

**Original Article:****Effect of WiFi waves (2.45 GHz) on aminotransaminases (ALP, ALT and AST) in liver of rat****Mehdi Pooladi<sup>1\*</sup>, Alireza Montzeri<sup>1</sup>, Niloofar Nazarian<sup>1</sup>, Bitta Taghizadeh<sup>2, 3</sup>, Mohsen Odoumizadeh<sup>1</sup>**<sup>1</sup> Department of Biology, School of Basic Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran<sup>2</sup> Department of medical biotechnology, school of advanced medical sciences, Tabriz university of medical sciences, Tabriz, Iran<sup>3</sup> Laboratory of molecular biophysics, Institute of biochemistry and biophysics (IBB), University of Tehran, Tehran, Iran\*Corresponding Author: email address: [mehdi.pooladi7@gmail.com](mailto:mehdi.pooladi7@gmail.com) (M. Pooladi)**ABSTRACT**

Different disorders and diseases are associated with liver thus liver enzymes are commonly evaluated. Amino transaminases are among the most important enzymes in the liver, which their serum levels can indicate liver's health or abnormality. Environmental stimuli including electromagnetic field affect different cells and organs in the body including the liver. WiFi networks are among the most common inducers of electromagnetic field. In the present study, serum levels of three liver aminotransaminases including Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are assessed and histopathological evaluations are performed for four weeks in six groups of mice following WiFi<sub>2.45GHz</sub> exposure. Then, we have analyzed the data (*t*-test and one-way ANOVA; *P*<0.05). Our results show that amino transaminase levels are changed following WiFi<sub>2.45GHz</sub> exposure compared to control group and that these changes are increased with time. Respectively, AST, ALT and ALP levels showed 11.38%, 18.63%, and 4.85% increase on average, during these four weeks of the experiment. ALT and AST sustain more changes compared to the ALP in the liver. Electromagnetic induction is related to AST, ALP and ALT catabolism. The WiFi exposure time is an important factor that affects the maximum amount of absorbed electromagnetic energy in a specific period.

**Keywords:** WiFi; AST; ALP; ALT; liver**INTRODUCTION**

Liver is a sensitive organ in the body and studying its condition can be very useful in the case of diseases and abnormalities since a great number of these conditions in the human body are related to liver cell's metabolism and liver enzymes. Liver enzyme malfunction can be harmful to other tissues and organs as well. Environmental factors including electromagnetic fields are involved in cellular/enzymatic disorders of the liver [1-4]. Aminotransaminases are a group of enzymes that catalyze the transamination of amino acids and a  $\alpha$ -keto acid which is an important reversible reaction in amino acid synthesis process. Aminotransaminases also provide

essential and semi-essential amino acids in different tissues in the body. ALP, AST and ALT are among the aminotransaminases of the liver that their serum level indicates the condition of liver hepatocytes [5-7]. There are different sources of electromagnetic waves in our environment, including wireless communications, radars, satellites and TV and radio antennas. The electromagnetic field is also widely used in medical instruments and the hospitals. WiFi is a cheap common technology which exposes its users to a spectrum of short wavelength electromagnetic waves (2.45 GHz). The adverse effects of WiFi exposure on metabolic and reproductive system is not clear yet [8-10]. In the present study, we have

evaluated the risk of enzymatic disorders of the liver in rats following WiFi<sub>2.45GHz</sub> exposure. For this purpose, rats were exposed to WiFi<sub>2.45GHz</sub> waves and serum levels of three liver minotransaminases (AST, ALP and ALP) were studied.

## MATERIALS AND METHODS

Six groups of 20 male Wistar rats (three months old) with body weight ranging from 200±20 g provided by Laboratory Animal Center of Tehran University, were chosen randomly. Animals had free access to food and water, maintained on a 12 h light/12 h dark cycle in a temperature-controlled environment (22°C). All the animals in this study received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23 revised 1985). Usage of animals was in accordance with the ethical committee of the Science and Research Branch, Islamic Azad University, Tehran, Iran. Ethics approval was made for this research.

### Six experimental groups include

- First control group (C<sub>1</sub>): consisted of rats exposed to powered-off electromagnetic field (No WiFi<sub>2.45GHz</sub> exposure) and were evaluated after a week.
- Second control group (C<sub>2</sub>): consisted of rats exposed to powered-off electromagnetic field (No WiFi<sub>2.45GHz</sub> exposure) and were evaluated after four week.
- First experimental group (G<sub>1</sub>): healthy rats exposed for seven consecutive days (one week, 8 hours daily) to WiFi<sub>2.45GHz</sub> waves.
- Second experimental group (G<sub>2</sub>): healthy rats exposed for 14 consecutive days (two weeks, 8 hours daily) to WiFi<sub>2.45GHz</sub> waves.
- Third experimental group (G<sub>3</sub>): healthy rats exposed for 21 consecutive days (three weeks, 8 hours daily) to WiFi<sub>2.45GHz</sub> waves.
- Forth experimental group (G<sub>4</sub>): healthy rats exposed for 28 consecutive days (four weeks, 8 hours daily) to WiFi<sub>2.45GHz</sub> waves.

### WiFi exposure system

A room (2m×2m×2m) was prepared and WiFi exposure was provided by 4 antennas (NanoStation Loco M2, 2.45 GHz, 8.5 dBi, Ubiquiti Networks, Inc. USA) placed on each side of the room. Rat's Plexiglas cages (40cm×40cm×20cm) was placed at the middle of the room while the animals had movement freedom. The safe level of Specific Absorption Rate (SAR) and surface power density for two experimental groups (T+ WiFi<sub>2.45GHz</sub> and S+ WiFi<sub>2.45GHz</sub>) was calculated according to regulations published by the Atomic Energy Organization of Iran and the International Commission on Non-ionizing Radiation Protection (ICNIRP). The following equation was used for SAR calculations:

$$SAR = \sigma \cdot E^2 / \rho$$

In which E is the electric field (v/m),  $\sigma$  is conductivity (s/m) and  $\rho$  is tissue's mass density (Kg/m<sup>3</sup>). This equation shows that SAR value can be determined by knowing the magnitude of the electric field and that SAR has a direct relationship with electric conductivity. Surface power density and electric field magnitude was determined using a field meter (NARDA, N-550, USA). Our evaluations showed that during WiFi<sub>2.45GHz</sub> exposure the values of surface power density and SAR in rat's brain was 0.8 mW/cm<sup>2</sup> and 5.174406171 W/Kg, respectively.

### Experimental procedure

After WiFi exposure, blood samples were collected from all animals in six experimental groups. Blood samples (1 CC) were taken from the hearts of rats fasting for 8 hours by cardiac puncture following general anesthesia. The studies were performed in accordance with guidelines established by the university research center (Department of Biology, School of Basic Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran) and all animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institute of Health (NIH publication 86-23 revised 1985). All samples were collected in the morning after 8 hours of WiFi<sub>2.45GHz</sub> exposure. Blood samples were then centrifuged (in 4°C, 3000 g for 5 minutes) and the serum was collected.

In our study, ALP level was determined biochemically (DGKC opt, 1970, 37°C) using an auto-analyzer and ALT and AST levels were determined biochemically by IFCC without pyridoxal phosphate at 37°C liquid stable, with an auto-analyzer.

**STATISTICAL ANALYSIS**

The *t*-Test was performed for statistical analysis of data with SPSS software (version 19) and the *P* <0.05 was considered significant.

**RESULTS**

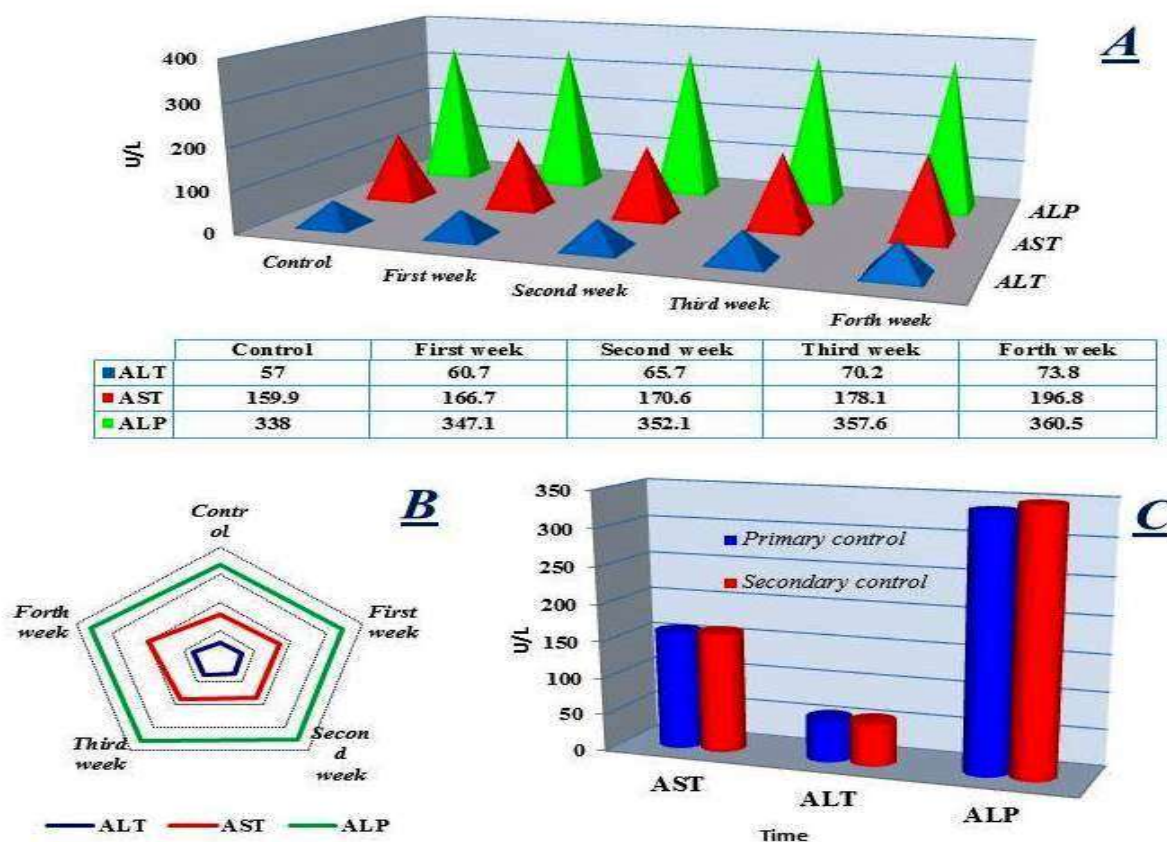
Our results showed significant elevations in serum levels of liver aminotransaminases following WiFi<sub>2.45GHz</sub> exposure compared to control groups (C<sub>1</sub> and C<sub>2</sub>). The observed elevation in enzyme levels was time dependent and transaminases levels increased with WiFi<sub>2.45GHz</sub> exposure time (first to fourth week) compared to control groups. At the fourth week of the experiment, liver aminotransaminases reached their maximum levels, while in the first

three weeks a slight change in enzyme levels was observed. AST evaluation showed 4.3%, 6.7%, 11.4% and 23.1% increase in the G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub> and G<sub>4</sub> of the experiment respectively, compared to C<sub>1</sub> [*P* <0.05 (7.978e-009)]. While C<sub>1</sub> and C<sub>2</sub> showed no significant (less than 0.11%) changes during these four weeks of the experiment.

ALT levels showed 6.5%, 15.3%, 23.2% and 29.5% increase in the G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub> and G<sub>4</sub> of the experiment respectively, compared to C<sub>1</sub> [*P* <0.05 (8.115e-008)]. While C<sub>1</sub> and C<sub>2</sub> showed no significant (less than 1%) changes during these four weeks of the experiment.

Finally, ASP results showed 2.7%, 4.2%, 5.8% and 6.7% increase in the G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub> and G<sub>4</sub> of the experiment respectively, compared to C<sub>1</sub> [*P* <0.05 (5.367e-004)]. While C<sub>1</sub> and C<sub>2</sub> showed 3.1% increase in ASP level during four weeks of the experiment.

Comparative diagram and statistical analysis of AST, ALT and ALP levels is presented in figure 1 and table 1, respectively.



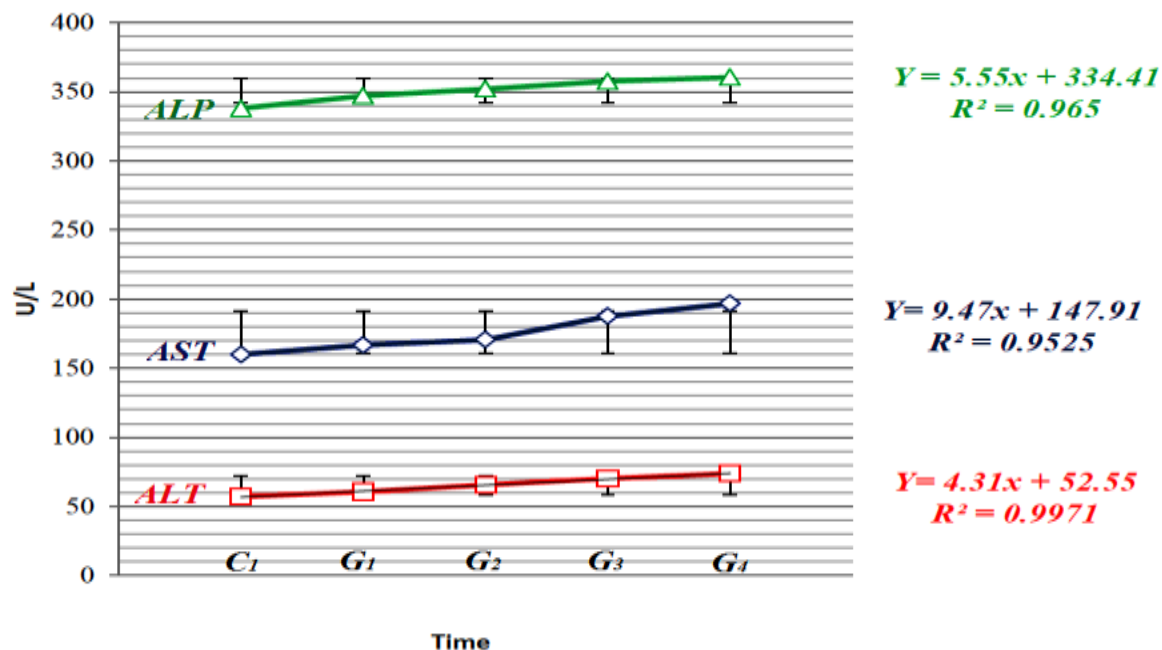
**Figure 1.** (A) Liver aminotransaminases levels during four weeks of the experiment compared to control group, (B) radar chart of enzyme level changes during four weeks, (C) comparison of C<sub>1</sub> (control of first week) and C<sub>2</sub> (control of fourth week)

**Table 1.** Statistical analysis (*t*-test and one-way ANOVA; *P* <0.05) of AST, ALP and ALT levels in six experimental groups following Wi-Fi<sub>2.45GHz</sub> exposure.

	Group	Valid	Missing	Mean	Median	Error Of Mean	Variance	Min	Max	Rande
<b>AST</b> (U/L)	C1	20	0	159.9	161.5	15.33	234.98	132	181	49
	C2	20	0	161.6	162	9.31	86.71	147	175	28
	G1	20	0	166.7	168.5	16.74	271.34	137	188	51
	G2	20	0	170.6	172	16.46	370.93	144	195	51
	G3	20	0	178.1	179	17.00	288.98	147	203	56
	G4	20	0	196.8	198	15.52	306.84	163	223	60
<b>ALT</b> (U/L)	C1	20	0	57	56	6.20	38.44	48	69	21
	C2	20	0	57.5	58.5	6.24	38.94	44	66	22
	G1	20	0	60.7	60	6.33	40.01	50	70	20
	G2	20	0	65.7	67	5.75	33.12	55	73	18
	G3	20	0	70.2	69	6.79	46.17	61	81	20
	G4	20	0	73.8	75	3.24	11.73	68	78	10
<b>ALP</b> (U/L)	C1	20	0	338	339	22.95	526.88	299	367	68
	C2	20	0	348.6	347.5	11.12	123.6	332	369	37
	G1	20	0	347.1	350	21.2	453.21	310	373	63
	G2	20	0	352.1	357	17.03	290.1	322	371	49
	G3	20	0	357.6	357.5	15.64	244.48	330	377	47
	G4	20	0	360.5	361.5	13.94	194.27	335	381	46

Figure 2 represents Wi-Fi<sub>2.45GHz</sub> effects on AST, ALP and ALT levels during four weeks of Wi-Fi<sub>2.45GHz</sub> exposure. AST and ALT showed more increase in their serum level compared to ASP during this time. The obtained results presented in this work indicate that the intensity

of Wi-Fi<sub>2.45GHz</sub> waves and time of the exposure is two main parameters involved in enzyme elevations. The observed increase in enzyme levels was time-dependent and showed further increase with time. While, after 28 days of Wi-Fi<sub>2.45GHz</sub> exposure, enzyme levels elevated significantly.



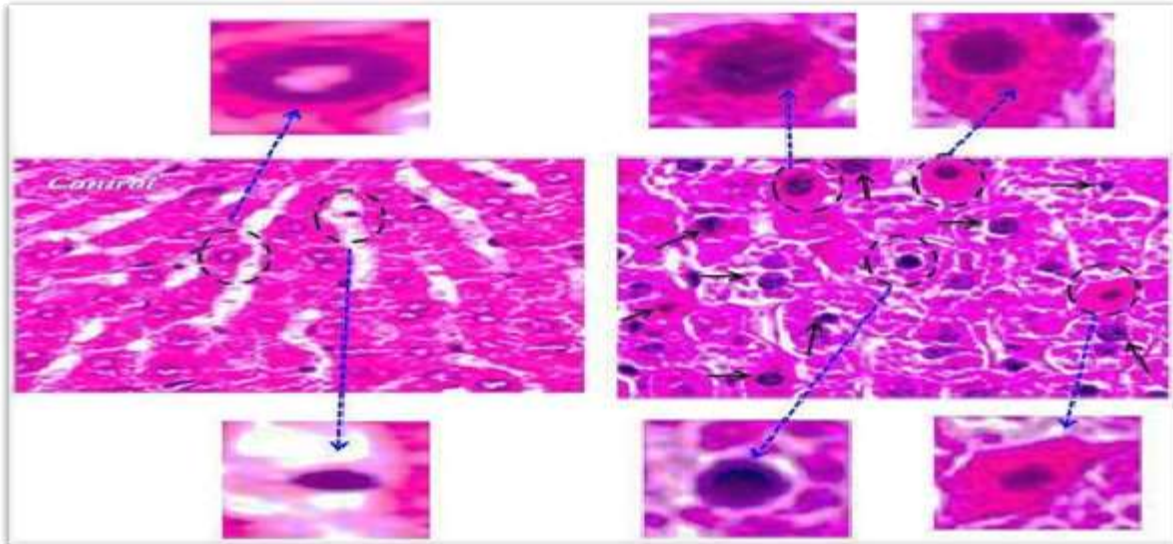
**Figure 2.** Comparison of ASP, ALT and AST levels during four weeks (G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub>, and G<sub>4</sub>) of the experiment with C<sub>1</sub> group. (C<sub>1</sub>: control of first week, G<sub>1</sub>: first week, G<sub>2</sub>: second week, G<sub>3</sub>: thirt week, G<sub>4</sub>: fourth week).

Figure 3 demonstrates the results of histopathological studies of the liver following Wi-Fi<sub>2.45GHz</sub> exposure and the control group.

Histopathological studies indicate Wi-Fi<sub>2.45GHz</sub> induced changes including increased necrosis of liver parenchyma, hepatocytes displaying an

eosinophilic cytoplasm along with the appearance of polymorphism and hyperchrome chromatin, which may eventually result in cancer cell transformation. Pathological studies showed liver hyperemia in the portal vein and

central veins of the liver and observed near single nucleated cells. Overall, WiFi<sub>2.45GHz</sub> exposure resulted in the appearance of irregular and fibrotic cellular and tissue structures in the liver.



**Figure 3.** Histopathologic images of liver tissue, (a) normal tissue with no WiFi<sub>2.45GHz</sub> exposure, (b) liver tissue following WiFi<sub>2.45GHz</sub> exposure.

## DISCUSSION

Our results confirm the harmful effects of the waves. This effect was significantly additive. We are exposed to different sources of low energy electromagnetic field every day. There is no doubt that continuous exposure of the body to these electromagnetic fields significantly affects our cells [11, 12]. Here, we decided to study one of the most important and sensitive tissues of the body under the exposure of electromagnetic waves. The liver controls the body's metabolism and contains important enzymes. The electromagnetic field can alter the normal life cycle of cells and tissues. There are a large number of studies in the literature that confirm the effects of WiFi and electromagnetic waves exposure on the body, however, no standard scale is exists to determine the threshold in which electromagnetic waves start affecting cells and tissues in the body. It is critical to determine the risk threshold and maximum safe absorbed energy of the body induced by an electromagnetic field. These effects should be further studied and compared in different tissues in the body [13-15].

There are a large number of studies including M. Markov and Y.G. Grolier's research on the

possible effects of electromagnetic fields on cells, metabolism and cancer initiation. In 2013, M. Markov and Y.G. Grolier studied non-ionizing electromagnetic waves that a large portion of human population is exposed to and showed that electromagnetic fields from mobile phones can contribute to initiation of cancer [16, 17]. Most of the biological and clinical effects of electromagnetic fields involve oxidative stress and its action on cellular proteins. Liver enzymatic proteins play a key role in cellular metabolism [18]. The liver has a high metabolic rate that is normally dependent on the oxygen flow. In this regard, Amare and colleagues in 2009 suggested the use of antioxidants such as zinc and selenium to resolve electromagnetic field induced oxidative stress [19]. Our results also show changes in liver metabolism enzymes. In a similar study by N. Gumral and colleagues in 2009 on the effects of selenium administration on WiFi-induced oxidative stress revealed that WiFi-induced lipid peroxidation is decreased with selenium administration. More research in this area by G. Aynali and colleagues and M. Naziroglu and colleagues in 2013, 2012 and 2017 respectively, showed similar results. However, each group studied a different aspect

of WiFi wave's contribution in oxidative stress induction. It can be concluded that a part of change induced by electromagnetic fields in the body is related to generation of oxidative stress in the tissues [20-23]. The research on WiFi waves and its effects is not limited to metabolism and cancer. Kumar and colleagues showed that exposure to WiFi waves with 2.45 GHz energy increases caspase 3 and creatine kinase in sperms which results in low levels of testosterone and melatonin in mice [24]. Our group published a similar research in 2015 in the Journal of Paramedical Sciences which showed significant adverse effect of WiFi waves on sex hormones in male rats. A large number of studies are on the effects of electromagnetic fields on genetic makeup, physiology, immune system, metabolism and the structure of cellular components [25, 26]. High levels of AST, ALP and ASP are present in the liver, and their serum level elevates following liver injury [27]. In this work, changes in the serum levels of these three liver enzymes is an indication of WiFi-induced abnormalities in liver cells. Inflammatory abnormalities of liver cells results in acute increase of aminotransaminases serum levels that subsequently increases glucose renovation, alters liver aminotransaminase's (ALP, AST and ALT) activity. The activity of these three enzymes can be altered by different chemical, biological and physiological compounds and even by the changes in the Krebs cycle. Reduced Krebs cycle activity results in reduced production of intermediate compounds and AST, ALP and ALT activity [28-31]. Based on the results of enzymatic and pathological studies, it can be concluded that WiFi<sub>2.45GHz</sub> waves induce adverse effects on the liver which increases liver aminotransaminases levels. One of the effective factors in this research is the time limit. By increasing the time of receiving the WiFi<sub>2.45GHz</sub> waves, it will be possible to see more changes in the amount of enzymes. Finally, the importance of liver activity on other tissues and total metabolism of the body should also be considered. Despite all the data and reports on biological effects of electromagnetic fields, the range and rate of these effects has not been determined yet. More research is needed in this area to achieve this goal.

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*"The authors declare no conflict of interest"*

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