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GLUTATHIONE SCAVENGING EFFECT ON CELL PHONE AND SODIUM NITRATE INDUCED-OXIDATIVE STRESS

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ABSTRACT

The excessive use of mobile phones in the last decades has created much concern on the effect of emitted electromagnetic fields and specific absorption rate on human health particurly, head absorption rate. Also Sodium nitrate ($NaNo_3$) an inorganic compound can also affect neural tissue. Glutathione a non-essential amino acid which is known also as the brains master antioxidant, was recently found as a potent free radical scavenger and antioxidant. The objective of this study was to examine $NaNo_3$ and 900MHz mobile phone-induced oxidative stress that promotes production of reactive oxygen species (ROS) in neural tissue damage in cerebrum and cerebellum and the role of glutathione against possible oxidative neural tissue damage in these organs. Twenty –four rats were randomly grouped as follows: Group 1(normal control) Group 2($NaNo_3$ 50mg/kg given intra-peritoneal). Group 3(900MHz EMR exposure 30min/day for 10days, Group 4(NaNo3 50mg/kg given intraperitoneal + Cellgevity 500mg/70kg before the daily $NaNo_3$ injection). Group 5 (900MHz EMR exposure +Cellgevity before the daily EMR exposure). Group 6 (Cellgevity 500mg/kg given orally). Malondialdehyde (MDA), Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSH-Px), evaluated for the changes of antioxidant status. Sections of the tissues were demonstrated by H and E. In the study group, SOD was significantly increased in group 5 while MDA, GSH-Px, and Catalase activities decreased. There were also changes in histological architecture of the cerebrum and cerebellum. In conclusion, exposure to 900MHz EMR and injection of NaNO3induced oxidative stress supplement GSH (Cellgevity) reversed the effect of oxidative stress exhibiting neuroprotective effect.

KEYWORDS: Glutathione, Sodium Nitrate, Oxidative Stress, Neurotoxicity, Mobile phone, Neuroprotection.

INTRODUCTION

The uses of mobile phones in recent times have raised several questions about their safety. Mobile or cell phones are the most frequently used and widely acceptable means of communication. It is often held near the skull the anatomical bony cast of the brain and designed to emit specific range(s) of electromagnetic radiation (EMR) that affects the brain via the induction of oxidative stress (1). Oxidative stress is the specific cellular stress where the ideal physiological ratio of oxidants to antioxidant is altered in favour of oxidants (2). Essentially, it reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage (1). Consequently, it is one of the causative agents of increased production of free radical species and /or decrease in the effectiveness of antioxidant defences systems such as glutathione (2, 3).

Mobile phone- induced free radicals formation in tissues depend on the amount of emitted radiation absorbed and its frequency of exposure (4-6).The rate of exposure to electromagnetic field (EMF) is in the ultra-high frequency ranges between 300-3000 MHz (7). The effect on any part of the body depends also on its frequency of exposure and power. Evidence from previous study shows that exposure to the radio frequency radiations from mobile telephones or their base station could affect health, since biological systems interact resonantly (7).This damages cell components including proteins, lipids and DNA (7).

The specific absorption rate which is an expression of the amount of radio frequency power deposited in the head or body when device is transmitting. Exposure to non-ionizing radiation such as EMF causes headache, poor concentration, memory failure, drowsiness and anxiety in humans (8-10). In addition, food preservatives or additive have been shown to induce

oxidative stress as well (11). Sodium Nitrates ($NaNo_3$) compounds are toxic inorganic chemicals commonly found in food, environment and in the bodies of aquatic animals (11). The wide spread use of nitrate salts in agricultural fertilizers and produce, processed foods and pharmaceutical industries coupled with high rates of air-borne nitrogen compounds emission from industries and automobiles make the chances of exposure to these chemicals even more inevitable (12-13). In respective of the sourceof the sodium nitrate, the released reactive oxygen species (ROS) have been consistently implicated in tissue injury (1).Particular, amongst these ROS are superoxide anion (O_2), which is predominantly generated by the mitochondria, hydrogen peroxide (H_2O_2) produced from O_2 by the action of superoxide dismutase (SOD) and peroxyonitrite ($ONOO^-$), generated by the reaction of O_2 with nitric oxide (NO) (1). The ROS are scavenged by superoxide dismutase (SOD), glutathione peroxide (GSH-Px), Catalase (CAT) and Malondialdehyde (MDA) (14). These serve also as biomarkers for assessing oxidative stress and lipid peroxidation.

Furthermore, exogenous and endogenous antioxidants such as glutathione, vitamins C and E, and selenium are potent oxidant scavengers. Glutathione (GSH) exists as reduced or oxidized form is by far the most important antioxidant in most mammalian cells (15). Its thiol group is a potent reducing agent denoting electron to both enzymatic and non enzymatic reactions (16). Cellgevity has been identified in recent times as a new dietary supplement aimed at enhancing natural glutathione levels in the human body and targets removal of damaging toxins from the body, increase base energy levels and promote healthy joints. The major ingredients of cellgevity are the new nutritional compound known as Riboceine. This nutrient enables the body to produce an optimal amount of glutathione. Apart from Riboceine, cellgevity also contains several other ingredients to fight aging and promote health including alpha lipoic acid, broccoli seed extract, turmeric root extract, grape seed extract, quercetin, milk thistle, vitamin C, selenomethionine, Cordyceps, black pepper and aloe extract. Glutathione has the important function of destroying reactive oxygen intermediates and free radicals that are constantly being formed during metabolism (17- 20).

The major effect of oxidative stress is on the brain because it depends on oxygen as a major fuel to drive its activity (21). The cerebrum is the largest part of the brain in terms of structure, energy and oxygen utilization and responsible for perception, coordination and interpretation of bodily activities. Next to it is the cerebellum which controls voluntary movement of the body. Oxidant readily assesses and impact effect on these parts. Thus, this study was designed to access glutathione (cellgevity) scavenging effect on cell phone EMR and sodium nitrate induced oxidative stress in rats, evaluate oxidative stressbiomarkers and histological effect on the selected brain regions.The study may reveal a platform to understand the toxic effects and can further be used for the amendment in current guidelines of mobile radiation and agricultural product preservation practices. In furtherance, glutathione can be recommended for implementation as supplement in routine primary health care.

MATERIALS AND METHODS

Drug/Chemicals

Cellgevity (Cornerstone Research and Development, Inc. USA) was obtained from a drug store in Enugu, Nigeria. West Africa. Cellgevity (1000mg) was dissolved in 100ml of distilled water. Sodium Nitrate ($NaNO_3$) PubChem CID: 24268 and histological reagents were obtained from a chemical store in Ebonyi State, Nigeria.

Experimental Animals

Twenty-four (24) Wistar albino rats of both sexes weighing (150-200g) were used for the experiment. Rats were maintained in the Animal facility of the Department of Anatomy, Faculty of Medicine. Ebonyi State University, Abakaliki. The animals were kept in standard netted cages under controlled temperature (24-26ºC), humidity (55-60%), and photoperiod (12h of light and 12h of dark) for two week prior to the experiment. The rats were allowed free access to a commercial balanced animals chow diet manufactured by Top Feeds Nigeria limited and water *ad libitum* throughout the study period. Thereafter, the animals were randomly divided into six (6) groups, comprising four (4) animals per group and treated as shown below. The protocol for the study was reviewed and approved by the Institutional Animal ethical committee (IAEC) Ebonyi State University Abakaliki. Nigeria.

Experimental Design

- Group1 (control group) received 0.1ml saline 10 days orally
- Group2 Sodium nitrate ($NaNO_3$) induced with 50mg/kg for 10days intraperitoneally
- Group3 (EMR induced) 900MHz at 30min/day for 10days exposure
- Group4 50mg/kg of $NaNo_3$ and treated with 500mg/kg of Cellgevity for 10days
- Group5 EMR induced (900MHz) and treated with 500mg/kg of Cellgevity for10 days
- Group6 500mg/kg of Cellgevity for 10 days orally

Induction of Oxidative Stress:

Induction by Sodium Nitrate ($NaNO_3$)

Oxidative stress was induced using 50mg/kg of sodium nitrate ($NaNO_3$) per body weight. It was dissolved in 10ml of distilled water (vehicle) and administered intraperitoneally to the rats for 10days to groups 2 and 4.

Induction by Mobile Phone Emitting Electromagnetic Radiation (EMR)

In order to expose the animals to cell phone stimulated waves we constructed a plexi ruber cylinder of external (radius 15cm, height 30cm) and internal (radius 5cm, height 30cm). The animals were placed between the internal and external space during the experiment. The internal space was intended to prevent the animals from entering the mobile phone chamber. The phone (Nokia 3310) was placed on vibration mode while the animals were exposed to 900MHz for 30mins daily exposure time for 10days.

Analysis of Oxidative Parameters

At the end of the experiment blood was collected from the left ventricle of each animal in a vial containing 0.5 M EDTA under inhaled ether anaesthesia. Commercially available kits were used according to the respective manufacturer's protocol for the measurement of biomarkers of oxidative stress including Malondialdehyde (MDH, Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate the changes in antioxidant status.

Estimation of superoxide dismutase (SOD)

The assay mixture contained 1ml of pyrogallol-tris-DEPTA, 0.2ml of suitably diluted tissue and 0.8ml of water. The rate of pyogallol autoxidation is taken from the increase in absorbance at 420nm. The activity of SOD was expressed as units/min/mg proteins. One unit of the enzyme is defined as the amount of enzyme which inhibits the rate of pyrogallol auto oxidation.

Estimation of catalase (CAT)

Homogenize the tissue with phosphate buffer at 1 to 4ºC and centrifuge buffer and allow standing in cold condition with occasional shaking. The supernatants were combined and used for assay.H_2O_2-phosphate buffer was taken in one curette containing enzyme solution without H_2O_2 phosphate buffer at 240nm. It was noted for a decrease in the optical density from 0.450 to 0.400. This value was used for the calculations.

Estimation of glutathione peroxidase (GSH-Px): The reaction mixture consisting of 0.2ml each of EDTA, sodium azide and 0.4ml of phosphate buffer, 0.1ml of suitably diluted tissue was incubated at 37ºC at different time intervals. The reaction was arrested by the addition of 0.5ml of TCA and tubes were centrifuged at 2000rmp. To 0.5ml DTNB were added and the colour developed was read at 420nm immediately. The activity of GPx is expressed as µmoles of glutathione oxidized/minutes/mg protein.

Estimation of Malondialdehyde (MDA): MDA levels were estimated by double heating, the principle is the spectrophotometric measurement of the colour generated by the reaction of thiobarbituric acid (TBA) with MDA. 2.5 ml of supernatant in each centrifuge tube and the tubes were placed in a boiling water bath for 15min. after cooling in tap water, the tube were centrifuged at 100min and 2ML of the supernatant was added to 1ml of 6.7 gt^{-1} TBA solution in a test tube and allowed in a water bath for 15min. the solution was cooled in tap water the concentration of MDA was calculated by the absorbance coefficient of the MDA – TBA complex (absorbance co-efficient = $1.56 \times 10^5 cm^{-1} M^{-1}$) and is expressed as nano moles pergram units ($nM\ g^{-1}$) wet tissue.

Tissue collection

At the end of the blood sample collection, each rat was humanely sacrificed by chloroform inhalation and the brain was dissected, immediately rinsed with normal saline and fixed in 10% neutral buffered formal saline. Thereafter, it was manually processed for H and E staining for light microscopy investigation.

Statistical analysis

Data on the study were analyzed by one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 23, USA, followed by the student T test. Data are represented as the mean ± standard deviation (SD), $P<0.05$ was considered significant.

RESULTS

	MDA	SOD	GPX	CATALASE
Group I	3.70±0.44	16.77±1.43	21. 13 ± 2.12	20. 65 ± 0.91
Group II	3.27±0.12	17.81 ± 0.90	20.01 ± 0.49	20. 02 ± 0.52
Group III	3.28±0.18	17.14 ± 1.54	19. 70 ± 1.01	19. 48 ± 1. 48
Group IV	3.68±0.38	18. 46 ± 0.99	20. 19 ± 0.07	20. 18 ± 0.06
Group V	3.33±0.35	18. 76 ± 0.59*	20. 48 ± 0.52	20.69 ± 0.61
Group VI	3.46±0.10	18. 32 ± 1. 33	19.84 ± 0.81	19 93 ± 0.47
***P*=.05**	.172	.169	.461	.308

Table 1: Oxidative Stress Parameters, Means Value with Their Standard Deviation

The above table showed that the values of MDA, GSH-Px and CAT decreased insignificantly ($P \leq .05$) while the values of SOD increased insignificantly ($P \leq .05$). However, significant increase in SOD with value of .028 was observed in group 5 when compared to control ($P \leq .05$) in the post Hoc Test

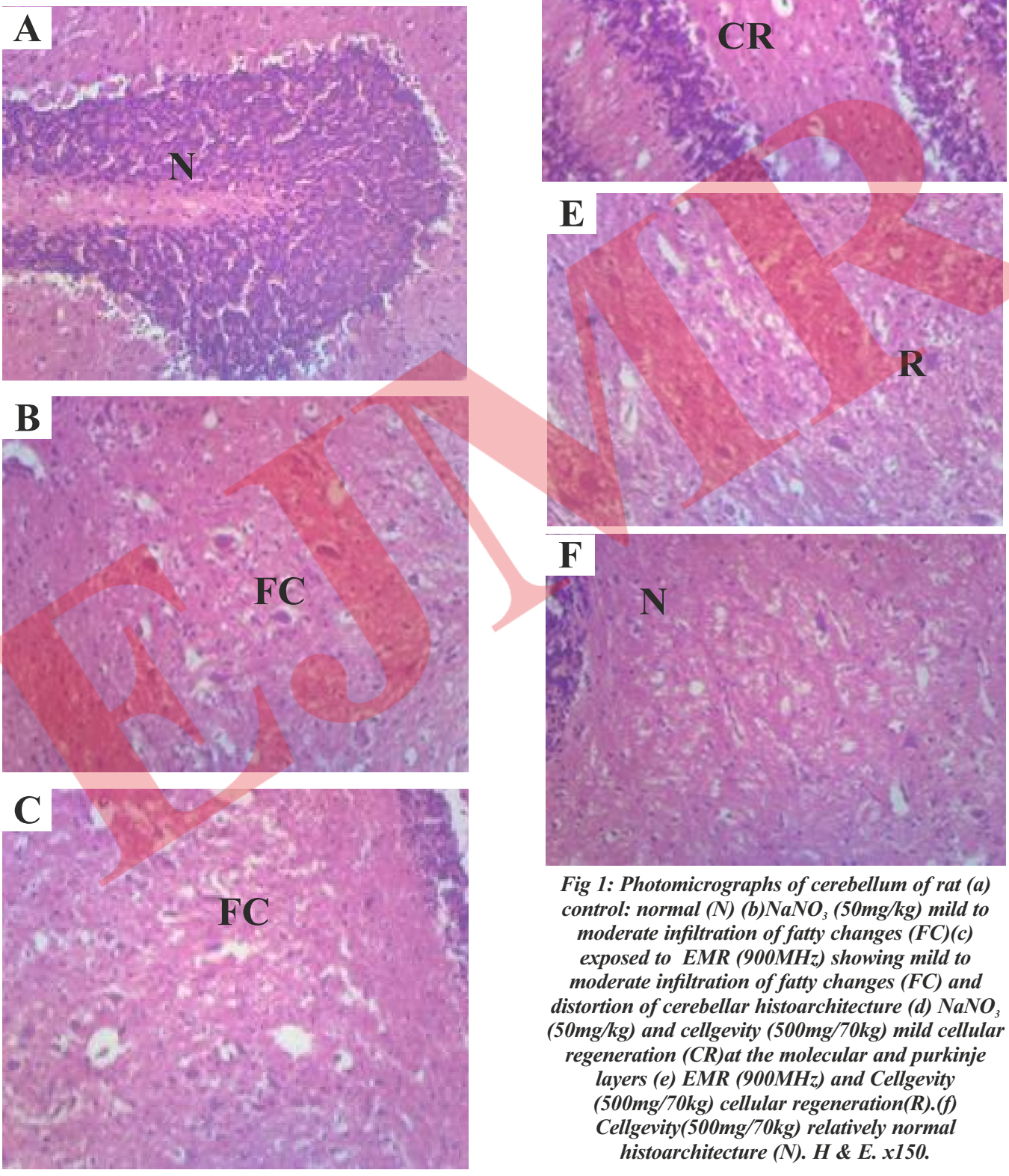

Fig 1: Photomicrographs of cerebellum of rat (a) control: normal (N) (b)$NaNO_3$ (50mg/kg) mild to moderate infiltration of fatty changes (FC)(c) exposed to EMR (900MHz) showing mild to moderate infiltration of fatty changes (FC) and distortion of cerebellar histoarchitecture (d) $NaNO_3$ (50mg/kg) and cellgevity (500mg/70kg) mild cellular regeneration (CR)at the molecular and purkinje layers (e) EMR (900MHz) and Cellgevity (500mg/70kg) cellular regeneration(R).(f) Cellgevity(500mg/70kg) relatively normal histoarchitecture (N). H & E. x150.

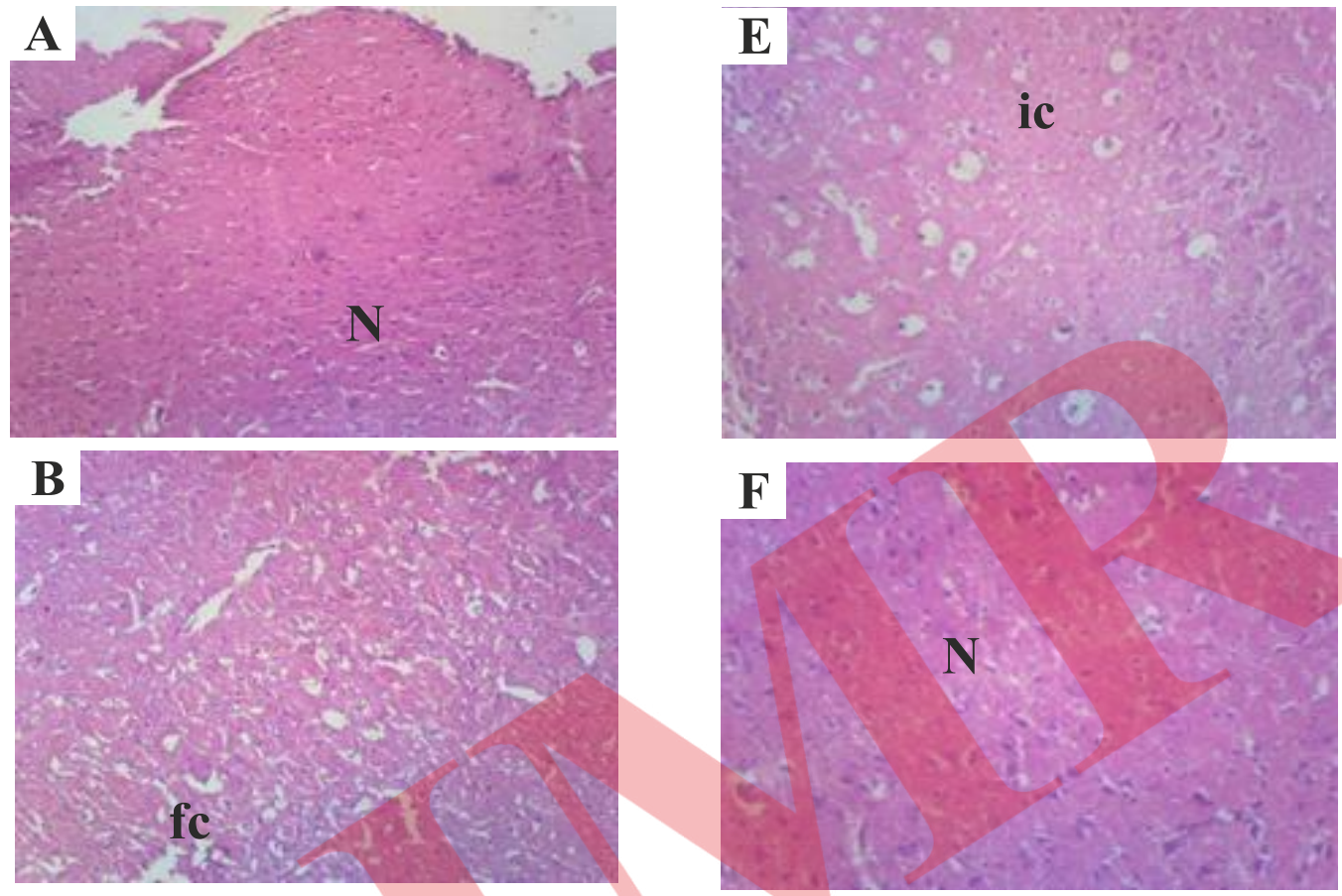

Fig 2: Photomicrographs of cerebrum of rat control (a) normal architecture. $NaNO_3$ (50mg/kg) induced (b) fatty changes (FC) and loss of brain architecture (NC). EMR (900MHz) fatty changes (FC) (c). $NaNO_3$ (50mg/kg) and Cellgevity (500mg/70kg) (d)EMR (900MHz) and Cellgevity (500mg/70kg) (e) prominent fatty changes (FC), mild to moderate infiltration of inflammatory cells(IC) and loss of brain tissue (necrosis) (NC). Cellgevity (500mg/70kg)(f) normal. H & E. x150

DISCUSSION

Sodium nitrate has been extensively used for the production of fertilizers, pyrotechnics and smoke bombs, glass and pottery enamels and food preservatives (especially meats) and can be absorbedinto the brain, liver, kidney and lungs where it produces cellular effect. Cell phones are also the most common device for communication that contributes to the pathogenesis of neurodegenerative diseases, affects some enzymes and their molecules. The close proximity of the mobile phone antenna to the brain has raised concerns about the biological interactions between EMR and brain regions such asthe cerebellum and cerebrum. Beside the use of mobiles phones, exposure to EMF from sources like base

stations and high voltage power lines have led to adverse health effects. The common effects include: eye irritation and cataracts, fatigue and exhaustion, anxiety, sleep disruption and depression. A few studies have reported serious effects like brain tumors, Alzheimer's disease, Parkinson's disease, eye cancer, leukemia and bleeding in Brain (16). The main mechanism identified with the environment oxidant is the induction of oxidative stress pathways.

This study evaluates the effect of oxidative stress on the brain using oxidative and lipid peroxidation markers as well as the histology of the selected brain regions. The constant exposure of the human brain to environmental pollutants alter the normal physiological state thus causing oxidative stress which occurs with increase in production of ROS or decrease in concentration of antioxidant (20). The decreased in the values of MDA, GSH-Px, and CAT indicate protection against lipid peroxidation and can be attributed to the supplementation with glutathione.Hence, it implies exposure to the inductors (sodium nitrate and EMR), actually causes oxidative imbalance in the region and Cellgevity ameliorate the effect. This was consistent with previous reports indicating that free radicals are involved in EMR induced tissue injury (4, 6). Glutathione treatment provided protection against free radical generation acting as an efficient free radical scavenger (15).

The histological effect on both the cerebrum and cerebellum signified the exposure to $NaNO_3$ and EMR exhibit similar effects which was characterised by fattychanges and necrosis following distortion of the cerebral and cerebellar histoarchtecture. Previous studies have implicated oxidative stress in the pathogenesis of neurodegenerative diseases (21, 22). Meanwhile, administration of Cellgevity in the treated groups revealed ameliorating effect with regeneration of neurons. It is known that neuroprotection is a relative preservation of neuronal structures and /or function which implies reduction in the rate of neuronal loss over time. This neuroprotective effect was attributed to the increase availability of glutathione in the form of Cellgevity (15). It is well reported that glutathione is an important neuroprotective molecule in the brain and increase neuronal GSH level has been viewed as primary approach to the treatment of neurodegenerative disease. Often, increased oxidative stress is a common mechanism behind neurodegeneration and neuroprotective treatments targeted at oxidative stress or excitotoxicity. The oxidative stress triggers neural cell death, neuroprotection is achieved by treatment regimens that include a glutamate antagonist and/or antioxidants. The glutathione antioxidant was able to limit oxidative stress induced by sodium nitrate and electromagnetic field radiation.

CONCLUSION

The study has established that exposure mobile phone electromagnetic radiation and sodium nitrate could cause release of free radicals. Treatment regimen with antioxidant was able to restore structural integrity of neurons in the brain regions. Put together it can be concluded that Cellgevity offered antioxidant and neuroprotective effects against oxidative stress. In furtherance, glutathione can be recommended for implementation as supplement in routine primary health care while we advocate the amendment in current guidelines of mobile radiation and agricultural product preservation practices.

CONFLICT OF INTEREST

All the authors do not have any possible conflicts of interest.

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