the form of rupture of the basement membrane dropped off spermatogenic cells, inter tubular fibrosis and Leydig cells hyperplasia .
Interestingly, presentation to radiation of 50-Hz recurrence for 8 h/day for eight months caused histological varieties of the testicles that included weight reduction and a decrease in the normal diameter of the seminiferous tubule **(27).**
Regarding the oxidative stress markers, the present study revealed that mobile radiation exposed rats showed significant increase in testicular malondialdhyde with significant decrease in testicular antioxidant SOD and GPx activity.
La Vignera et al. **(28)** revealed that electromagnetic radiation-incited injury prompted increased creation of ROS. With respect to antioxidant consumption, electromagnetic radiation may overpower defensive systems and result in cell injury and apoptosis **(1).**
Besides, Gautam et al. **(29)** found an expansion in lipid peroxidation and neurotic degeneration and a diminishing in GPx antioxidant enzymes in the rat testis.
As opposed to the outcomes revealed over some studies discovered advantageous effects of ELF-EMF on male reproductive potential. Hori & coworkers **(30)**. Moreover, Darbandi et al. **(31)** hypothesized that exposures of a few hours to 50 Hz ELF-EMF may be associated to beneficial effects of sperm morphology and motility, whereas longer periods of exposure or ELF-EMFs with different characteristics may negatively influence sperm quality or have no effects.
One of outstanding features of the present study was the observation that epidydmal sperm count, sperm motility and serum testosterone, FSH and LH levels were significantly increased by vitamin D3 treatment. In addition, oxidative stress markers MDA activity was reduced and the antioxidant SOD and GPx activity were increased significantly in the testis these are associated

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with marked improvement of light photo microscopic features of testicular tissue

Also, Merino et al. **(32)** and Ciccone et al. **(33)** clearly demonstrated a direct and positive relationship between serum vitamin D level and overall semen quality, male reproductive potential, and testosterone levels. Interestingly, in a model of Vitamin D deficiency diet-fed mice testicular weight and the number of spermatozoa in the cauda epididymis are significantly decreased, and the percentage of mature seminiferous tubules is decreased **(10)**.

Interestingly, the normal levels of calcium have positive correlation with testosterone production in endoplasmic reticulum **(34)**. Moreover, it was found that Vitamin D is able to influence testosterone synthesis by a genomic vitamin D-induced expression of osteocalcin **(35)**.

Our current study confirmed the antioxidant effect of vitamin D through decreasing the testicular MDA activity which is an important marker for lipid peroxidation in addition to increasing antioxidant enzyme activity in the testis.

In a similar line, Kutuzova& DeLuca **(36)** demonstrated that vitamin D and its receptor are engaged with securing the tissues against oxidative harm. Calcitriol reduces cell calcium, inhibits the synthesis of inducible nitric oxide synthase, and builds levels of the antioxidant glutathione **(37)**.

Calcium homeostasis is controlled by VD, and have a significant role in spermatogenesis in sertoli cells. So, VDD may alter the function of reproductive system by calcium dependent mechanism **(38)**. Vitamin D is additionally important to keep up the level of calcium in the leydig cells in order to regulate the testosterone levels and spermatogenesis **(39)**.

Moreover, the high levels of ROS and RNS induce adverse effects on the DNA and RNA **(40)**. Vitamin D improved antioxidant free radical scavenging activity and decreased the creation of free radicals **(41)**. Furthermore,Jueraitetibaike et al. **(42)** reported that vitamin D advance the synthesis of ATP both through the cAMP/PKA pathway and the increase in intracellular calcium ions.

The discrepancy between our results and those of others may be due to differences in the duration of exposure to mobile radiation and the method of exposure with the difference in frequencies, duration of VD administration, species difference and / or nutritional status.

Conclusion

Taking the present findings together, we confirmed the harmful effect of mobile phone radiations on testicular function in male albino rats, this radiation exposure induced oxidative stress which led to testicular tissue structural and functional changes that appeared in form of alteration in sperm parameters and sex hormonal disturbance in addition, the effect of VD administration on modulating these effects was confirmed by the current histopathologic alteration in exposed rats. We recommend further studies to assess the effect of mobile radiation and vit d treatment on the testicular hypophyseal axis even at the hypothalamic level.

Declaration ofInterest Statement

There is no conflict of interests regarding the publication of this research

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