Protective Role of Hispolon and its Derivatives in Apoptosis Induced by Exposure of Mobile Phone Radiation to Primary Culture of Cortical Neurons

Vara Prasad Saka
Vignan Pharmacy College
Chitra Vellapandian
SRM College of Pharmacy
Damodharan Narayanaswamy (damodhan@srmist.edu.in)
SRM College of Pharmacy https://orcid.org/0000-0003-4246-5351

Research Article

Keywords: Electromagnetic radiation, Hispolon, Inonotus hispidus, Mobile Phone, Reactive Oxygen Species, Apoptosis

Posted Date: June 17th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1644043/v1

Abstract

The enormous advancement of technology in communication systems and vast growth in the utilization of mobile phones alarms the effects of over exposure to radiations emitted from mobile phones. This study investigated the effect of Hispolon (HIS), a biologically active polyphenol compound isolated from *Inonotus hispidus* and its derivates such as hispolon pyrazole (HP), hispolon mono methyl ether (HME), and hispolon monomethyl ether pyrazole (HMEP) against the deleterious effects of mobile phone radiation on primary cultured cortical neurons. Cortical neuronal cells were isolated form a day-old neonate rats, cultured, and treated with HIS and its derivatives while exposed to mobile phone (2100 MHz, 1.6 W/Kg SAR) on call answered mode for 2 hrs. Post exposure the cells were analysed for cytotoxicity, reactive oxygen species (ROS), mitochondrial membrane potential (MMP), apoptosis using propidium iodide (PI) assay through flowcytometry, genotoxicity through DNA ladder assay, and gene expression analysis of p53, Bcl2, and Bax. The cytotoxicity analysis indicated that pyrazole derivatives were less toxic among the treated compounds. Pyrazole derivatives such as HP and HMEP significantly decrease radiation induced ROS levels in the cells, reduced MMP, apoptosis, and DNA damage effectively, while they didn't alter gene expression of p53, Bcl2, and Bax. From the results, it can be concluded that the antioxidative and anti-apoptotic activity of the pyrazole derivatives might be attributed to the phenolic and diketo groups present in them. Hence the pyrazole derivatives can be further evaluated their pathway of neuroprotection which may gain importance as lead molecules in the development of neuroprotective formulations.

1. Introduction

The revolutionary rise in the utilization of mobile phones led to the development of new technologies for better connectivity. The development has reached the fifth generation (5G) wireless communication technology, ranging from 600 MHz to 54 GHz, which offers a very high internet speed (Yu et al. 2017). Along with the advancement in communication technology, there are increased levels of emission of higher frequency electromagnetic fields (EMF) into the atmosphere from the devices and related equipment. The deleterious effects of the generated EMF on the health of human beings are raisin' public concern globally (Hasan et al. 2021). Many scientists have reported that EMF has negative effects on various organs, including the brain (Grafström et al. 2008; Nittby et al. 2009; Hasan et al. 2022), kidney (Hasan et al. 2021), ear (Colletti et al. 2011), heart(Azab 2017), liver(Koyu et al. 2009), and reproductive organs(Hasan et al. 2021). During the conversation, the mobile phone is used mostly near the head. Therefore, the brain is the most exposed organ to the EMF emitted from mobile phones, which can contribute to neurological and behavioural abnormalities in both children and young people. There have been several studies reporting the damage to the brain and creating cognitive impairment and behavioural deterioration (Cassel et al. 2004; Hasan et al. 2022). Many other studies reported that there was a negative impact on the antioxidant system in the body against oxidative stress caused by the EMF emitted from mobile phones, especially in the brain. The oxidative stress developed due to EMF affected many biochemicals and organelles in the nerves, such as enzymes, proteins, mitochondria, and DNA,

which led to neuronal damage and apoptosis (Kannan and Jain 2000; Reimertz et al. 2003; Ilhan et al. 2004; Zhao et al. 2007; Joubert et al. 2008; Carvour et al. 2008; Khan et al. 2011; Megha et al. 2012; Avci et al. 2012; Bilgici et al. 2013; Lombardi et al. 2014; Tohidi et al. 2015; Xing et al. 2016; Narayanan et al. 2018; Gupta et al. 2018; Jeong et al. 2018; Alkis et al. 2019; Asl et al. 2020; Dauda Usman et al. 2020; Singh et al. 2020; Schuermann and Mevissen 2021). In contrast, a few studies reported that exposure to mobile phone radiation didn't affect memory, learning, and task performance as well as cellular activities such as cell apoptosis, proliferation, and cell cycle (Banaceur et al. 2013; Chen et al. 2014; Son et al. 2018). However, there have been several reports on the detrimental effects of mobile phone EMF. However, the use of mobile phones is unavoidable, and most people are unaware of the consequences of excessive use. Although there are no specific drugs to alleviate the effects of EMF, we can use a few drugs to reverse the mechanism of its negative effects. Many previous studies have attributed the antioxidant mechanism, and many researchers are working on natural and synthetic compounds to see if the deleterious effects of EMF can be reversed (Ilhan et al. 2004; Koyu et al. 2009; Imge et al. 2010; Ahmed et al. 2017; Altun et al. 2017; Asl et al. 2020).

Hispolon (HIS), a polyphenol bioactive compound available in several medicinal mushrooms such as *Inonotus hispidus*, *Phellinus linteus* and *Phellinus igniarius*(Nasser et al. 1996; Mo et al. 2004; Wang et al. 2014). It has been reported for various pharmacological activities such as anti-oxidant (Chang et al. 2007), anti-tumor (Hsin et al. 2017), antiviral (Ali et al. 2003), analgesic and anti-inflammatory (Ravindran et al. 2010; Huang et al. 2011), hepatoprotective (Chang et al. 2007), immunomodulatory (Gröndemann et al. 2016), cerebroprotective (Prasanth et al. 2021) and anti-diabetic (Chen et al. 2013). Several of its new derivatives have been synthesised and evaluated for various activities, based on the reported pharmacological activities. Recently, HIS and its derivatives such as hispolon pyrazole (HP), hispolon monomethyl ether (HME), and hispolon monomethyl ether pyrazole (HMEP) have been tested for strong antioxidant and reactive oxygen species (ROS) scavenging activity in cell free systems (Shaikh et al.) and genotoxicity in irradiated cells(Chethna et al. 2018a). The exposure to the radiation causes increase in the levels of intracellular ROS and damage to DNA thus inducing cell death(Zhao et al. 2007). However, any agent that can decrease the levels of ROS or reverse the mechanism of ROS induced DNA damage can protect cells form mobile phone radiation. This prompted us to carry out the study to explore the possibility of HIS and its derivatives in reversing the cellular damage induced by mobile phone radiation in neuronal cells. With this background, HIS and its derivatives HP, HME, and HMEP were evaluated for cytotoxicity, mechanism of cytotoxicity and genotoxicity in the normal and radiation exposed neuronal cells. The HIS and its derivatives used in the study are shown in the figure. 1.

2. Materials And Methods

2.1. Chemicals and Reagents:

HIS, HP, HME, and HMEP were procured from Natsol pvt ltd, vizag. Propidium iodide (PI), diethyl pyrocarbonate (DEPC), agarose, sodium dodecyl sulphate (SDS), dimethyl sulfoxide (DMSO), triton X-100, SYBR Green-II dye, cell culture medium (RPMI), Neurobasal TM-A, fetal calf serum (FCS), penicillin,

streptomycin, EDTA, trizol reagent, cDNA synthesis kit, 10× SYBR green polymerase chain reaction (PCR) mix and RNAase were purchased from Sigma Chemical Company (St. Louis, MO, USA). Dichloro fuorescein diacetate (DCF-DA), 5, 5', 6, 6'-tetrachloro-1, 1', 3, 3'-tetraethyl benzimidazolo carbocyanine iodide (JC-1) were obtained from Molecular probes, USA. The gene-specific primers for RT-PCR were custom synthesized from the local agents. All other chemicals with maximum available purity were purchased from reputed local manufacturers/suppliers.

2.2. Animals:

Timed pregnancy induced Female Sprague Dawley rats (6–8 weeks, 150–200 g) were housed each in a cage and fed with standard pellet diet and water *ad libtum*. Appropriate environmental conditions in compliance with CPCSEA guidelines. All the procedures involved in the animal experimentation were approved by IAEC of Vignan Pharmacy College, Vadlamudi, Guntur.

2.3. Preparation of Primary Cortical Neuronal Cell Culture:

Primary cortical neuronal cell culture was prepared from the cortical cells of new-born fetuses within 24 hours of delivery from Sprague – Dawley rats as per the existing protocols (Dai et al. 2007; Sahu et al. 2019). Briefly, two 0.5-2 mm^3 pieces of cortices were sectioned from the posterior dorsal surface of the isolated brains from new-born fetuses. The cortical sections were placed in 0.125% (W/V) trypsin to dissociate cells through intermittent shaking for 15 minutes at an interval of 5 min. The dissociation process was terminated by adding 5% serum. The dissociated cells were triturated with a fine-tipped pipette, pelleted, and resuspended in a DMEM medium with 10% NCS and 10% HS plus 1% penicillin-streptomycin. The resulting cell suspension was incubated for 3 min, again pelleted, resuspended in DMEM medium, and incubated at 37◦C in CO_2 humidified incubator for 30 min after plating in uncoated 90mm culture dishes; same was repeated twice. During this incubation, glial cells and other unwanted debris adhere to the bottom of the plate, and the cortical cells were also recovering from the trituration. Afterward, the supernatant solution was collected, pelleted, resuspended, and plated into poly-L lysine-coated 25-cm^2 cell culture flasks (Nunc, Roskilde, Denmark) at a density of 1.25×10^6 cells/cm^2 in a plating medium. All cultures were maintained in an incubator set at 37 ºC with 5% CO_2. After two hours, the DMEM medium was replaced with fresh Neurobasal TM-A medium-plus B27 supplements, 100 U/ml penicillin, 100 µg/ml of streptomycin, and 0.5 mM L-glutamine. Viable cells were counted manually using a Neubauer chamber (10×objective) stained with 0.4% trypan blue. This approach produced substantially enriched cultures for neurons (> 95 percent purity) with very little astrocyte and microglial content. The neuronal cells were cultured for seven days before being treated with freshly made serum-free Neurobasal TM-A medium containing B27 supplements but no antioxidants. Cells were pre-treated with hispolon and its analogues (10 µM) prepared in 0.1% DMSO in all *in-vitro* studies.

2.4. Exposure Protocol:

All the primary cortical neuronal cells except for sham control were treated with hispolon and its analogues (10 µM), incubated at 37°C with 5% CO_2 for 30 min, and then irradiated to mobile phone radiation for 2 h by placing mobile phone with its antenna over the center and on top of the coverless

Petri dishes containing cells, while incubated at 37°C with 5% CO_2. A 4G mobile phone with a signal for GSM at 2100 MHz system was used. The peak specific absorption rate (SAR) OF 1.6 W/Kg. after 2 h of exposure, cells were harvested for further evaluation.

2.5 Cytotoxicity assay:

2.6 A colorimetric MTT assay was used to determine cytotoxicity, as previously described (Aras et al. 2008). After irradiation, primary cortical neuronal cells were treated with MTT solution (0.5 mg/ml^{-1} in PBS) in triplicates for 4 h at 37 °C. The formazan metabolites formed by living cells reducing MTT were solubilized with 10% SDS in 0.01 N HCl, incubated overnight, and the absorbance at 550 nm was measured. The reduction in absorbance of treated samples when compared to control cells was used to calculate the percentage (%) cytotoxicity.

2.7 Measurement of ROS:

After irradiation, cells were washed in PBS and loaded with 5 M DCHF-DA, a cell-permeable and oxidation-sensitive fluorescence probe, for 30 minutes at 37°C. The fluorescence intensity of 2',7-dichlorofluorescein (DCF) after excitation at 488 nm and emission at 530 nm on a multimode plate reader (Multiskan FC, Thermo Scientific) was used to represent the levels of intracellular ROS and superoxide anion (Kunwar et al. 2012; Raghuraman et al. 2017).

2.8. Mitochondrial membrane potential (MMP) assay

Primary cortical neuronal cultures were labelled with JC-1 (10 µg ml^{-1}, final concentration) for 20 minutes at 37°C in the dark after irradiation, and fluorescence emissions at 535 and 610 nm were measured using a multimode microplate reader after excitation at 485 and 565 nm, respectively. The MMP was defined as the ratio of fluorescence intensities at 610 and 535 nm (Cossarizza et al. 1993).`

2.9. Assessment of apoptosis by DNA fragmentation

Apoptotic cells produce distinct DNA ladders made up of nucleotide fragments after DNA-agarose gel electrophoresis, which can be seen by staining with ethidium bromide. DNA fragmentation was one of the criterions for apoptotic cell death that we used. and this was determined as previously stated (Giordano and Costa 2011). Before DNA isolation, primary cortical cell monolayers were washed in ice cold TBS (20 mM Tris-HCl, pH 7.6, 137 mM NaCl). On a 1.5 percent agarose gel, the DNA (5µg) was electrophoresed for 1.5 hours at 100 V. UV transillumination was used to detect the DNA fragments, which were stained with ethidium bromide.

2.10. Estimation of cell death by PI staining

As previously reported, propidium iodide (PI) staining was used to assess cell death in primary cortical neuronal cultures caused by mobile phone radiation (Van Engeland et al. 1996). In brief, primary cortical neuronal cells were stained overnight in the dark with a solution containing 50 µg ml^{-1} PI, 0.1% sodium citrate, and 0.1% Triton X-100. A flow cytometer (Partec, Germany) was used to collect the labelled cells,

which were then characterised for cell cycle phases using FlowJo software. Apoptotic cells were represented by the pre-G1 population.

2.11 Quantitative real-time polymerase chain reaction (RT-PCR)

Total RNA was extracted from primary cortical neuronal cells using a TRIzol reagent according to the manufacturer's instructions 24 hours post irradiation. 2 µg total RNA was used for reverse transcription (cDNA synthesis kit, Thermo Scientific, USA), and real-time PCR was performed in a Rotor-Gene Q (QIAGEN, Germany) using the template (cDNA), SYBR green master mix (Roche Applied Science, Germany), and gene-specific primers according to the standard protocol. The threshold cycle (CT) values for the target genes estimated from the above runs were normalised against a housekeeping gene, GAPDH, using the method described previously (Ghatei et al. 2017)(Kim et al. 2017). Table 1lists the forward and reverse primers used for cDNA amplification.

Table 1
List of specific gene primers

Gene	Forward (5´→3´)	Reverse (5´→3´)
Bcl2	ACCGTCGTGACTTCGCAGAG	GGTGTGCAGATGCCGGTTC
Bax	CGGCGAATTGGAGATGAACTG	GCAAAGTAGAAGAGG GCAACC
p53	GTATTTCACCCTCAAGATCC	TGGGCATCCTTTAACTCTA
GAPDH	GAGAAACCTGCCAAGTATG	GGAGTTGCTGTTGAAGTC

2.12 Statistical analysis

The results of an independent experiment are presented as means SEM, n = 3. To confirm the data's variability, ONE WAY ANOVA test with Tukey’s post hoc test was performed using Origin (version 9) software. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Cytotoxicity evaluation of Hispolon and its derivatives in Primary cortical neuronal cell:

To assess metabolic activity in cells, the MTT cell viability assay is commonly used. It measures how efficiently mitochondrial dehydrogenases reduce tetrazolium salts. Several factors influence the outcome of this essay, including cell number, cell metabolism, and mitochondrial activation. Cells were treated with 10 µM dissolved in 0.1% DMSO for 30 min before radiation and 2 hrs with and without radiation. Cell viability was determined using MTT assay after 24 hrs of post-irradiation. The results indicate that all the compounds analysed showed cytotoxicity in their respective group of cells, while hispolon and

monomethyl ether derivatives showed more toxicity when compared to pyrazole derivatives. The same was reflected in irradiated groups as shown in the Fig. 2. These indicate that hispolon pyrazole and hispolon monomethyl ether pyrazole showed significant ($P < 0.001$) protection when compared to radiation control (~ 20% and ~ 16% decrease respectively). Since these were found to be protective, further investigations were conducted in this line.

3.2. Effect of HIS and its Derivatives on Radiation-Induced ROS Generation in Primary Cortical Neuronal Cells

The generation of ROS was assessed using the DCHF-DA probe to determine the cause of the cytotoxicity seen in the MTT assay. When compared to the sham control group, ROS levels significantly increased after 2.5 hours of pretreatment with 10 μM Hispolon and its derivatives and mobile phone radiation. When comparing the HP, HME, and HMEP groups to the radiation control group, the pretreatment resulted in a significant decrease in ROS levels at $p < 0.001$. Pyrazole derivatives had shown the greatest reduction in ROS levels. The outcomes are depicted in the Fig. 3.

3.3 The Impact of HIS and its Derivatives on Radiation-Induced Mitochondrial Damage in Primary Cortical Neuronal Cells

The generated ROS due to irradiation may involve the oxidation of mitochondrial membrane proteins, increasing membrane permeability of mitochondria, and loss of MMP. The effect of generated ROS on MMP in primary cortical neuronal cells was assessed by the JC-1 probe as shown in Fig. 4. The control cells emitted more red fluorescence, indicating high MMP. On treatment with hispolon and its derivatives, the fluorescence of JC-1 shifted towards green and was significant. When the cells were irradiated with mobile phone radiation, the cells in radiation control showed a shift of fluorescence towards green because of the ROS generation due to radiation, which damaged the mitochondrial membrane proteins and led to a decrease in the MMP. On pre-treatment with Hispolon and its derivatives, hispolon and hispolon monomethyl ether could not protect the cells indicated by the shift from red to green, whereas hispolon pyrazole and hispolon monomethyl ether pyrazole showed significant protection.

3.4. Effect of HIS and its Derivatives on Radiation-Induced Apoptosis in Primary Cortical Neurons.

The damage incurred by the increase in ROS and decrease in MMP to the nucleic acids in primary cortical neuronal cells was validated by assessing the fragmentation of DNA through a DNA ladder assay. The formation of a ladder represents the damage to DNA which results in fragmentation. In the radiation control, the results revealed significant fragmentation of genomic DNA. Pre-treatment with HIS compounds did not show any protection from radiation induced ladder formation. Furthermore, these results were validated by quantitatively estimating the number of cells undergoing apoptosis through a PI assay. The radiation control group showed a significant increase in the pre-G1 peak compared to the control group. The groups pre-treated with pyrazole derivatives of HIS showed a significant decrease in the counts of pre G1 cells, suggesting the ability of these compounds to protect primary cortical neuronal cells from radiation induced apoptosis. Figures 4A and 4B show representative gated histograms of the

PI stained cells and the counts of pre-G1 cells, respectively. The fragmentation of DNA was represented in Fig. 5C. The control groups themselves showed basal levels of DNA fragmentation in Fig. 5C and a pre-G1 gated population in Fig. 5B, indicating that cortical cells undergo apoptosis with the passage of time, perhaps because of being primary cells.

3.5. Effect of Hispolon and its derivatives on apoptotic gene expression in primary cortical neurons.

Furthermore, to evaluate the pathway of apoptosis observed in PI assay as well as in MMP assay, apoptotic gene expression analysis was carried out. From the results it was found that there was significant shift in the apoptotic gene expression in radiation exposed cells when compared to normal control cells. Whereas in the pre-treated cells, there found a difference in the expression of apoptotic gens (Bax, Bcl 2, and p53). As shown in Fig. 6, pyrazole derivatives of HIS, viz., HP and HMEP exhibited protection against the radiation but not significant when compared to radiation control.

4. Discussion

Mobile phones are the devices known for the release of EMF into the atmosphere can impact the cellular metabolic process and wield several biological effects of thermal or non-thermal mechanisms (Balawender and Orkisz 2020). Head is the most exposed region of the body to the radiation during the conversation. EMF in the frequency range of 900–2200 MHz can penetrate the cranium and reach the deep brain, altering brain function and behaviour (Ntzouni et al. 2011, 2013; Aldad et al. 2012; Li et al. 2012; Saikhedkar et al. 2014; Narayanan et al. 2015, 2018; Ahmadi et al. 2018; Singh et al. 2020). Several other studies reported stress and associated problems such as headache, tiredness, impairment of behavioural and cognitive functions due to the exposure of mobile phone radiation (Behari 2010; Kivrak et al. 2017; Narayanan et al. 2019). It was observed form previous studies that, the exposure to mobile phone radiation can lead to ROS generation (Singh et al. 2020), impairment of antioxidant system (Kesari et al. 2011; Singh et al. 2020), genotoxicity leading to apoptosis (Motawi et al. 2014; Hussein et al. 2016) in the regions of brain. The slow release of EMF from the electronic devices especially from mobile phones accumulate in the living organism and may disrupt the functionality of systems. There were no protective drugs over radiation effects, but numerous synthetic and natural substances were studied for their ability to reverse the effects of mobile phone radiation. (Ahmed et al. 2017; Altun et al. 2017; Asl et al. 2020).

Because of its structural similarities to curcumin, HIS, a natural polyphenolic derived from several mushrooms, piqued the interest of researchers to investigate for its therapeutic actions and is also considered a curcumin derivative (Ravindran et al. 2010; Amalraj et al. 2017; Chethna et al. 2018b) and has been reported for various pharmacological activities such as anti-bacterial, anti-oxidant, anti-tumour, antiviral, analgesic and anti-inflammatory, hepatoprotective, immunomodulatory, cerebroprotective and anti-diabetic (Ali et al. 2003; Chang et al. 2007; Ravindran et al. 2010; Huang et al. 2011; Chen et al. 2013; Gröndemann et al. 2016; Hsin et al. 2017; Prasanth et al. 2021). This made the researchers to synthesise

the new derivatives to explore the spectrum of pharmacological activities (Shaikh et al.; Balaji et al. 2015, 2017). Considering the reported antioxidant activity of the HIS and its derivatives such as HP, HME, HMEP were studied for their protective role in primary cortical neuronal culture cells against the impact caused by the mobile phone radiation.

In the present study, HIS and its derivatives were assessed for their protection against radiation induced cytotoxicity. Mobile phone radiation exposure lead to cell death in various cell types including primary cultured neuronal cells (Cotgreave 2005; Xu et al. 2010; Nageshwar Rao and Satish Rao 2010a; Tohidi et al. 2021). The results of the present study are in line with the previous studies, exhibited the cell death in the primary cortical neuronal cells when exposed to the mobile phone for 2 hours. Among the treated groups, HP and HMEP administered groups showed significant decrease in cytotoxicity indicating protection when compared to the cells exposed to radiation alone which were in line the previous study attributed to the pyrazole groups present in diketo region of HIS and HME (Chethna et al. 2018b). The cell cycle analysis revealed significant increase in pre G1 peak indicating apoptosis in all treated groups. HP and HMEP significantly augmented pre G1 peak when compared to the radiation alone exposed group of cells. To understand the mechanism of protection of the pyrazole derivatives of HIS and HME, ROS levels were assessed hypothesizing that HP and HMEP alleviate the oxidative stress concentrated the neurons due to EMF from mobile phone radiation. Radiations emitted from mobile phones were reported develop oxidative stress by elevating the levels of ROS intra cellularly (Motawi et al. 2014). Nerve cells were more sensitive to ROS because of their weak antioxidant system, high metabolic rate, and decreased cellular turnover, ROS accumulates in neuronal cells (Lin and Beal 2006). Moreover, EMF also inhibit mitochondrial respiratory chain and prolong the half-life of ROS in the cells may lead to the cellular damage (Köylü et al. 2006). It can be observed from the results of the current study that, ROS levels were elevated in the primary cortical neuronal cells which are in line with the previous studies (Zhao et al. 2007; Nageshwar Rao and Satish Rao 2010b; Motawi et al. 2014). However there are many studies claimed no effect of mobile phone radiation on the development of oxidative stress (Hook et al. 2004; Zeni et al. 2007a). This incongruities between the studies may be due to the different systems of radio frequency (RF) generators, exposure protocols, and cell types (Zeni et al. 2007b; Simko 2007; Schwarz et al. 2008). HP, HME, and HMEP significantly reduced the levels of ROS in primary cortical neuronal cells when treated along with the radiation. They could be able to reverse the oxidative stress due to radiation as they were the derivatives of a bioactive polyphenolic compound HIS and are in line with the previous studies reported of their ROS scavenge in cell free systems(Shaikh et al.).

An increase in the rate of ROS production causes the build-up of ROS-associated damages in DNA, proteins, and lipids, which can lead to progressive cell dysfunction and, as a result, apoptosis, raising the overall probability of pathological conditions in an organism (Tsao and Deng 2004a; Suski et al. 2018). Mitochondria have been implicated in apoptosis regulation (Grebeňová et al. 2003). The membrane permeability of mitochondria is increased in presence of ROS and release its contents which could activate apoptosis (Guo et al. 2013). The results of the current study revealed the same that the ROS developed inside the cell upon mobile phone exposure of primary cortical neuronal cells indicated by green shift in JC-1 assay indicating the raise of mitochondrial membrane permeability might leak the

contents of the mitochondria and activate apoptosis (Sivandzade et al. 2019). HIS, HP and HMEP significantly increased red/green ratio in comparison with radiation control and indicating protection of mitochondrial membrane potential under mobile phone radiation. Furthermore, extent of DNA damage and protection of HIS derivatives against the radiation was assessed in the primary cortical neuronal cells. In the brain cells of rats exposed for 2 hours to a 2450 MHz field at 0.6–1.2 W/kg, Lai and Singh found an increase in single and double-strand DNA breakage. They also reported that EMR exposure enhanced apoptosis and generated DNA-protein and DNA-DNA crosslinks in biological samples from rats (Lai and Singh 1995, 1997, 2004; Lai 1996). The current study reveals that there was DNA fragmentation indicating damage when exposed to 2100 MHz mobile phone with 1.6 W/Kg SAR for 2 hours. Pre-treatment with HIS compounds resulted in the significant change in the DNA fragmentation, and the compounds like hispolon monomethyl ether and hispolon showed significant change in the fragmentation indicates the apoptotic nature. The control groups itself showed basal level of DNA fragmentation. Cells has an intrinsic mechanism to detect and repair the breaks in DNA(Chatterjee and Walker 2017). DNA damage leads to the activation of p53, a tumour suppression gene that raises its levels with an increased ability to bind DNA to mediate transcriptional activation, which in turn activates various genes that prompt cell-cycle arrest, apoptosis, or DNA repair (Lakin and Jackson 1999). The results reveal that there was significant difference in the levels of p53 in the radiation control which are in line with the previous studies (Yilmaz et al. 2014). The tumour suppressor protein p53, which induces cell cycle arrest or apoptosis in response to DNA damage, targets both Bcl2 and Bax. Overall, the coordination of these molecules are crucial for directing a cell's life and death (Basu 1998). In the current study, the relative gene expression levels revealed the reason for apoptosis in radiation control group mediated by the upregulating the p53 as well as Bax and downregulating the Bcl2. HIS and its derivatives pre-treatment doesn’t change in the relative expression of apoptotic genes in the neuronal cells. According to Pamela et al. 2020, even short-term exposure to cell phone radiofrequency emissions can up-regulate elements of apoptotic pathways in cells derived from the brain, with neurons appearing to be more sensitive to this effect. The findings were also in par with Pamela et al indicating the upregulation of apoptotic pathways in primary cultured cortical neurons, when exposed to a 2100 MHz mobile phone with 1.6 W/Kg SAR for 2 hours, while pyrazole derivatives significantly altered the effects of mobile phone radiation, indicating their protective effect. At the same time these compounds did not alter gene expression levels opening a gateway to explore other pathways of protection incurred by these compounds.

It was previously recognised that phenolic compounds had antioxidant and cytoprotective properties against radiation-induced oxidative damage. They can act as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelating agents due to their high redox potential, giving them intrinsic antioxidant capabilities (Tsao and Deng 2004b; Soobrattee et al. 2005).

Conclusion

The current study found that using a mobile phone exposure for 2 hours straight could cause neuronal cells to die because of apoptosis caused by ROS generation. Among HIS and its derivatives, pyrazole

derivatives showed protective activity by diminishing apoptosis in neuronal cells via ROS inhibition, further demanding elucidation of the mechanism of protection against radiation. Several studies also reported the same effect on humans and animals, altering the physiology of the brain and behaviour when exposed for shorter and longer periods. There were a few studies that contradicted each other on the harmful effects of mobile phone radiation. The disparities among the studies can be attributed to the different exposure systems and protocols used to study the effects. Due to the rapid and continuous advancement of communication technology, it is necessary to continue evaluating its effects on living systems. Regardless of the manufacturer's regulations and instructions for safer use, there should be self-awareness of the negative consequences of continued technology use. These studies will help technology developers create systems that are less harmful. From this study, it was found that pyrazole derivatives of HIS might become drug candidates of interest to formulate medicines that could reverse the effects of mobile phone radiation.

Declarations

Acknowledgements

Authors are thankful to Dr. G.V. Subbaraju garu, Natsol Laboratories Pvt Ltd, Vishakapatnam for providing Hispolon and its derivatives, Department of Biotechnology, VFSTR-Deemed to be the university for the support rendered for molecular biology techniques, Guntur, and the management of Vignan Pharmacy College for providing necessary infrastructure to conduct the experiment.

Ethics approval and consent to participate

IAEC approval was taken for the study from Vignan Pharmacy College.

Consent for publication

Not applicable for this study

Availability of data and materials

Yes, they are available.

Competing interests

The authors declare that they have no competing interests

Funding

No Funding support was obtained from any institution or organisation

Authors' contributions

All the authors contributed to the study conception and design. Mr. Vara Prasad Saka contributed for the experimentation, data collection, and result analysis. The first draft was compiled by Mr. Vara Prasad Saka and Dr. Narayanaswamy Damodharan improvised the draft. Dr. Chitra Vellapandian commented on the previous manuscript. The final manuscript was read and approved by all authors.

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Figures

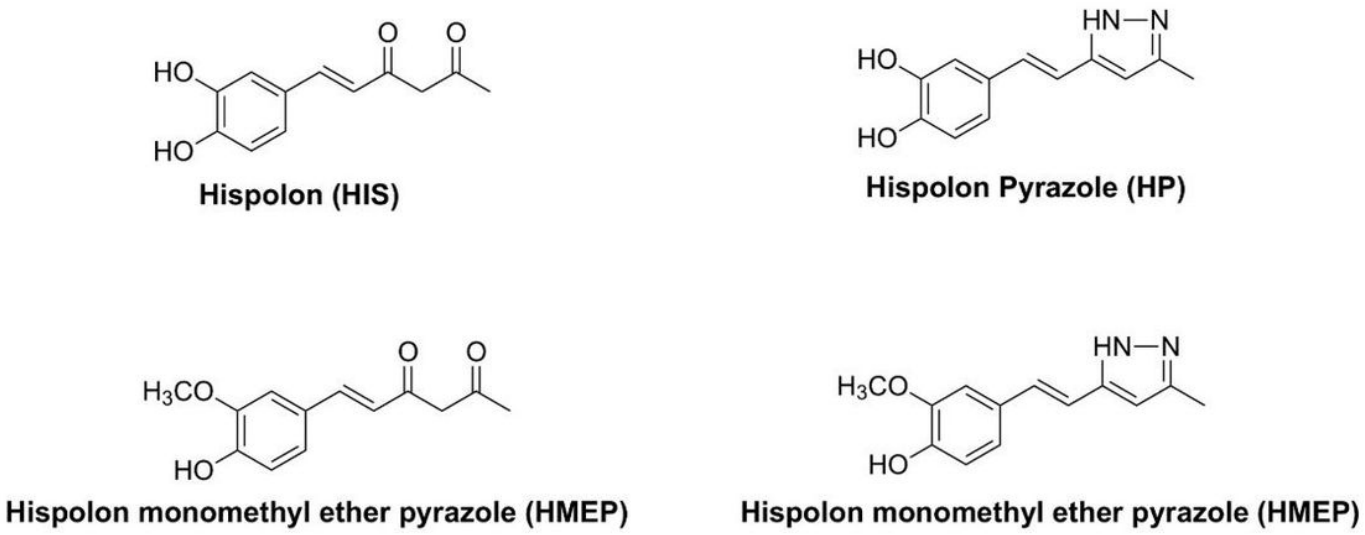

Figure 1

Chemical structures of Hispolon and its derivatives

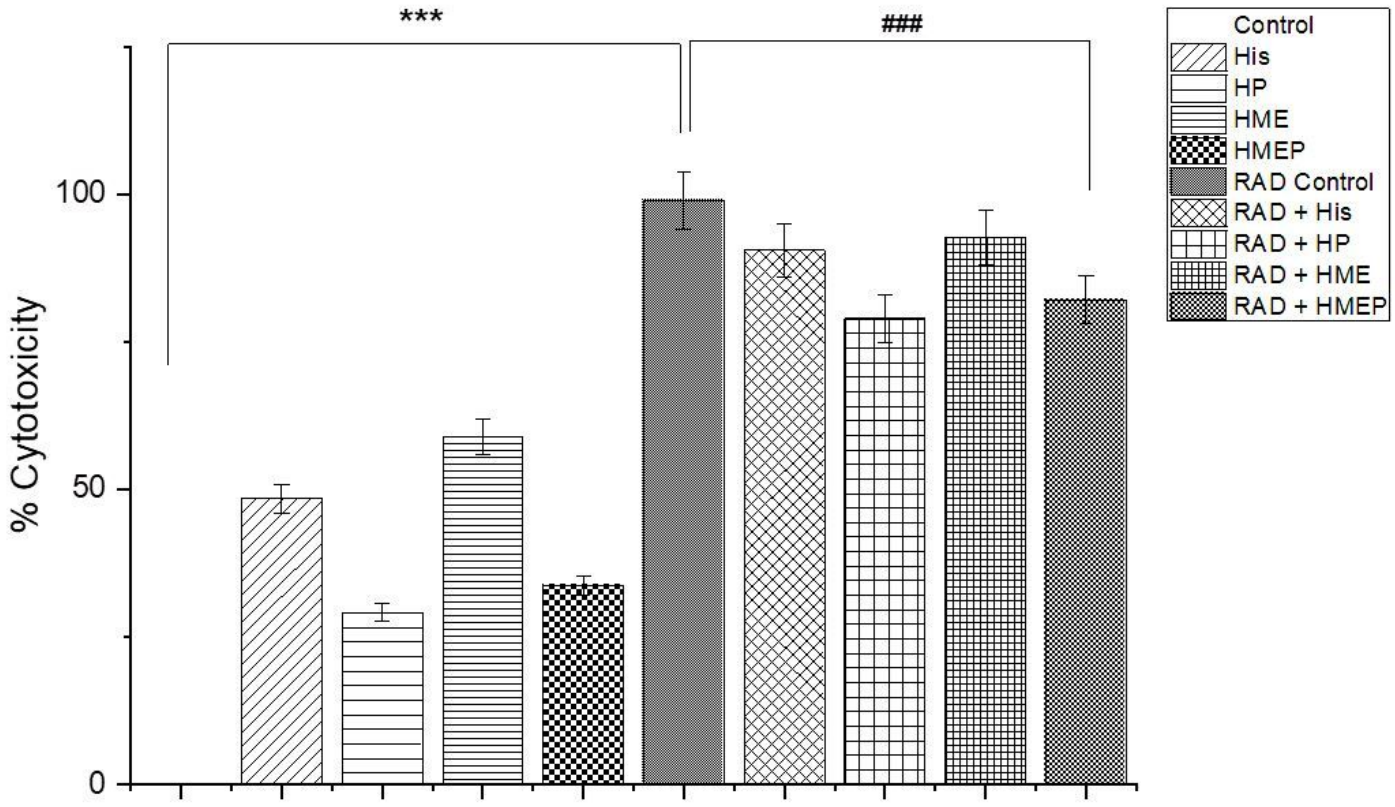

Figure 2

*Cytotoxic effect of Hispolon and its derivatives after 24 hrs of treatment by MTT assay. Data represented as percentage toxicity concerning control cells (DMSO, 0.1%). The results are presented as mean ± SEM (n=3). ONE WAY ANOVA test was done with Tukey's post hoc test was used and considered P<0.05 as significant. *** P<0.001 when compare Control; ###P<0.001 when compared to radiation control. His-Hispolon, HME- Hispolon Mono Methyl Ether, HP-Hispolon pyrazole, HMEP- Hispolon monomethyl ether pyrazole.*

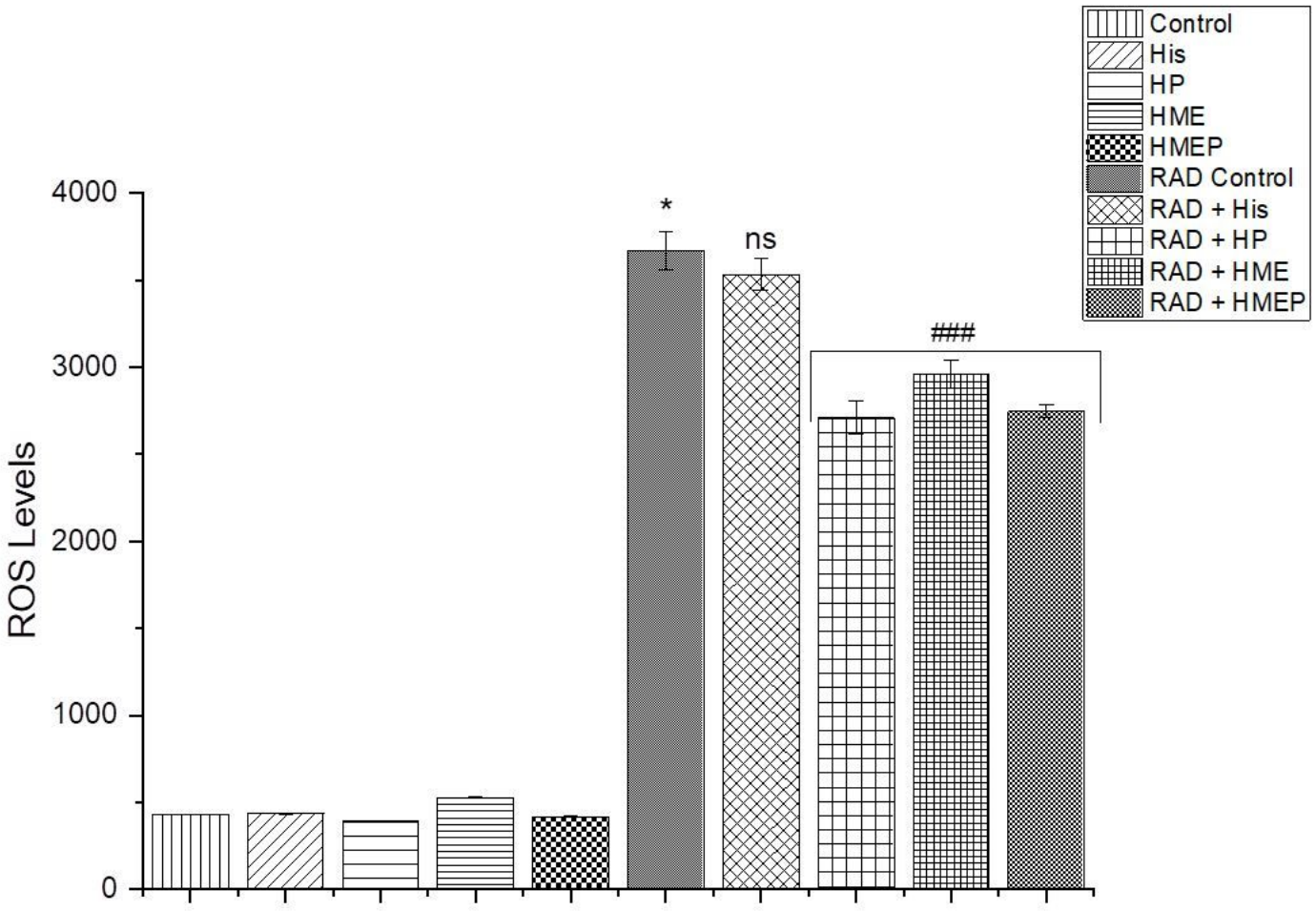

Figure 3

*Effect of Hispolon and its Derivatives on the Radiation-Induced ROS Generation in Primary cortical neuronal cell. The data represented as mean ± SEM (n=3). ONE WAY ANOVA test was done with Tukey's post hoc test was used and considered P<0.05 as significant. * P<0.05 when compare Control; ns- not significant, ###P<0.001 when compared to radiation control. His-Hispolon, HME- Hispolon Mono Methyl Ether, HP-Hispolon pyrazole, HMEP- Hispolon monomethyl ether pyrazole.*

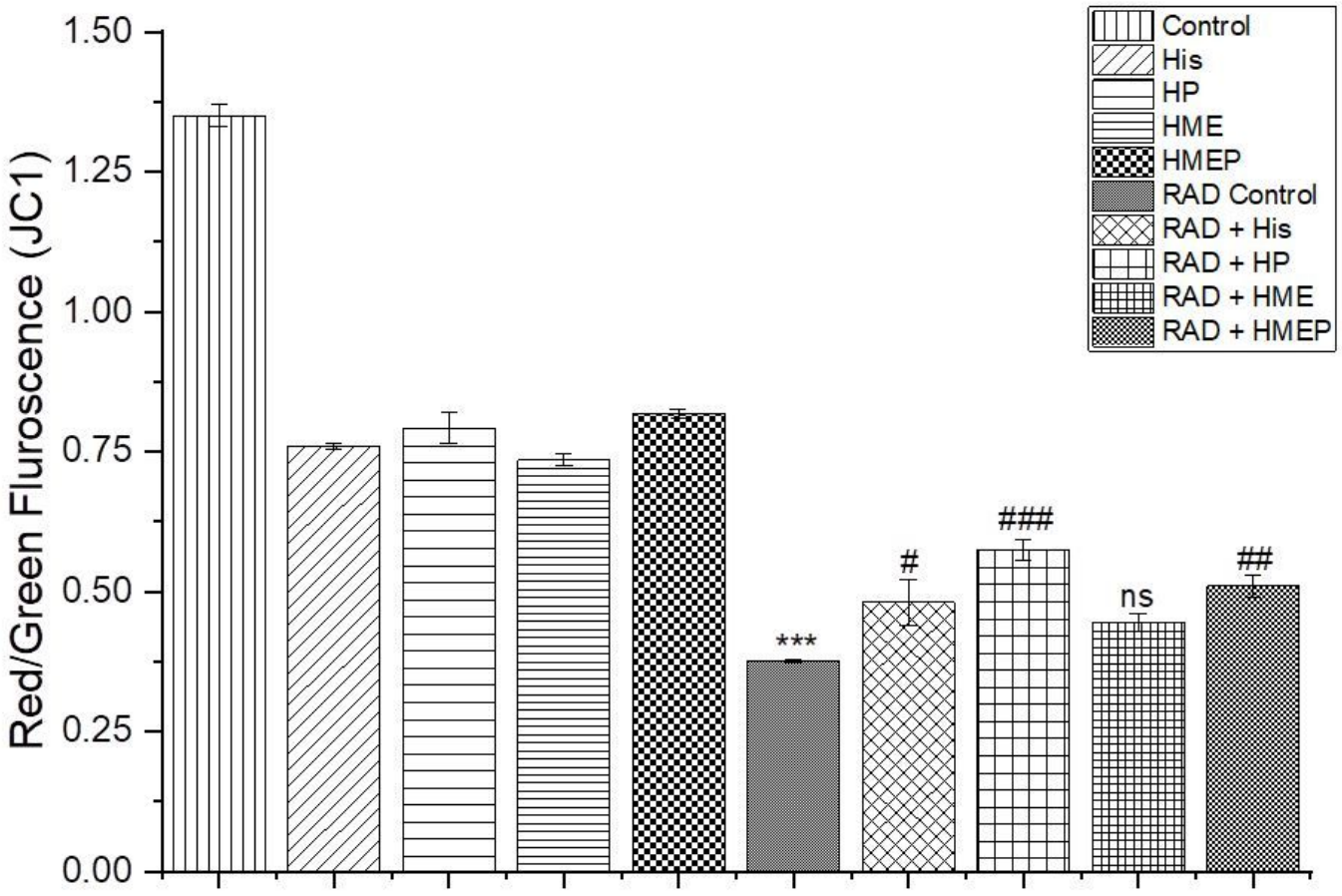

Figure 4

*Radiation-Induced Mitochondrial Damage in Primary Cortical Neuronal Cells: Effect of His and its Derivatives. The results are presented as mean ± SEM (n=3). ONE WAY ANOVA test was done with Tukey's post hoc test was used and considered P<0.05 as significant. *** P<0.001 when compare Control; ns- not significant, #P<0.05, ##P<0.01, ###P<0.001 when compared to radiation control. His-Hispolon, HME-Hispolon Mono Methyl Ether, HP-Hispolon pyrazole, HMEP- Hispolon monomethyl ether pyrazole.*

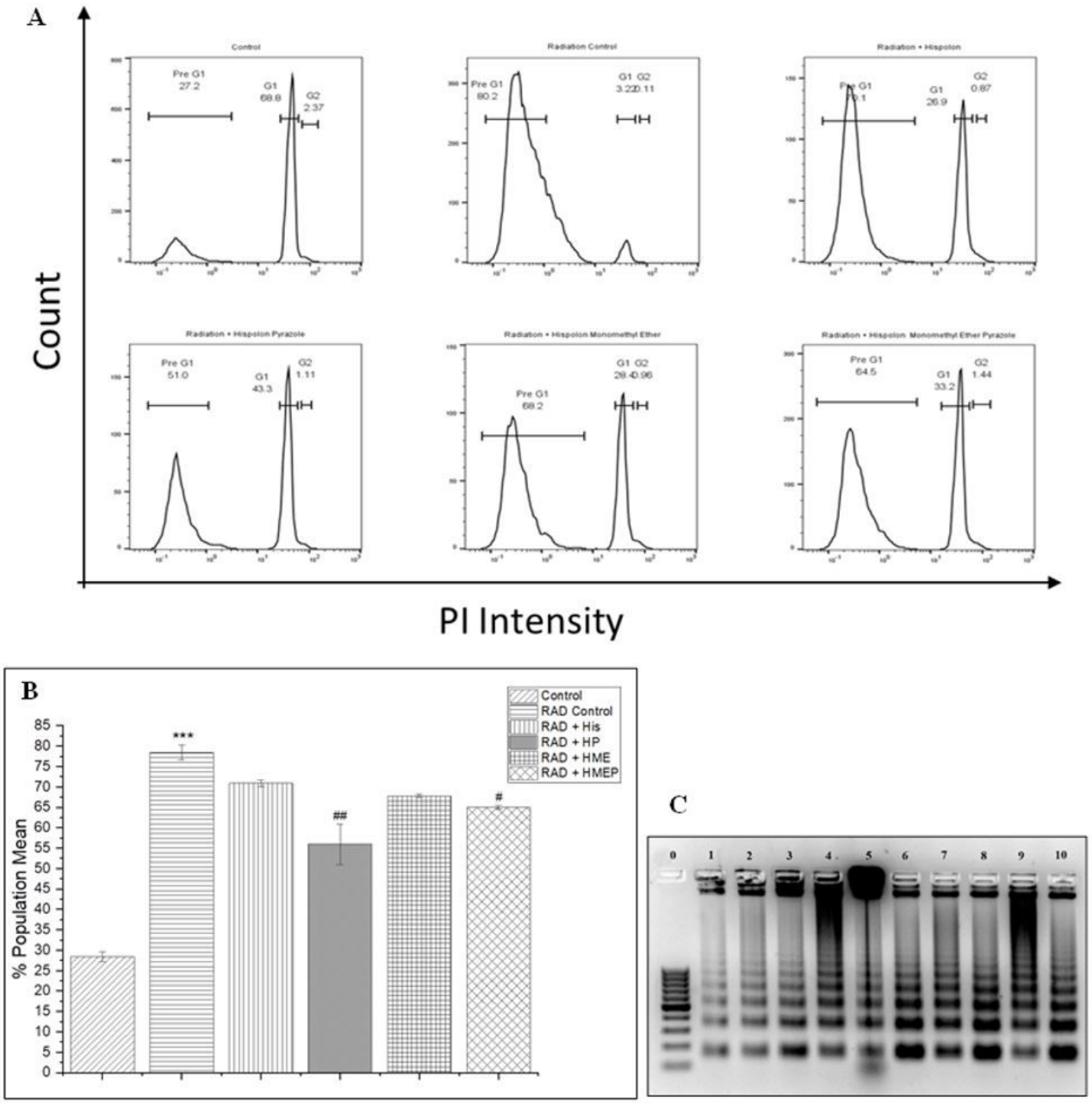

Figure 5

Effect of pre-treatment with Hispolon and its derivatives on radiation induced apoptosis in Primary cortical neuronal cells**.** *A. Pre G1 gated population showing apoptosis in different treatment groups; B. Quantification of pre G1 gated population in different treatment groups; C. DNA Fragmentation in Primary cortical neuronal cells. The results are presented as mean ± SEM (n=3). ONE WAY ANOVA test was done with Tukey's post hoc test was used and considered P<0.05 as significant.* *** *P<0.001 when compare*

Control; #P<0.05, ##P<0.01, when compared to radiation control. 0- control, 1-DMSO control, 2-Hispolon Control, 3-Hispolon Pyrazole Control, 4-Hispolon Monomethyl Ether Control, 5- Hispolon Monomethyl Ether Pyrazole Control, 6-Radiation Control (RAD), 7-Radation and Hispolon (RAD + His), 8- Radiation and Hispolon Pyrazole (RAD + HP), 9- Radiation and Hispolon Monomethyl Ether (RAD + HME), 10- Radiation and Hispolon Monomethyl Ether Pyrazole (RAD + HMEP).

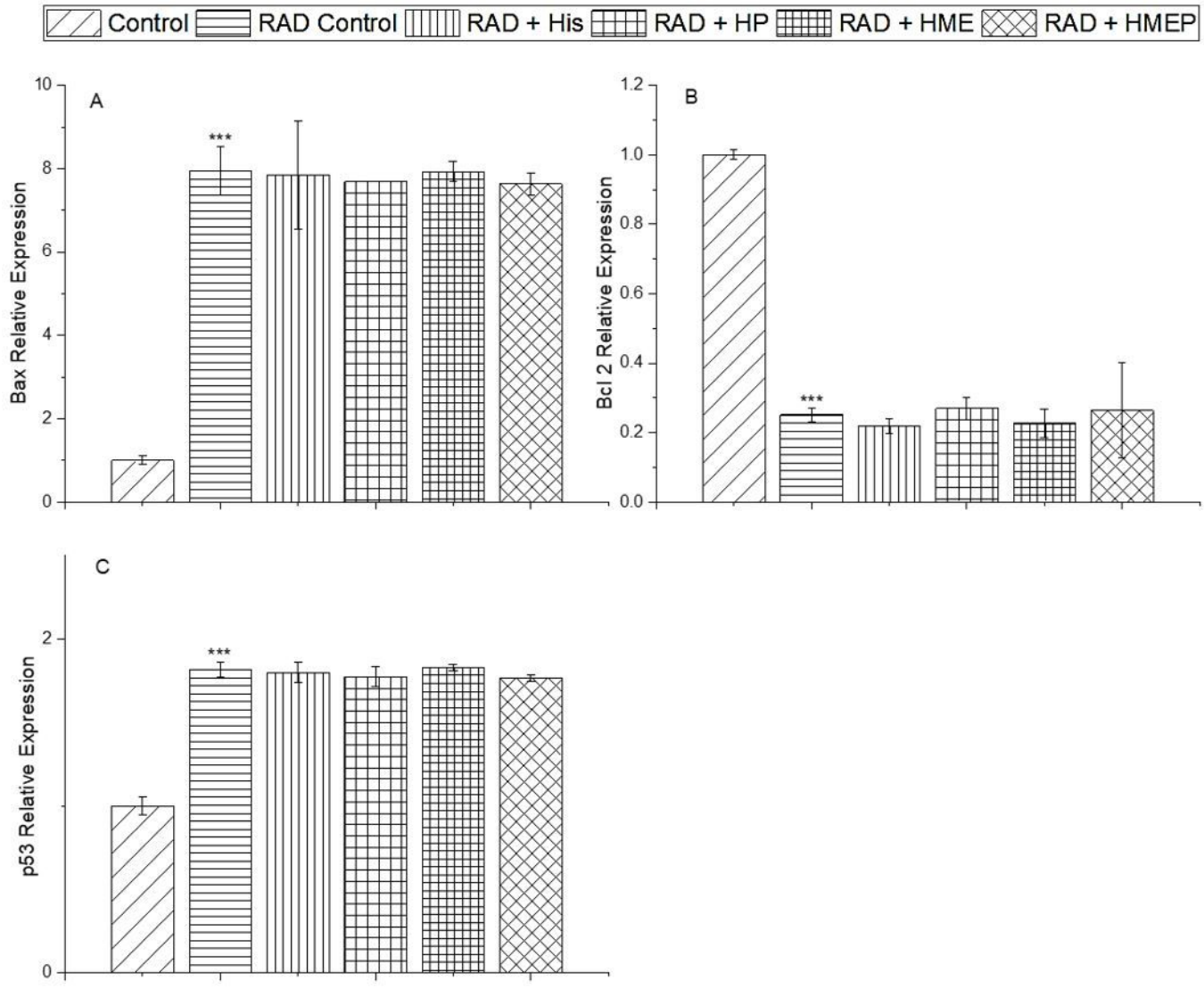

Figure 6

*Effect of Hispolon and its Derivatives on the Radiation-Induced Apoptotic Gene Expression in Primary Cortical Neuronal cell. The results are presented as mean ± SEM (n=3). ONE WAY ANOVA test was done with Tukey's post hoc test was used and considered P<0.05 as significant. *** P<0.001 when compare Control; ns- not significant, His-Hispolon, HME- Hispolon Mono Methyl Ether, HP-Hispolon pyrazole, HMEP- Hispolon monomethyl ether pyrazole.*