Cell Biochem Biophys
DOI 10.1007/s12013-013-9715-4

ORIGINAL PAPER

Effect of 3G Cell Phone Exposure with Computer Controlled 2-D Stepper Motor on Non-thermal Activation of the hsp27/p38MAPK Stress Pathway in Rat Brain

Kavindra Kumar Kesari · Ramovatar Meena · Jayprakash Nirala · Jitender Kumar · H. N. Verma

Abstract Cell phone radiation exposure and its biological interaction is the present concern of debate. Present study aimed to investigate the effect of 3G cell phone exposure with computer controlled 2-D stepper motor on 45-day-old male Wistar rat brain. Animals were exposed for 2 h a day for 60 days by using mobile phone with angular movement up to zero to 30°. The variation of the motor is restricted to 90° with respect to the horizontal plane, moving at a pre-determined rate of 2° per minute. Immediately after 60 days of exposure, animals were scarified and numbers of parameters (DNA double-strand break, micronuclei, caspase 3, apoptosis, DNA fragmentation, expression of stress-responsive genes) were performed. Result shows that microwave radiation emitted from 3G mobile phone significantly induced DNA strand breaks in brain. Meanwhile a significant increase in micronuclei, caspase 3 and apoptosis were also observed in exposed group ($P < 0.05$). Western blotting result shows that 3G mobile phone exposure causes a transient increase in phosphorylation of hsp27, hsp70, and p38 mitogen-activated protein kinase (p38MAPK), which leads to mitochondrial dysfunction-mediated cytochrome *c* release and subsequent activation of caspases, involved in the process of radiation-induced apoptotic cell death. Study shows that the oxidative stress is the main factor which activates a variety of cellular signal transduction pathways, among them the hsp27/p38MAPK is the pathway of principle stress response. Results conclude that 3G mobile phone radiations affect the brain function and cause several neurological disorders.

Keywords 3G mobile phone · Heat shock proteins · Cell signaling pathway · Apoptosis · DNA strand break

Introduction

Over three billion people worldwide currently using mobile phones, with demand expected to increase continuously in coming years (GSM World 2008). Most of the research studies mainly focused on the mobile phone (MP) exposure employed at 2nd generation (2G) technologies, and comparatively less research has been examined the newer 3rd generation (3G) technologies [1–5]. Most 2G phones use the Global System for Mobile Communication's (GSM) standard, which pulses 890–960 MHz and 1710–1880 MHz (GSM900 and GSM1800, respectively) signals at 217 Hz, whereas most 3G phones use the Universal Mobile Telecommunications System's (UMTS), W-CDMA2 air interface standard, which operates at a higher frequency range of 1900–2170 MHz and is without periodic pulsed modulation content. There is evidence that the frequency components may be very important for mobile phone-related biological effects [6].

Mobile phones transmit and receive signals via electromagnetic fields (EMFs) that are partly absorbed by the MP user. As MPs are commonly used in close proximity to the head, this feature has led to concerns about possible adverse effects on brain system. The effects of EMFs

Kavindra Kumar Kesari and Ramovatar Meena have contributed equally to this study.

K. K. Kesari (✉) · H. N. Verma
School of Life Sciences, Jaipur National University, Jaipur 302017, Rajasthan, India
e-mail: kavindra_biotech@yahoo.co.in

R. Meena · J. Nirala · J. Kumar
School of Environmental Sciences, Jawaharlal Nehru University, New Delhi 110067, India

Published online: 15 August 2013

emitted by mobile phones on the central nervous system (CNS) have become a particular focus of concern owing to the fact that mostly mobile phones are kept near head during talking mode and are in close proximity to the brain [7–9]. During these operations, the antenna of a cellular phone emits radio frequency electromagnetic fields that can penetrate 4–6 cm deep into the human brain [10, 11].

Thus, investigation of potential effects of EMF irradiation from MPs is an important health issue. Numerous studies have investigated the potential impact of Global System for Mobile Communication (GSM) MPs EMF on various brain functions in animal (in vivo) as well as in vitro model with both positive and negative findings [12–15]. In contrast, there have been few systematic investigations reported to date on the possible effects of Universal Mobile Telecommunication System (UMTS) EMFs emitted by the new generation (3G) of MPs with present exposure model [4, 5, 16, 17].

Previous studies on 2G phones from our group investigated the causative effects of these radiations on brain function, where decreased melatonin level, changes in antioxidant enzymes (SOD, GPx, CAT), decreased creatine kinase activity, DNA double-strand breaks, decreased kinases (Protein kinase C and histone kinase) activities, were reported [14, 18–20]. Although at proteomics level, Nylund and Leszczynski [21] have reported that exposure to human endothelial cell line EA. hy926 from mobile phone radiation changes the proteins expression in various cells.

Among these proteins whose expression is induced by mobile phone irradiation are transcription factors, including c-Jun and c-Fos [22, 23], and HSPs (heat shock proteins) such as Hsp27 [24] and Hsp70 [25, 26], but not Hsp90 levels that are actually reduced [26]. The elevated expression of these proteins may participate in the induction of various cellular processes that appear to be affected by mobile phones radiations [27], which include replication [21], cell-cycle progression [28], and apoptosis [26, 29]. Major mechanism that regulates transcriptional activity in response to extracellular stimuli is the activation of the MAPK (mitogen-activated protein kinase) cascades. These cascades are a group of signal transduction pathways which mediate the effects of various stimuli to regulate essentially stress response [30, 31]. Indeed, it has been shown that long exposure to mobile phone irradiation can activate the p38MAPK, c-Jun amino-terminal kinases (JNK), and extracellular signal-regulated kinases (ERK) cascades [21, 32]. These changes in activity of MAPKs can consequently regulate the physiological response of the exposed cells and organisms, due to the effects of electromagnetic fields at mobile phone frequencies. By comparing with previous data, present study found more significant biological effects of 3G MPs in whole brain function. This is because biological effects mainly depend on various factors, i.e., frequency, power density, exposure system, distance, time, etc. Present study has been focused on 3G mobile phone radiations effects on brain functions with 60 days of exposure. Numbers of parameters have been proposed to identify the effect of 3G cell phone on brain by comparing with our previous studies on 2G [14, 15, 17, 18]. At this time, the exposure setup of 3G mobile phone has also been designed by keeping in mind that the exposure should be in natural condition as we are exposed during talking mode. With this setup, several molecular and biochemical parameters (Induction of oxidative stress, expression of stress-responsive genes, DNA fragmentation, DNA double-strand break, micronuclei formation, and apoptosis) have been performed. This is an important study to find out the possible mechanism of the stress-induced adverse effects of 3G mobile phone on brain functions.

Materials

RNase, proteinase K, low melting point agarose, dimethyl sulfoxide (DMSO), (*N*,*N*-diethyl-*para*-phenylenediamine (DEPPD), and propidium iodide (PI) were purchased from Sigma-Aldrich Co., USA. Primary monoclonal antibodies including antiphospho-p38MAPK, antiphospho-Hsp-27, antiphospho-Hsp-70, anti-Bax, anti-Bcl-2, anti-cytochrome-c, anti-β actin, and AP-labeled goat anti-rabbit immunoglobulin and AP-labeled goat anti-mouse immunoglobulin were obtained from Santa Cruz Biotechnology. Polyvinylidene difluoride (PVDF) membranes were purchased from Millipore Corporation, USA. The rest of the chemicals were purchased from a local chemical company.

Methods

Animals Exposure

Forty-five-day-old male Wistar rats (200 ± 10 g) were used in the present study. Animals were divided into three groups, cage control ($n = 6$), sham exposed ($n = 6$), and exposed group ($n = 6$) and all the experiments were repeated thrice. The animals were maintained as per guidelines and protocols, approved by the Institutional Animal Ethics Committee. The animals were housed in clean polypropylene cages in an air conditioned room, where the temperature was maintained on 25–27 °C, with constant humidity (40–50 %) and kept on 12L:12D photocycle. The animals were fed on standardized normal diet (Tetragon Chemie Private Limited, Bangalore) and water ad libitum.

Exposure Setup

Wistar rats were used in the present study to observe the effect of 3G mobile phone exposure. Each rat was kept separately inside the Plexiglas box for the exposure of 2 h/day up to 60 days. Exposure setup has been designed by keeping in mind that human operate (hold) the mobile phone actually is not in stationary condition. Human generally need to use the mobile phone by tilting it has reference to parallel axis. Such concept suggests that the maximum power received was depending on the mobile phone angle as per our exposure system designed. It means that the mobile phone tilting up to zero to 30° shows that the reflected power decreases due to change in increased mobile phone angle. Here, an induced field was measured using monopole antenna, immersed in the exposure rectangular cage. The mobile phone was stepping up and down from zero to 30°, which was connected with steeper motor control by the computer system. The movement of the motor is restricted to 30° with respect to the horizontal plane. Such command was set as per software programming with angular movement at a pre-determined rate of 2° per minute. This is an automatic exposure system designed to perform as stepper motor control. It was working for automation-based system where bipolar stepper motor moved at 1.8°/step (stepper motor minimum movement 1.8°/steps it means that 200 steps in the one revolution on 360° completion). 3G cell phones were used for the purpose of exposure which was energized by another active mobile phone on video calling mode (without vibration and ringing tone) in the sending (Tx) and receiving (Rx) conditions. In respect to sham-exposed group, the condition was same but without energizing the mobile phone. The measured data for induced fields in various locations are used to compute corresponding SAR values. Method of the power measurement inside the cage with and without animal measures the power using monopole antenna transmitted power of mobile phone (Fig. 1a–d). Automatic system which was fully computer controlled, requires several parameter to get enter the values in formulation for measurement as indicated in Fig. 1b.

1 Total exposure time
2 Set the maximum angle
3 Holding of the position of mobile phone
4 Set the value of cycle time rate

Power Measurement

Presently, we used an automated SAR measurement system along with mobile phone for compliance testing at 2115 MHz. The system is operated by its angular movement using a computer controlled stepper motor, fitted with a miniature tip monopole antenna, used to measure induced field, which in turn is used to compute spatial SAR variations.

Plexiglas material was used to fabricate an exposure cage (rectangular box) where, dimension of the cage (215 × 65 × 25 mm) with fixed holes (2 mm diameter, upper plate thickness of the cage 2 mm) was designed for the purpose that animals may stay stress-free and in air ventilated condition. Power measurement was carried out inside the cage with different angles. A monopole antenna was positioned to measure outputs at various angle of the mobile phone (with respect to mobile phone antenna positioned horizontal plane). Fields computed by Monopole antenna were recorded by Agilent power meter (Model-U2000 Series USB Power Sensors). Values of the induced fields so obtained were used to compute SAR values at various angles during the mobile phone movement. Exposure was given by mobile phones having a time average specific absorption rate of 0.9 W/kg, as mentioned by the manufacturers. For the purpose of calibration, the emitted power of mobile phones was measured by using a monopole antenna. We recorded the experimental SAR value 0.26 W/Kg in 3G mobile phone-exposed groups. SAR has been calculated by equation ($SAR = \sigma.E^2_{RMS}/\rho$). The angle between mobile and the rat head is kept small and SAR value shows a significant variation in near field range.

Biological Parameters

Preparation of Tissue Homogenates and Cell Extracts

Immediately after the exposure period, one rat at a time was anesthetized then decapitated and its brain was dissected. The whole brain was washed four times with phosphate-buffered saline (PBS) (1.37 mM NaCl, 4.3 mM Na_2HPO_4, 2.7 mM KCl, 1.4 mM KH_2PO_4, pH 7.4) to remove red blood cells (RBC). Then brain tissue was minced into small pieces by using a tissue press (Biospec Products Inc., USA) and a single cell suspension was made by using a pipette.

The single cell suspension of brain was centrifuged at 2000 rpm and pellet was rinsed with ice-cold PBS and once with ice-cold homogenization buffer (25 mM HEPES pH 7.5, 0.3 mM NaCl, 1.5 mM $MgCl_2$, 0.5 mM dithiothreitol, 20 mM β-glycerophosphate; buffer H). Cells were then centrifuged at 40,000 rpm for 30 min at 4 °C. The resulting supernatants were immediately frozen in liquid nitrogen and stored at −80 °C for further biochemical assays. Protein concentrations were estimated with BSA standards.

Total Reactive Oxygen Species (ROS)

0.1 g of whole brain tissue was homogenized with addition of 1:9 (W/V) phosphate-buffered saline (0.1 M Na_2HPO_4,

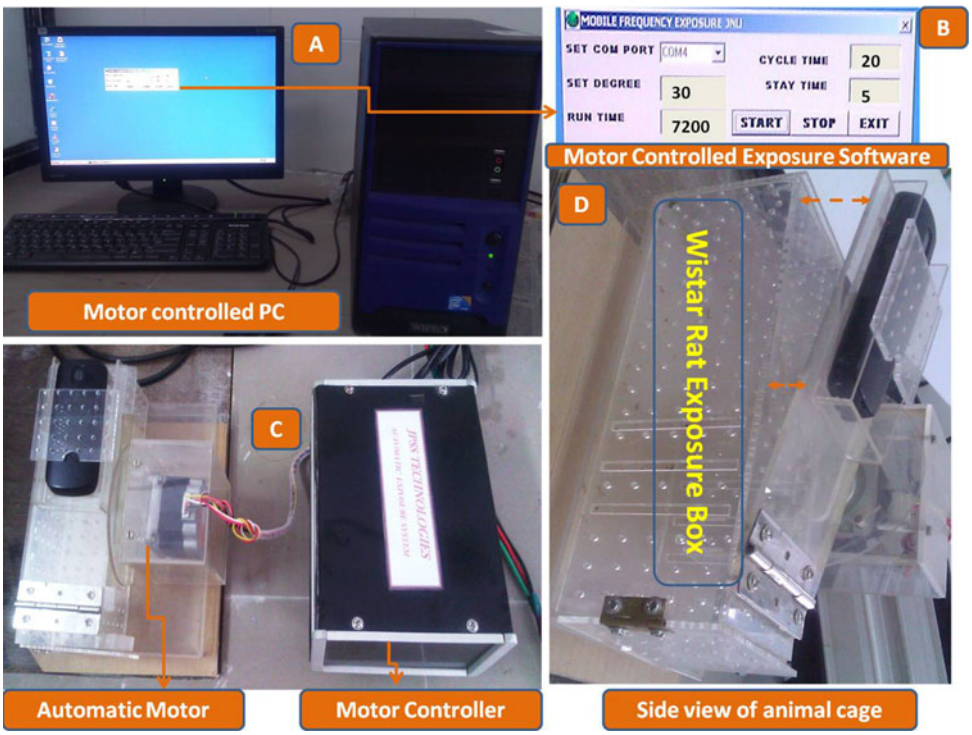


Fig. 1 The four different views and their functions (**a**, **b**, **c**, **d**) of automatic computer controlled stepper motor system for mobile phone exposure. **a** The computer system in which software has been installed and also indicating the several parameter to get enter the values (*visible on screen*) in formulation for measurement and controlling the motor fitted exposure system. **b** Indicating the required parameters which need to feed in software to operate the setup. **c** The *top view* of mobile phone exposure system where mobile phone got attached on the *top of the box* and connected with stepper motor controller system. **d** The *side view* of exposure box, indicating the angular movement from 0 to 30°

0.1 M KH_2PO_4, 1.37 M NaCl, 2.7 mM KCl, pH 7.4). The homogenates were centrifuged at 14,000 rpm for 10 min at 4 °C (Eppendorf centrifuge 5418 R, Germany) and supernatant was used for ROS measurement. ROS level was measured by employing DEPPD staining [33]. ROS levels in sample were calculated from the calibration curve of H_2O_2 and expressed as equivalent to levels of hydrogen peroxide (1 unit = 1.0 mg H_2O_2/l). Calibration curve for the standard solution was obtained by calculating slopes from optical density graph.

DNA Double-Strand Breaks

A comet assay, also known as single cell gel electrophoresis is used to determine DNA damage. From the brain cells suspension, 10 µl of its suspension was mixed with 0.2 ml of 0.5 % agarose. Agarose was suspended in phosphate-buffered saline (PBS) and was kept at 37 °C to maintain physiological condition. The mixture was pipetted out and poured on the glass slides which were precoated with 0.8 % agarose and immediately covered with coverglass (24 mm × 50 mm). These slides were kept in an ice-cold steel tray on ice for 1 min to allow the agarose to settle the gel. Again a layer was made over the gel with 100 µl of 0.8 % agarose as before, after removing the coverglass. These slides were immersed in ice-cold lysing solution (2.5 M NaCl, 100 mM EDTA disodium salt, 1 % Triton-X 100, 10 mM Tris–HCl, pH 10) and kept overnight at 4 °C. After lysing overnight, the slides were removed and subjected to DNA unwinding in electrophoresis buffer (0.3 M NaOH, 2 % DMSO, 10 mM EDTA, pH <13.0) for 20 min in the dark at room temperature in a horizontal slab of an electrophoresis assembly. The slides were electrophoresed at 0.7 V/cm and 250 mA for 30 min. The slides were neutralized in neutralization buffer (0.5 M Tris–HCl, pH 7.5) for 20 min followed by dehydration in 70 % ethanol. All slides were air dried at room temperature and stained with 20 µg/ml EtBr (ethidium bromide) dye. The tail length, tail moments, and tail migration were measured by using Comet Assay IV software (Perspective Instruments, Haverhill, Suffolk, UK) as a function of DNA damage. For measurement, 100 comets were scored and analyzed for each sample [18].

Micronucleus Assay: Flow Cytometry

Micronuclei assay followed as per Kesari et al. [34]. Blood sample was collected after 60 days of exposure. Blood samples were washed with 5 mL of PBS and centrifuged at 300×*g* for 5 min. The pellet was suspended in 100 mL PBS and the cells were fixed in 5 mL fixative for 5 min.

Sample was stained with acridine orange [35]. After staining, the samples were centrifuged at 3000 rpm for 5 min. The supernatant was aspired and the pellet was dissolved in 1 mL PBS. Samples were analyzed on Flow Cytometer (Beckman Coulter) equipped with 15 mW argon laser. All the events were recorded on forward scatter (FSC, linear scale, related to cell size), side scatter (SSC, log scale, related to cellular complexity), DNA fluorescence (FL1 log green fluorescence, 530 nm), and RNA fluorescence (FL4 log red fluorescence, 675 nm).

Micronucleus Assay: Confocal Microscopy

Whole blood smears were collected on the day following the mobile phone exposure. Whole blood smears were prepared on clean microscope slides, air dried, fixed in methanol, and stained with acridine orange (125 μg/ml in pH 6.8 phosphate buffer) for 1 min just before the evaluation with laser scanning confocal microscope (CSLM). The frequency of poly chromatic erythrocytes (PCEs) per total erythrocytes was determined using a sample size of 1000 erythrocytes per animal. The number of micro nucleated poly chromatic erythrocytes (MNPCEs) was determined using 1000 PCE per animal. Briefly, immature erythrocytes, i.e., PCEs were identified by their orange–red color, mature erythrocytes by their green color, and micronuclei by their yellowish color.

Measurement of Caspase 3 Activity

The activity of caspase 3 was measured using the colorimetric caspase-3 assay kit. Briefly, the homogenized whole brain was centrifuged at 3000 rpm for 10 min at 4 °C. The pellet was then resuspended in lysis buffer for 20 min and centrifuged at 20,000×*g* for 20 min at 4 °C, and the supernatant was collected. The assays were conducted in 96-well plates, and all the measurements were carried out in triplicate. The caspase 3 colorimetric assay is based on the hydrolysis of the peptide substrate acetyl-Asp-Glu-Val-Asp p-nitroanilide (Ac-DEVD-pNA) by caspase 3, resulting in the release of p-nitroaniline (pNA) moiety. To assess the specific contribution of caspase 3 activity, Ac-DEVD-pNA substrate (2 mM) was added to each well, according to the manufacturer's protocol. The plates were incubated overnight at 37 °C to measure caspase 3 activities. Absorbance was measured with a microplate reader (Spectromax M2) at 405 nm. Caspase 3 activity was expressed in μmol pNA released per min per ml of cell lysate at 37 °C.

DNA Fragmentation Assay

DNA ladder assay was conducted according to the protocols [36], with a little modification. Genomic DNA was isolated from brain sample of cage control, sham exposed and exposed rat according to method of Sambrook et al. [37] with slight modification. DNA samples were analyzed by horizontal electrophoresis for 5 h in 1.2 % agarose gel prestained with 1 mmol/l ethidium bromide (EB). The DNA was visualized under UV light and photographed [38].

Quantification of Cell Death

FACS analysis using PI staining detects cell death by means of the dye entering the cells along with changes in the target cell membrane and DNA damage. For the cell death assessment, the brain single cell suspension with cell density of 2×10^5 cells per ml was stained with PI (2.5 μg/ml) and incubated for 5 min at room temperature. Then, cells (10,000 per sample) were analyzed on a FACScan flow cytometer, using Cell Quest software.

Western Blotting

Samples (20–40 mg of protein) from cell lysates were separated by SDS-polyacrylamide (10 %) gel electrophoresis and electrophoretically transferred to polyvinylidene difluoride membranes (Millipore Corporation, USA). After blocking with 5 % nonfat dry milk, the membranes were incubated with dilute solution (1:1000) of primary monoclonal antibodies including antiphospho-p38MAPK, antiphospho-Hsp-27 & Hsp70, anti-Bax, anti-Bcl-2, anti-cytochrome-c, and anti-β actin (Santa Cruz, USA) overnight at 4 °C. Then, the membranes were exposed to the secondary antibodies (1:1000 dilution), directed at a species-specific portion of the primary antibody. AP-labeled goat anti-rabbit immunoglobulin and AP-labeled goat anti-mouse immunoglobulin were purchased from Santa Cruz Biotechnology (Santa Cruz). The protein bands were observed using BCIP/NBT color development substrate (Sigma Aldrich, USA).

Statistical Analysis

All data were expressed as mean ± standard deviation (SD). The statistical software SPSS for windows 13.0 was used to perform a post hoc multiple comparison test such as Fisher's least significant difference (LSD) and *t* test following a one-way analysis of variances (ANOVA). $P < 0.05$ was considered as statistically significant difference between two groups.

Results

Oxidative Stress (OS)

The mean ROS value in brain of the 3G mobile phone-exposed group (37.52 ± 4.39 U/l) was significantly higher

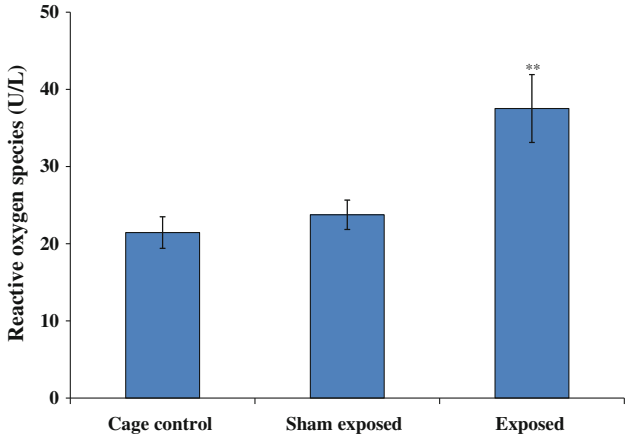


Fig. 2 Comparative reactive oxygen species level in brain of sham exposed, cage control, and 3G mobile-exposed Wistar rat measured by DEPPD assay. ROS is expressed in units. One unit is equal to 1 mg/l H_2O_2. ROS value is calculated from standard curve. *Asterisks* indicates statistically significant results with statistical variation of mean ± SD

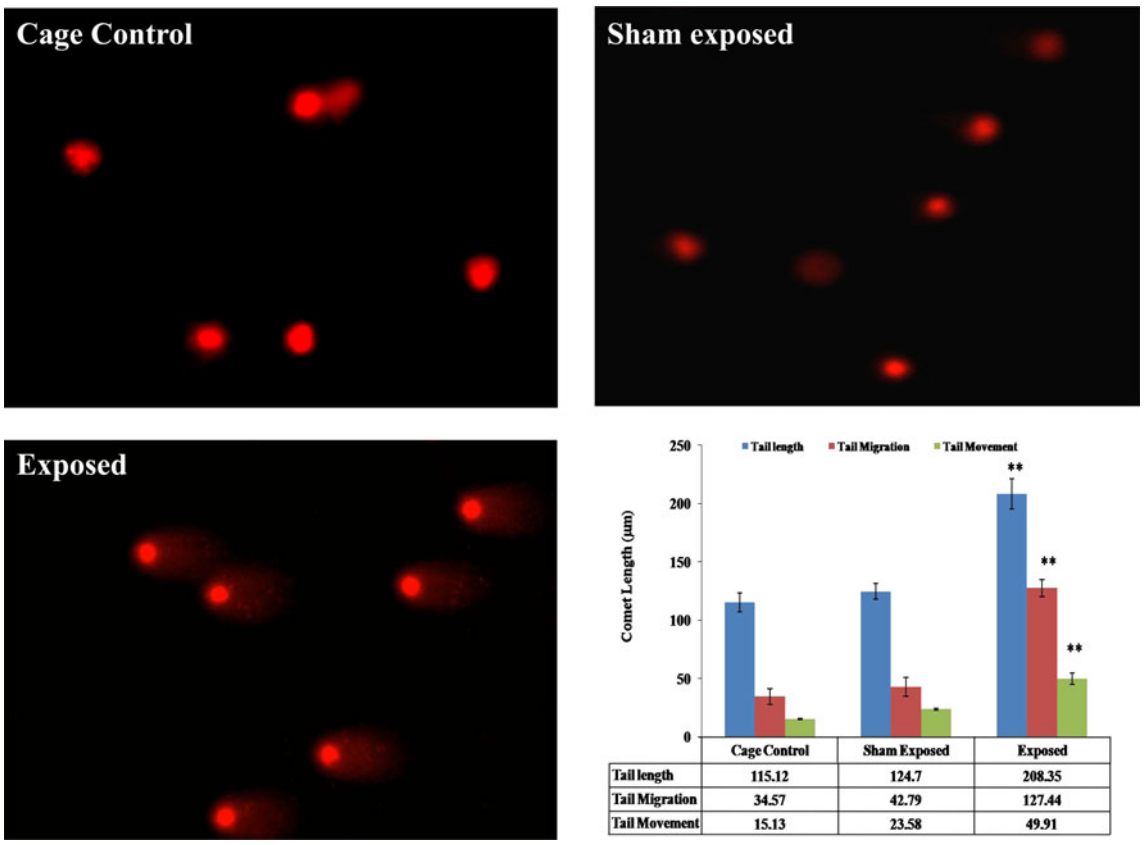
than the sham-exposed (23.75 ± 2.04; $P < 0.005$) and cage control (21.44 ± 1.97; $P < 0.001$) groups (Fig. 2). These results indicate that 3G mobile phone-induced oxidative stress in brain, which leads to adverse effects.

DNA double-strand break

In the qualitative picture of DNA double-strand break, larger tail length in brain cells was observed in 3G mobile phone-exposed group of animals as compared to cage control and sham-exposed ones (Fig. 3). The quantitative result shows that there is a significant increase in tail length, tail migration, and tail movement of DNA in exposed group as compared to the sham exposed (where P values for tail length $P < 0.002$; tail migration $P < 0.001$; and tail movement $P < 0.003$) and cage control (where P values for tail length $P < 0.001$; tail migration $P < 0.003$; and tail movement $P < 0.002$) groups. These results indicate that a prolonged exposure to cell phone radiation enhances the DNA double-strand breaks in brain cells and causes neurological disorders.

Determination of Micronuclei

The micronucleus test results obtained for male Wistar rats exposed with 3G mobile phone for 60 days: the mean number of MNPCE was significantly increased (132.66 ± 8.62) in mobile phone-exposed group as compared to cage



	Cage Control	Sham Exposed	Exposed
Tail length	115.12	124.7	208.35
Tail Migration	34.57	42.79	127.44
Tail Movement	15.13	23.58	49.91

Fig. 3 Comet images of brain cells among the 3G mobile phone exposed, sham exposed, and cage control groups. The *graphical representation* of quantitative analysis of DNA strand breaks in brain cells of mobile phone exposed to Wistar rats as indicated in figure. *Asterisks* indicates statistically significant results with statistical variation of mean ± SD

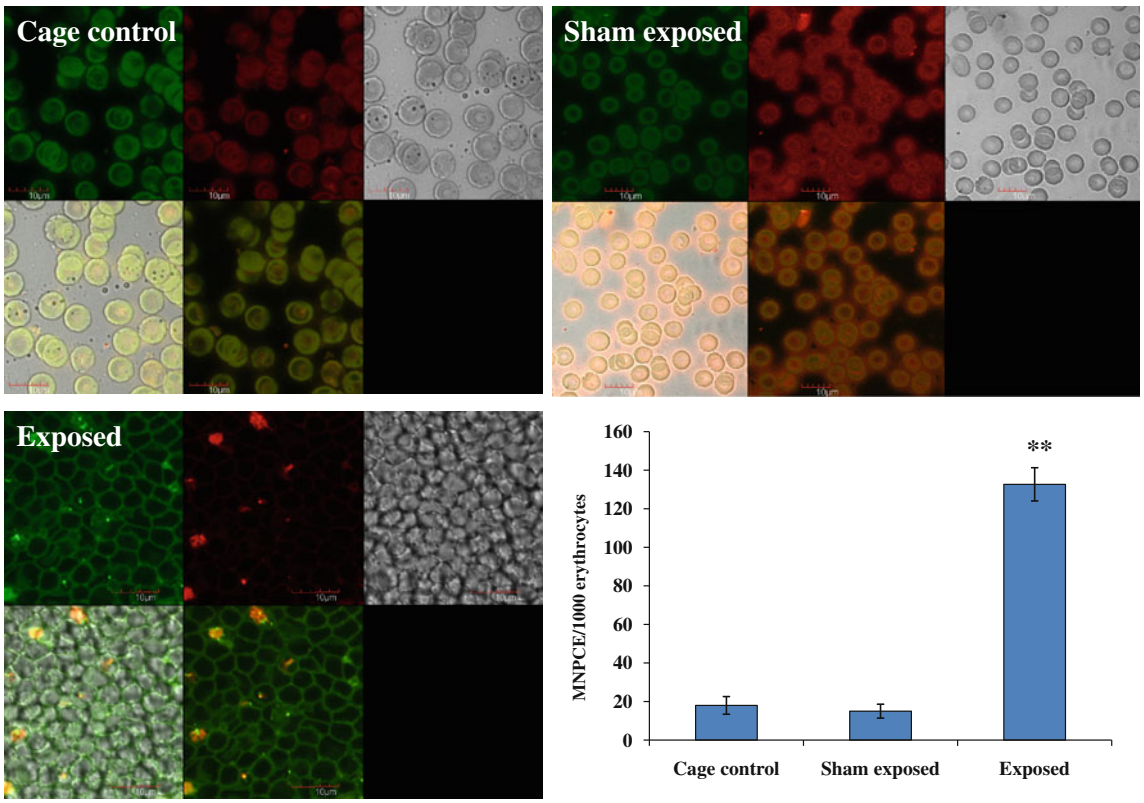


Fig. 4 Micronucleated polychromatic erythrocytes (MNPCE) cells of 3G mobile phone exposed, sham exposed, and cage control stained with Acridine orange. The *graph* indicates number of MNPCE among all three groups. *Asterisks* indicates statistically significant result with statistical variation of mean ± SD

control (18 ± 4.58; $P < 0.005$) and sham-exposed (15 ± 3.56, $P < 0.002$) groups (Fig. 4). On the other hand, ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) as the indicator of micronuclei induction was determined by flow cytometer (Fig. 5). The ratio of PCE/NCE in 3G mobile phone-exposed group (0.24 ± 0.02) was significantly lower as compared with cage control (0.60 ± 0.04; $P < 0.002$) and sham-exposed group (0.56 ± 0.05; $P < 0.001$).

Caspase 3 Activity

A statistically significant activation of caspase 3 was observed in 3G mobile phone-exposed animals as shown in Fig. 6. Brain caspase activity showed significant increase in the exposed group (88.83 ± 7.35) as compared with the cage control (47.16 ± 5.84, $P < 0.01$) and sham-exposed ones (52.83 ± 8.17; $P < 0.05$).

Measurement of Apoptosis

To confirm the occurrence of apoptosis in the brain cells after 3G cell phone exposure, we used fluorescence-activated cell sorting (FACS) analysis by propidium iodide (PI) staining and DNA ladder assay. Results show a significant increase ($p < 0.001$) in cell apoptosis in exposed group as compared with cage control and sham exposed (Fig. 7). The mean value in exposed group was recorded (29.9 ± 2.38) as compared with cage control (2.90 ± 0.64; $P < 0.001$) and sham exposed (5.44 ± 1.34; $P < 0.003$).

Cell apoptosis was also confirmed by DNA fragmentation assay (Fig. 8). 3G mobile phone-exposed group of 60 days showed typical DNA ladder patterns of discontinuous DNA fragments and sham-exposed group showed little bit DNA ladder, while cage control group still remained genomic DNA without any fragmentation.

Western Blot

In the western blot analysis, a similar amount of heat shock proteins (Hsp 27, 70) were found in the cage control and sham-exposed animals, whereas 3G mobile exposure induced the stress which activated the heat shock proteins (Hsp 27, 70). The activated Hsp 27 and Hsp 70 were detected with phospho antibody. However, activated p38MAPK was basal levels in the cage control and sham-exposed group and an increased level of activated

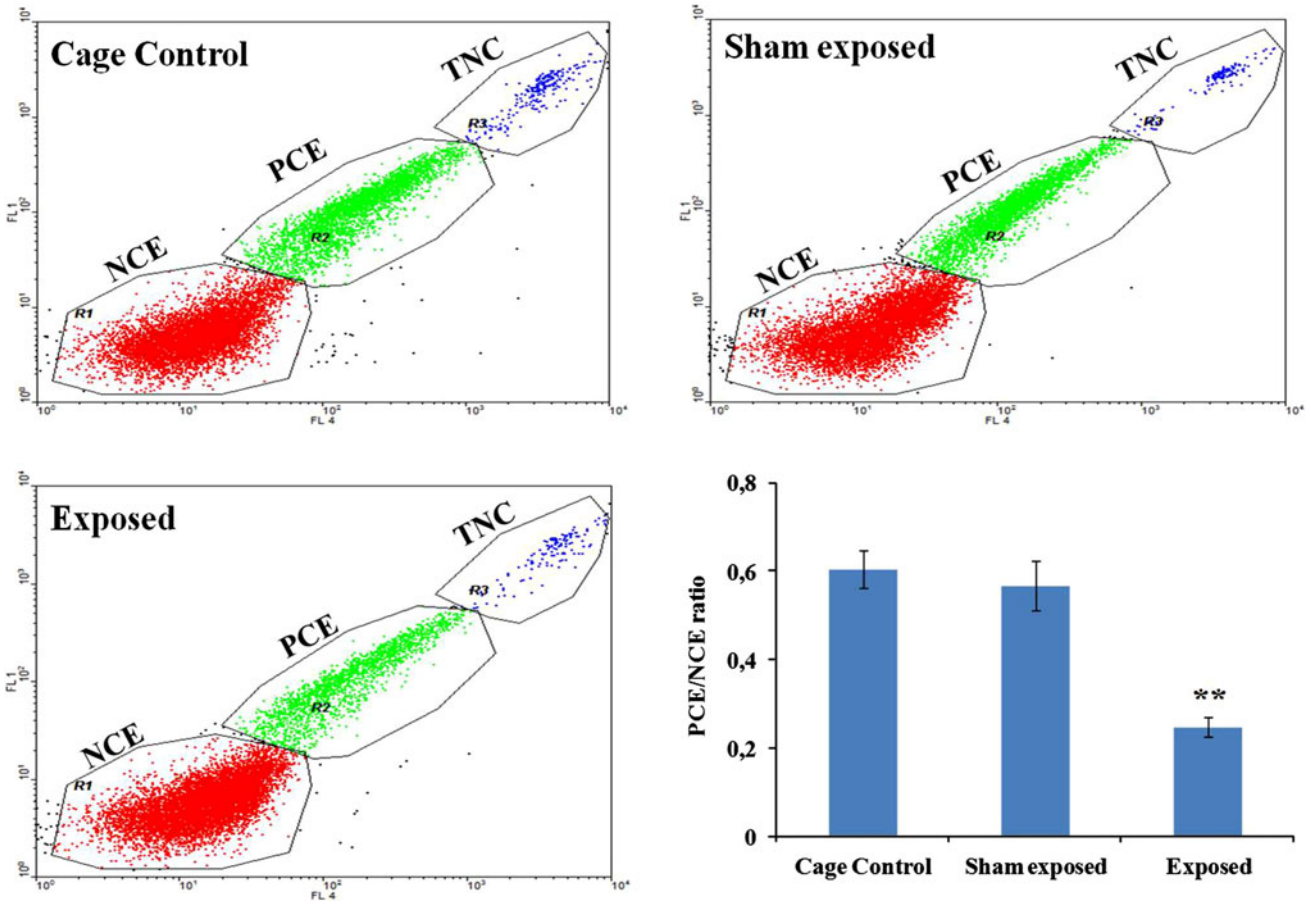


Fig. 5 Comparison of PCE/NCE ratio of percentage gated among the 3G mobile phone exposed, sham exposed, and cage control groups was determined by flow cytometry. Where PCE is polychromatic erythrocytes, NCE is normochromatic erythrocytes, and TNC is total nucleated cells. *Asterisks* indicates statistically significant result with statistical variation of mean ± SD

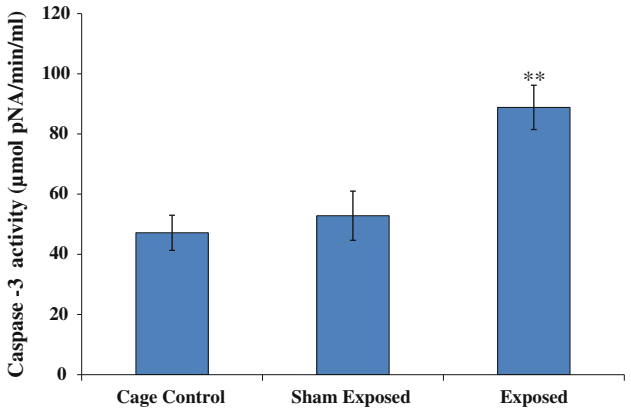


Fig. 6 The comparison of caspase 3 activity among the cage control, sham exposed, and exposed groups. Caspase 3 activity is expressed as µmol pNA/min/ml. *Asterisks* indicates statistically significance result with statistical variation of mean ± SD

p38MAPK was observed in exposed group (Fig. 9). These results indicate that heat shock proteins, which induce cell death MAPK signaling [39, 40], were strongly activated in 3G mobile exposure.

To evaluate the contribution of the mitochondrial pathway to the induction of apoptosis by non-ionizing radiation, we examined changes in expressions of pro-apoptotic genes, such as Bax, which were responsible for dissipation of mitochondrial membrane potential, and cytochrome *c* release from mitochondria. Exposure of 3G mobile phone radiation significantly increases the activity of Bax (Fig. 9) and release of cytochrome *c* to the cytosol (Fig. 9). 3G mobile phone radiations also cause activation of caspase 3 along with the cytochrome *c* release into the cytosol (Fig. 9), in agreement with previous studies suggesting that caspase 3 activation follows cytochrome *c* release [41, 42]. These results indicate that mitochondrial dysfunction-mediated cytochrome *c* release and subsequent activation of caspases are involved in the process of radiation-induced apoptotic cell death.

D[redacted]

[redacted]