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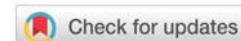
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Research Article

Behavioral impairments and biochemical alterations in brain following exposure to WiFi radiation and aluminum in rats

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Abstract

Today, WiFi radiofrequencies exposure is becoming almost unavoidable. Besides, aluminum is widely used in daily life despite its involvement in neurodegenerative diseases onset.

In this study, we investigated the effects of a 14 day exposure to WiFi radiation for 2 hours a day and aluminum chloride (AlCl₃) at 200mg/Kg/day alone and associated on behavior, oxidative stress, oligoelements homeostasis and metals accumulation in the brain of rats.

Results showed that WiFi radiation alone induced anxiety. Aluminum administration triggered anxiety, locomotor deficits and exploratory behavior impairments. WiFi radiation and aluminum association impaired emotional and exploratory behavior. At biochemical level, WiFi and aluminum co-exposure induced cerebral oxidative stress compared to other experimental groups. Moreover, aluminum intake increased cerebral aluminum content. WiFi radiation coupled with aluminum increased cerebral aluminum, iron and cadmium contents compared to control, WiFi and Aluminum groups, and lead content compared to WiFi and Aluminum groups. Our results reveal that WiFi radiation and aluminum, especially when associated, are harmful for the brain. Thus, it is prominent to limit the exposure to WiFi radiation and aluminum for healthy nervous system.

Abbreviations

Al: Auminum; AlCl₃: Aluminum Chloride; ANOVA: Analysis of Variance; °C: Celsius Degree; Ca: Calcium; CAT: Catalase; Cd: Cadmium; Cm: Centimeter; Cu: Copper; DNA: Deoxyribonucleicacid; EEG: Electroencephalogram; EDTA: Ethylenediaminetetraacetic Acid; Fe: Iron; GHz: Giga Hertz; GSH-Px: Glutathioneperoxidase; GST: Glutathione S-Transferase; h: hour; IARC: International Agency for Research in Cancer; Kg: Kilogram; LTE: Long-Term Evolution; MDA: Malondialdehyde; Mg: Magnesium; mg: milli gram; ml: milliliter; NO: Nitric Oxide; PLSD: Protected Least Significant Difference; Pb: Lead; rpm: rotation per minute; s: second;

SOD: Superoxide Dismutase; U: Unit; UMTS: Universal Mobile Telecommunications System; WiFi: Wireless Fidelity; Zn: Zinc

Introduction

The recent widespresad use of wireless devices and the emerging of the fifth technology generation are continuously rising worries about potential adverse health effects of the radiofrequencies emissions [1-3]. Wireless Fidelity (WiFi) technology operating at 2.45GHz is among the most important sources of these non ionizing electromagnetic radiation [4,5].

Radiofrequency radiation are reported harmful for immune [6-8], reproductive [9-11], cardiovascular [12],

endocrine [13] and other body systems. Although the brain is recognized among the most vulnerable organs to radiofrequencies [14,15], there are many controversies regarding neurological outcomes of these radiation in both laboratory animals [16] and humans [17]. Behavioral changes associated with radiofrequency radiation were due to several neural and molecular mechanisms [18] including changes in hippocampal lipidome and transcriptome [19] and an increase in vacuolization in brain tissues [13]. WiFi radiation are reported to be an important threat to human health [20]. They induced electrohypersensitivity and demyelinating syndrome leading to focal seizures, ataxia, vertigo and headaches in humans [21]. Radiofrequencies are classified as 2B agents (possibly carcinogenic to humans) by the International Agency for Research in Cancer (IARC) [22]. However, according to other studies, radiofrequency radiation do not increase brain tumor risk [23] and can be even used as a treatment of brain metastasis [24] and Alzheimer's disease [25-28].

Aluminum (Al) is among the most enriched metals in our biosphere. Despite its extensive use in daily life (adjuvanted vaccines [29] and other pharmaceuticals [30], food additives [31,32], cosmetics...), Al has unknown biological function [33]. This heavy metal is able to cross biological barriers, reach various body fluids, enter cells and accumulate in different organs, mainly in the central nervous system [33]. Al is associated with Alzheimer's disease [34], Down's syndrome [34], dialysis dementia syndrome [34], dementia [35] and epilepsy [36] in humans. Daily oral administration of aluminum chloride AlCl_3 (100mg/Kg) elicited cognitive impairments, anxiety and motor deficits in rats following a treatment of 15 days [37] and 42 days [38,39]. These aluminum-induced behavioral changes are due to several mechanisms including hippocampal and cortical oxidative stress [40], *in vitro* and *in vivo* neuronal and glial cell death [41], neurotransmission disruption [37,39,40], cerebral Al-catecholamine complexes generation [42] and amyloid plaques deposition [43].

In the present investigation, we were interested in neurological effects of concomitant exposure to WiFi radiation and aluminum for several reasons. Indeed, the brain is the target of both WiFi radiofrequencies and aluminum. Then, the daily and high exposure to WiFi and aluminum increases the risk of concomitant exposure to these two factors. Besides, coexposure effects of WiFi and aluminum have not been yet studied which emphasizes the interest of our investigation. Thus, the objective of this study is to evaluate the neurological effects of exposure to WiFi radiation (2 h/day) and aluminum chloride (AlCl_3) (200mg/Kg/day) alone and associated during 14 consecutive days in rats. For this purpose, we focused on emotional, locomotor and exploratory behavior (open field test), oxidative stress and oligoelements (iron, zinc and calcium) and metals (aluminum, lead and cadmium) contents in the brain.

Materials and methods

Animals and experimental design

Male Wistar rats weighing about 120 g were procured

from SIPHAT, Tunisia. The animals were kept under 12h/12h light-dark cycle conditions in a temperature- and humidity-controlled animal facility with free access to standard pellet diet and water. All procedures were approved by the Faculty Ethics Committee (Faculty of Sciences of Bizerte, Tunisia).

After an adaptive phase, the rats were randomised into four groups of six animals each:

- **Control group:** Rats received daily distilled water and were kept away from WiFi radiation sources during 14 days.
- **WiFi group:** Rats were daily exposed to 2.45GHz WiFi radiation for 2h/day and received distilled water (vehicle) along 14 days.
- **Chloride aluminum group (AlCl_3):** Rats were administrated daily AlCl_3 (200mg/Kg/day) without exposure to WiFi radiation for 14 days.
- **WiFi+ AlCl_3 group:** Rats were exposed daily to both WiFi radiation (2h/day) and AlCl_3 (200mg/Kg/day) during 14 days.

WiFi radiation are emitted for 2h/day from a D-Link® modem at 25cm away from exposure cage [44]. Chloride aluminum was dissolved in distilled water and administered to the animals *per os* at a dose of 200mg/Kg of body weight / day [45].

Behavioral assessment: Open field test

The next day (15th day), open field test was performed. The apparatus was a clear plastic circular area (1m in diameter) with a 50cm wall and divided into 1 central and 6 peripheral parts of equal surface [46]. Three identical objects were located in three peripheral parts of the apparatus. The rat is placed in the open field for one session of 5 minutes. Centre entries and time reflected anxious behavior. Locomotor abilities were evaluated through the locomotion time and the number of peripheral crossed quadrants. Objects exploration number and time serve for exploratory behavior assessment. Object exploration was defined as physical contact with an object with snout, vibrissae or forepaws. The open-field apparatus and the objects were wiped out using a 10% alcohol solution after every trial to eliminate olfactive clues.

Biochemical determinations

Oxidative stress markers assessment in brain: The lipid peroxidation was determined by Malondialdehyde (MDA) measurement according to the double heating method of Draper and Hadley [47]. The activity of Superoxide Dismutase (SOD) and Catalase (CAT) was assayed according to Misra and Fridovich [48] and Aebi [49] respectively. Protein concentration was determined by the method of Bradford [50].

Cerebral oligoelements and metals contents measurements

A part of each brain sample was incinerated at 50°C in the



oven until getting a residue of constant weight which was then cooled to room temperature. Concentrate nitric acid was added to the residue (in the rate of 3ml /100mg of dry weight and then 1ml for each extra 100mg) for sample recovery. Samples were then heated at 120°C in a thermoblock until mineralization. After cooling the resulting residue, its volume was increased to 10 ml with double distilled water. Aluminum (Al), iron (Fe), zinc (Zn), calcium (Ca), lead (Pb) and cadmium (Cd) concentrations were measured by inductively coupled plasma-atomic emission spectroscopy.

Statistical analysis

Statistical analysis of the obtained data were performed by Statview® software using analysis of variance (ANOVA) for comparison between groups, followed by a Fisher's Protected Least Significant Difference (PLSD) post-hoc test for multiple comparisons between all groups. Values for $p < 0.05$ and $p < 0.001$ were considered statistically significant. Data are given as mean ± standard error of the mean (SEM). Histograms were done by GraphPad Prism 5® software.

Results

Open field test

Emotional behavior: WiFi radiation and aluminum alone and associated induced anxiety by reducing ($p < 0.05$) center entries and time compared to control group (Table 1).

Locomotor behavior: Aluminum affected locomotor abilities by decreasing ($p < 0.05$) the locomotion time and the number of crossed peripherals parts of the arena (Table 1).

Objects exploration: Aluminum alone and associated with WiFi radiation decreased ($p < 0.05$) the number and the time of objects exploration compared to control group. Aluminum's effect on objects exploration time was even highly significant ($p < 0.001$). Aluminum diminished ($p < 0.05$) the number and the time of objects exploration compared to WiFi group (Table 1).

Oxidative stress in brain: The co-exposure to WiFi radiation and aluminum increased significantly cerebral MDA level compared to control ($p < 0.001$), WiFi ($p < 0.001$) and aluminum ($p < 0.05$) groups. WiFi exposure reduced SOD activity compared to control group ($p < 0.05$). WiFi and aluminum co-treatment, however, increased ($p < 0.05$) SOD activity compared to WiFi group reaching a comparable level as control one's. We

noticed no significant ($p > 0.05$) difference in CAT activity in all experimental groups (Figure 1).

Oligoelements and metals contents in brain

Following aluminum treatment, rats displayed a significant increase ($p < 0.05$) in cerebral Al content compared to control and WiFi groups (Figure 2). The co-exposure to WiFi and aluminum induced a significant increase in cerebral Al ($p < 0.001$), Fe ($p < 0.001$) and Cd ($p < 0.05$) contents compared to control, WiFi and aluminum groups. Besides, WiFi and aluminum association increased ($p < 0.05$) cerebral Pb content compared to WiFi and Al groups. We noticed no significant effect ($p > 0.05$) of the treatment on cerebral Zn and Ca levels (Table 2).

Discussion

Our results showed that a 14 day exposure to WiFi radiation (2h/day) induced anxiety. For a similar treatment period, aluminum administration (at 200mg/Kg/day) impaired emotional, locomotor and exploratory behavior. The latter was more altered by aluminum than by WiFi radiations. WiFi radiation associated with aluminum induced emotional and exploratory behavior deficits. At biochemical level, WiFi and aluminum co-exposure induced cerebral oxidative stress by increasing MDA level compared to control, WiFi and aluminum groups. WiFi exposure reduced SOD activity compared to control group. As for oligoelements levels and metals accumulation in the brain, Al administration increased Al content compared to control and WiFi groups. The co-exposure to WiFi radiation and Al increased Al, Fe and Cd contents compared to control, WiFi and Al groups and Pb content compared to WiFi and Al groups.

Behaviorally, we found that WiFi radiation (2h/day for 14 days) induced anxiety. Aluminum (200mg/Kg/day for 14 days) affected emotional, locomotor and exploratory behavior. The co-exposure to WiFi radiation and aluminum altered emotional and exploratory behavior. Exploratory behavior impairments were more important due to aluminum than WiFi radiation.

Previous studies proved hazardous impacts of radiofrequency radiation on behavior. WiFi exposure for 2h/day along 20 days induced an anxiety like behavior without impairing spatial learning and memory abilities in male rats [44]. Chronic exposure to 2.45GHz WiFi signal for 12h/day during 30 days impaired the discrimination between the novel and familiar objects in unimodal and multimodal object recognition tasks

Table 1: Open field test. Adult male rats (n=6) were exposed to none, WiFi radiation (2h/day), $AlCl_3$ (200 mg/Kg) and WiFi radiation + $AlCl_3$ for 14 consecutive days. Data are given as mean ± SEM.

	Control	WiFi	$AlCl_3$	WiFi+ $AlCl_3$
Number of center entries	4.33±1.84	0.83±0.40 a	0.33±0.21 a	1.00±0.52 a
Center time (s)	11.00±4.20	2.83±1.33 a	0.67±0.42 a	3.17±1.56 a
Number of crossed peripheral quadrants	36.50±9.14	27.50±5.05	19.00±2.38 a	21.50±4.01
Locomotion time in peripheral parts (s)	195.83±23.59	150.00±33.81	105.33±9.34 a	134.67±24.32
Number of objects exploration	18.17±3.66	14.00±3.23	4.50±0.85 ac	9.83±1.25 a
Time of objects exploration (s)	52.00±10.37	36.67±8.26	12.00±1.69 aac	19.83±2.65 a

a: $p < 0.05$ versus control group; aa: $p < 0.001$ versus control group; c: $p < 0.05$ versus WiFi group.

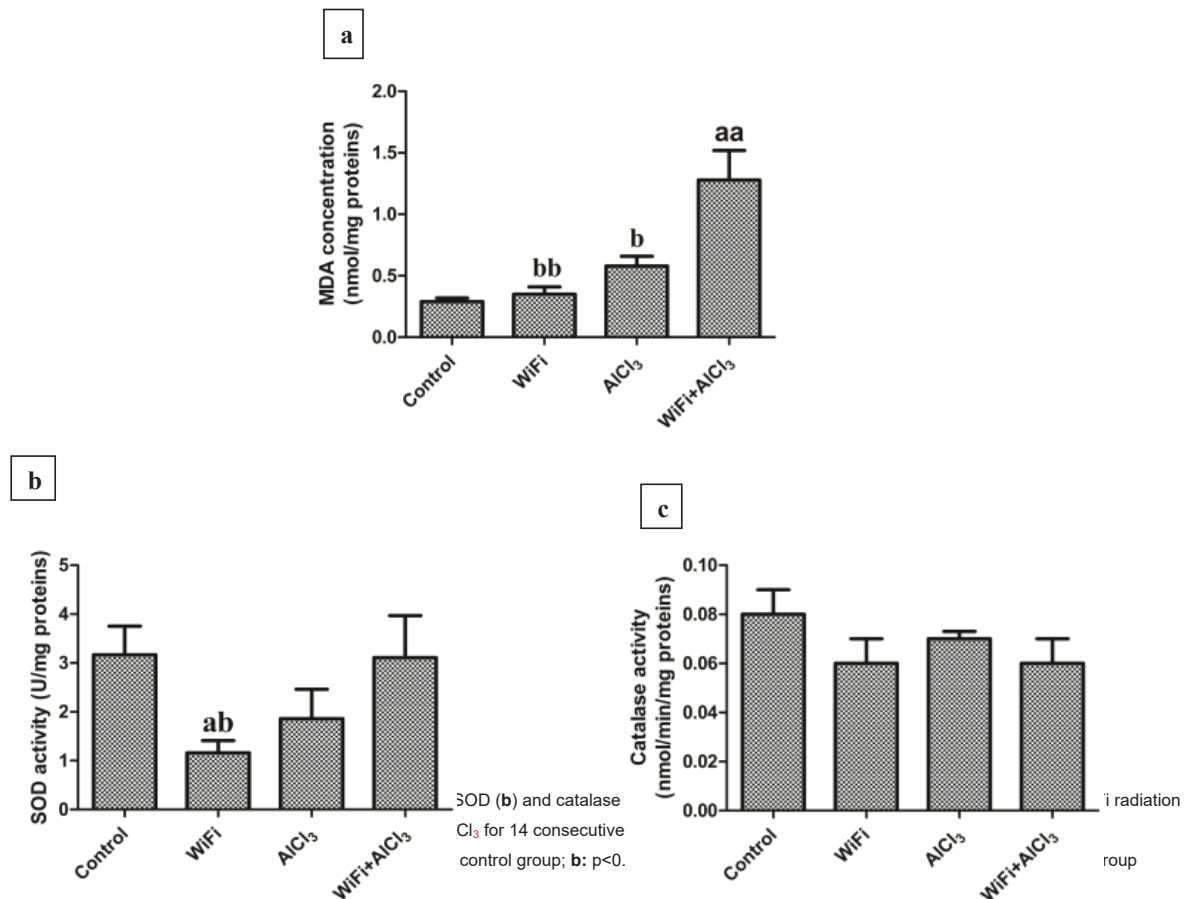


Figure 1: Oxidative stress in brain. MDA level (a), SOD (b) and catalase (c) activities. Adult male rats (n=6) were exposed to none, WiFi radiation (2h/day), AlCl₃ (200 mg/Kg) and WiFi radiation + AlCl₃ for 14 consecutive days. Data are given as mean ±SEM.

a: p<0.05 versus control group; aa: p<0.001 versus control group; b: p<0.05 versus WiFi+AlCl₃ group; bb: p<0.001 versus WiFi+AlCl₃ group

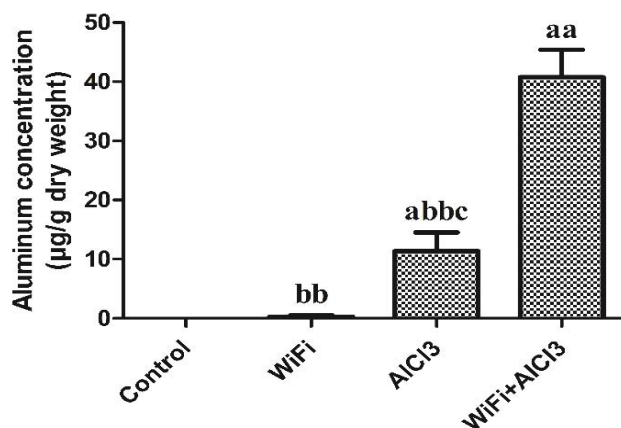


Figure 2: Aluminum concentration in brain. Adult male rats (n=6) were exposed to none, WiFi radiation (2h/day), AlCl₃ (200 mg/Kg) and WiFi radiation+AlCl₃ for 14 consecutive days. Data are given as mean ±SEM.

a: p<0.05 versus control group; aa: p<0.001 versus control group; bb: p<0.001 versus WiFi+AlCl₃ group; c: p<0.05 versus WiFi group

memory decline with anxiety in female rats [54] respectively. The exposure to 900 MHz radiofrequency radiation disrupted dendritic arborization pattern in basolateral amygdala leading to place preference alteration in adolescent male rats [55]. Radiofrequencies cause at least 13 neuropsychiatric effects including depression in humans [56].

However, according to a systematic review dealing with the effects of radiofrequencies exposure, mostly released by mobile phones, on cognitive behavior of laboratory animals, among 62 studies having been published since 1993, 21 studies reported significant impairments, 20 studies reported no significant effects, and 4 studies reported beneficial impacts [16]. Short-term radiofrequency exposure from new generation of mobile phones (Universal Mobile Telecommunications System (UMTS) and Long-Term Evolution (LTE)) reduced EEG alpha power with no effects on cognitive performance in healthy young-adult university students [57]. Emotional and behavioral problems in 5-year-old children were induced by radiofrequency radiation emitted by mobile phone base stations and indoor sources (contributing very little to radiofrequencies exposure) rather than mobile phone radiofrequencies (leading to an exposure peak in the head) [58]. Long-term exposure (2h/day for 8 months) to 1950MHz radiofrequencies exerted beneficial effects on Alzheimer's disease by rescuing hyperactivity-like

in male rats [51]. Long term exposure to 2.5GHz WiFi radiation (4, 6 and 8 weeks) increased anxiety and affected locomotor function in male rats [52]. The exposure to 2.45 GHz WiFi radiation for 2h/day along 40 days and for 4h/day during 45 days elicited learning and memory deficits in male rats [53] and



Table 2: Oligoelements and metals levels in brain. Adult male rats (n=6) were exposed to none, WiFi radiation (2h/day), AlCl₃ (200 mg/Kg) and WiFi radiation+AlCl₃ for 14 consecutive days. Data are given as mean ±SEM.

Oligoelements and metals (x10 ⁻³ µg/mg dry weight)	Control	WiFi	AlCl ₃	WiFi+AlCl ₃
Iron	3.00±1.00	1.00±0.32 bb	6.00±1.00 bb	21.00±4.00 aa
Zinc	1.00±0.47	1.00±0.46	2.00±1.00	0.39±0.02
Calcium	2.00±0.30	6.00±5.00	2.00±0.16	2.00±0.26
Lead	20.00±6.00	13.00±4.00 b	10.00±3.00 b	31.00±3.00
Cadmium	2.00±0.28	1.00±0.44 b	2.00±0.42 b	3.00±0.22 a

a: p<0.05 versus control group; **aa:** p<0.001 versus control group; **b:** p<0.05 versus WiFi+AlCl₃ group; **bb:** p<0.001 versus WiFi+AlCl₃ group; **c:** p<0.05 versus WiFi group.

and anxiolytic behaviors, improving cognitive deficits and increasing glucose metabolism in hippocampus and amygdala regions of the brain in Alzheimer's model mice [28].

Our results are in line with several previous investigations proving behavioral impairments of aluminum exposure. Indeed, AlCl₃ treatment at 50 mg/Kg for 15 days and 28 days resulted in spatial learning deficit in mice [59] and rats [43], respectively. Daily oral administration of AlCl₃ (100mg/Kg) for 15 days induced rat model of Alzheimer's disease by triggering anxiety, frontal-dependent motor deficits and cognitive decline [37]. AlCl₃ oral administration (100mg/Kg) for 42 days induced anxiety, spatial memory performance deficits and motor dysfunction, signs of Alzheimer's disease-like pathology in rats [38,39]. Memory impairments were also associated with chronic AlCl₃ exposure at 40mg/Kg and 175mg/kg for 60 days in mice [60] and rats [61] respectively, and at 150 mg/kg for 90 days in rats [62]. Chronic aluminum exposure at 12 and 72mg/Kg for 270 and 360 days resulted in learning, memory and exploratory behavior deficits in a dose and time dependent manner in rats [63]. Occupational aluminum exposure for a long time could be associated with an increased risk of cognitive impairments in aluminum workers [64,65]. Moreover, chronic aluminum (0,3%) exposure in drinking water during 4 months since the intra-uterine age led to locomotor impairments in rats [66].

As for brain oxidative stress, WiFi radiation and aluminum co-exposure increased cerebral MDA level compared to control, WiFi and Al groups. WiFi radiation alone reduced SOD activity compared to control and co-exposed WiFi and Al groups. According to several previous studies, WiFi radiation altered behavior through inducing brain oxidative stress especially at brain level. Indeed, WiFi is an important threat to human health causing oxidative stress [20]. For instance, repeated 2.45GHz WiFi exposure for 2h/day during 20 days increased cerebral MDA level and CAT activity in male Wistar rats [44]. The exposure to 2.45GHz radiation for 4h/day during 45 days elicited the depletion of brain antioxidant enzyme systems (SOD and CAT) and reduced glutathione levels leading to memory decline and anxiety behavior in female Sprague Dawley rats [54]. Long term exposure (2h/day for 6 months) to 900, 1800, and 2100 MHz mobile phone radiofrequencies increased lipid peroxidation and oxidative deoxyribonucleic acid (DNA) damage in the frontal lobe of the brain in male rats [67]. Continuous exposure to 2.45GHz radiofrequencies (24h/day for two months and a half) affected oxidative defense system

in male Wistar rats by decreasing total antioxidant capacity of plasma and the activities of several antioxidant enzymes, including CAT, glutathione peroxidase (GSH-Px) and SOD, and increasing glutathione S-transferase (GST) activity [68].

Regarding aluminum effects on oxidative equilibrium, AlCl₃ treatment 50mg/kg for 15 days increased the level of oxidative stress markers such as MDA, Advanced Oxidation of Protein Products (AOPP) and Nitric Oxide (NO) in various brain regions (prefrontal cortex, striatum, parietal cortex, hippocampus, hypothalamus and cerebellum) in mice [59]. Daily oral administration of AlCl₃ 100 mg/Kg for 15 days induced lipid peroxidation and SOD activity depletion in the prefrontal cortex and the hippocampus in rats [37]. Oral intake of AlCl₃ 100mg/Kg for 42 days caused oxidative stress in rat brain tissues leading to Alzheimer disease like symptoms [38,39,69]. AlCl₃ exposure at a dose of 17mg/Kg and 84mg/Kg for 4 weeks reduced brain total antioxidant status (decrease in GSH level and inhibition of GPx and SOD activities) with a subsequent increase in lipid peroxidation in male rats [70,71]. AlCl₃ treatment 175 mg/Kg for 60 days induced oxidative stress in the hippocampus and the frontal cortex in rats [61]. AlCl₃ administration 10mg/Kg and 150mg /Kg for 3 months increased lipid peroxidation and decreased GSH, CAT, and SOD activities in the brain [72] and mainly in the hippocampus in rats [62]. AlCl₃ exposure 10mg/Kg from day 6 to 64 of age induced hippocampal and cortical oxidative stress by reducing catalase activity and increasing lipid peroxidation in adult male rats [40]. Moreover, in vitro investigations showed that AlCl₃ (50µM) exposure caused reactive oxygen species generation in primary hippocampal neuronal cells [73] and hippocampal synaptosomes of rats [74]. AlCl₃ (500mg/L) added to the deionized drinking water starting from day 6 of gestation until just after weaning or until the age of 70 days postnatal life reduced tissue contents of vitamin C, GSH, total proteins and the activities of Na⁺/K⁺-ATPase and SOD and increased cerebral lipid peroxidation and NO level in rats [75].

The assessment of oligoelements and metals levels in the brain showed that aluminum treatment increased Al content compared to control and WiFi groups. The co-exposure to WiFi and aluminum increased Al, Fe and Cd levels compared to control, WiFi and Al groups. Furthermore, WiFi and Al co-exposure increased Pb level compared to WiFi and Al groups. There was no significant effect of WiFi radiation and aluminum neither alone nor associated on cerebral Zn and Ca levels. In



line with our deleterious effects of Al, a Camelford resident died following late-onset epilepsy development due to aluminum consumption in potable water. The case report showed aluminum-loaded glial cells and neuronal debris, aluminum accumulations in the occipital lobe and the hippocampus and abnormal calcifications in the occipital lobe [36]. Sub-chronic AlCl₃ administration at a dose of 2mg/Kg/day for 1 and 3 months induced dose-dependent Al accumulation in the brain and the blood of male rats. Surprisingly, the strong positive correlation between Al content in the brain and the blood for 1 month exposure disappeared by the third month probably due to neural barrier adaptation to Al exposure or the saturation of brain Al transport [76]. AlCl₃ exposure 50 μM caused Al accumulation in primary hippocampal neuronal cells of rats [73]. Aluminum measurements in brain tissues from 12 donors diagnosed with familial Alzheimer's disease revealed extremely high aluminum concentrations which proves aluminum's role in Alzheimer's disease development [77]. Besides, AlCl₃ administration 10mg/Kg for 3 months decreased seric Zn, Copper (Cu), Magnesium (Mg) and increased seric Fe in rats [72]. Mobile phone radiofrequencies exposure induced Fe and Al accumulations in the stratum granulosum of rabbit's cerebellum [78].

Conclusion

The present experiment demonstrated that exposure to WiFi radiation and aluminum alone and especially when combined altered emotional, locomotor and/or exploratory behavior through oxidative stress induction, oligoelements homeostasis disruption and metals accumulation in the brain. Therefore, the public should be aware and limit its exposure as much as possible to WiFi radiation and aluminum.

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