Summary

Mobile phone is one of the technological miracles of this century. The number of mobile phone users is continuously increasing and the total number has surpassed 4.6 billion. That means two third of the world population is using this technology. Growth of the number of mobile phone users is fast in newly industrialized and developing countries. Mobile phones are the radio devices, transmit and receive radio frequency radiation at 800-1800 MHz. The growing development progress of electronic industries and the increasing use of electrical appliances have led to higher rate of exposure of people to electromagnetic field (EMF). There is widespread scientific and public interest in possible health hazards from exposure to electromagnetic fields (EMFs) associated with radiofrequency (RF) and microwave (MW) radiation. This interest has resulted in various studies designed to assess both the occupational and residential health risk of EMFs. Some recent epidemiologic studies have suggested that the exposure to extremely low frequency (ELF) electromagnetic fields affect human health and there are plenty of incidences of certain types of health problems which have been increased among individuals living or working in environments exposed to such fields.

According to the studies, due to the tropical climatic conditions in Indian subcontinent, radiation effects are more in Indians. Children, adolescents and pregnant women are specially recommended to avoid excessive use of mobile phones. The reports advise that the people, having active medical implants, should keep their cell phone at a safe distance away from the implant. The use of hands-free technology is also recommended to lower physical contact with the body and the cell phone and the mobile phones not adhering to standard levels of energy radiation should be banned. Schools, playgrounds, high-density residential areas and hospitals should be restricted from installing mobile phone towers. Apart from health risks, the study also indicates that mobile phone radiation creates environment hazards like disappearance of sparrows, butterflies, bees and insects from big cities.

Extremely low frequency electromagnetic fields interact with an animal by inducing electric fields inside the body. These induced fields represent the internal exposure or "dose". In living animals, normal physiological activities are resulting

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into a variety of natural endogenous electric fields and also extend into rest of the body. The endogenous fields will combine by simple addition with any field induced by external exposure to electromagnetic fields. Mostly animal studies are used in the experiments to evaluate the biological effects of EMF, since *in situ* measurement of induced field cannot be performed in humans, therefore. Several contradictory results have been found in various studies using rat and mice as experimental models to elucidate the reproductive toxic effects of exposure to weak magnetic fields.

The radiation exposure can be quantified in terms of the amount of energy absorbed by a unit mass of the object. This is expressed as the specific absorption rate (SAR) which is measured in Watt/kg. The localized specific absorption rate as per the Indian guidelines standard is 2 Watt/kg, averaged over a six minute period. With higher SAR values of mobile phones, the users could potentially receive much higher radio frequency exposure. It is recommended that SAR levels be lowered down to 1.6 Watt/kg. In tissue, SAR is proportional to the square of the internal electric field strength. The SAR value is independent from the duration (or repetition) of exposure. Values of SAR depend on the incident field parameters (frequency, intensity, polarization, source-object configuration), the characteristics of the exposed body (size, internal and external geometry, dielectric properties of tissues) and ground effects and reflector effects of other objects in the field near the exposed body.

Exposure to high density microwaves can cause detrimental effects on biological systems through thermal action. With regard to localized heating and the susceptibility of individual tissues to heat, the central nervous system (CNS), the testis and the lens of the eye seem particularly sensitive. Electromagnetic field radiation emitted from cell phones may interfere with normal reproductive parameters and result in male infertility. Since all established biological and health effects of microwave exposure are related to thermal effect, safety guidelines do protect against excessive microwave heating.

However, there are many reports over the last three decades that microwave (or radiofrequency) exposure can exert non-thermal influences also, at intensities well below those necessary to cause any detectable heating. In previous investigations it

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was found that non-thermal microwave (or high frequency) exposure is able to induce several changes at the level of DNA and protein molecules including the increase in DNA single and double strand breaks in rat brain, induction of micronuclei in rat bone marrow, alteration of protein conformation without bulk heating or induction of oxidative stress in rat brain tissue. The molecular biological changes can evoke a number of alterations at the cellular level, such as increased Ca2+ efflux in cultured human cells after exposure. Hormones and receptors might be the prime targets for worst possible health hazards to the mobile phone users and their future generation.

Literature is available on the hazards of mobile phone radiation on various organs, however, with best of our knowledge, none of the electronmicroscopic study has been conducted yet to determine the effects of chronic exposure of mobile phone radiation on testicular tissue including the cells at various stages of spermatogenesis and other structures also. The research methodology adopted in our study has been already cited in highly ranked journals. The aim of this work was to monitor the time-course effects of chronic exposure to electromagnetic field emitted from domestic mobile phones on the reproductive organ of male rats (Group I- control, Group II- exposure of 3 hrs/day upto 6 months and Group III- exposure of 6 hrs/day upto 6 months). The animal model, used in this study, has been used previously by several workers to assess the adverse effects of toxicity due to various agents on reproductive outcomes.

The whole body exposure approach could stimulate any tissue as the animal body could act as an efficient antenna to pick up electromagnetic radiation, however, among the most susceptible tissues to EMF exposure were testes. Damage to these organs results in unorganized spermatogenic cells in seminiferous tubules and the reproductive hormone which must be produced in healthy manner. Such changes can have an almost immediate effect on fertility. In the present study, the genotoxicity in various cells in the testicular tissue was analyzed using various assays and the results were also supported by degenerative changes in histological, ultrastructural and hormonal aspects in experimental groups.
In present study, comet assay was performed using rat sperm cells to assess the DNA damage in various groups. It was observed that there was a significant increase in tail length, tail area, tail intensity, tail moment, olive tail moment, % DNA in comet tail, head diameter, head area, head intensity and decrease in % DNA in head in group II as compared to control group and in group III as compared to both control and group II, which revealed that consistent increases in DNA strand breaks in sperm was associated with the increasing duration of exposure. Maximum DNA damage in the sperms was observed in the group with maximum exposure duration from mobile phone.

In Halo assay, it was observed that the % of mildly damaged testicular cells was found to be increased in group II as compared to the group I (p<0.001) and group III (p<0.01). The difference between group I and group III was not found significant (p>0.05). The % of moderately damaged sperm cells was found to be increased in group II (p<0.001) and group III (p<0.001) as compared with the control group respectively. There was no significant difference between group II and group III (p>0.05). The % of extensively damaged sperm cells was found to be increased in group II (p<0.001) and group III (p<0.001) as compared with the control group respectively. There was also significant difference between group II and group III (p<0.001).

In histological studies, seminiferous tubule diameter, periphery and area significantly decreased in group II (p<0.001) and group III (p<0.001) as compared to the group I and also the tubule periphery significantly decreased in group III (p<0.001) as compared to the group II. The number of fused seminiferous tubules significantly increased in group II (p<0.001) and group III (p<0.001) as compared to the group I and also the number significantly increased in group III (p<0.001) as compared to the group II. Testicular sections of rats of group I were showing normal spermatogenesis within seminiferous tubule. Intact germinal epithelial layers of adjacent seminiferous tubules were clearly visible and the interstitium was confined to the angular spaces between the tubules and contained densely packed Leydig cells.

Testicular tissue of group II animals revealed detached adjacent seminiferous tubules and decreased number of Leydig cells at interstitial space. Large vacuoles,
condensed nuclei and free floating cells were also visible in the developing spermatogenic cells. Spermiated spermatozoa were invariably decreased and hence decreased density of spermatozoa in the lumen of seminiferous tubule was clearly observed in group II rat testes. In addition to the observations recorded from the testicular tissue of group II animals, fusion of adjacent seminiferous tubules and increased number of Leydig cells in the interstitial space were observed in group III animals. In this group, where exposure duration was maximum, many multinucleated giant cells (MNGCs) were also observed in seminiferous tubules. Such cells possessed 2 to 8 nuclei and the nuclei were either intact or had marginalized chromatin. The cytoplasm indicated little to extensive vacuolation. Some large cells were also appeared containing several micronuclei (arrow head). Such cells are designated as multiple micronucleate giant cells (MNGCs).

Transmission electron micrograph of group I testicular tissue illustrated the normal secondary spermatocytes and few early spermatids with acrosome cap formation. Copious cytoplasm was found containing the cell organelles. The micrographs of control testicular tissue revealed the secondary spermatocyte with granular cytoplasm and peripherally located mitochondria. The exposed testes showed variable degenerative changes within the seminiferous tubules. In group II animals, transmission electron micrograph was showing defective formation of acrosome cap. An amorphous electron-dense chromosomal aggregation was seen in the peripheral portion of nucleus and slight disruption of nuclear membrane was also observed in the spermatocytes. Dark, irregular shaped, vacuolated mitochondria with ill defined cristae were present in the spermatid cytoplasm. Vacuolated spermatids were present in the seminiferous tubules. The micrograph was showing degenerative vacuolated spermatid with defective acrosome cap.

In group III animals, transmission electron micrographs of testicular tissue were revealing degenerative vacuolated spermatids with acrosome cap formation. Secondary spermatocyte with dense granular cytoplasm and peripherally located mitochondria were also present. The micrograph was showing spermatids with dark mitochondria. Developing spermatogonia was observed with excessive accumulation of genetic material.

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of lipid droplets in the cytoplasm and the degree of cytoplasmic degeneration was triggered. The vacuoles of various shapes and sizes became prominent as the exposure duration increased. Annulated lamellae complex was present in a defective spermatid which was actually a degenerating endoplasmic reticulum. It was not observed in any of the other two groups.

Scanning electron micrograph of group I testicular tissue illustrated the furrow like depression running longitudinally on the outer surface of seminiferous tubules. Outside the seminiferous tubule, interstitial tissue was made up of connective tissue having Leydig cells, myoid cells and blood vessel. The germinal epithelium was located at the periphery of seminiferous tubule having a hollow lumen at the center. Larger cells occupied the basal part and smaller cells were situated towards the lumen. The spermatozoa tails were forming a network in the lumen and heads of spermatozoa showing a congression. Spermatogonia and spermatocytes were placed in the compartments surrounded by sertoli cells in the basal portion. Sertoli cells were characterized by their larger size than all other cells present in the seminiferous tubules.

In group II animals, scanning electron micrographs of testicular tissue were exhibiting asymmetrical shrinkage in the degenerating spermatogonia, spermatocytes, spermatids and sertoli cells after the exposure. The general appearance of the developing cells in the seminiferous tubule became rough with marked protrusion and depressions. Disorganized and different types of ruptured cells in the seminiferous tubule were also observed. Cluster of sperms with degenerated tails were noticed. The micrograph was showing the degenerated germinal epithelium residual of cytoplasm.

Scanning electron micrographs of testicular tissue of group III were showing deteriorated topography of various cells. Sharp edge craters and shrinkage induced on the surface of degenerating cells in the seminiferous epithelium. The cell membranes became spongy and necrotic. The residual cytoplasm, debris of degenerating cells in the epithelium, ruptured sperm head with distorted tail were also visible. The broken segments of degenerated cells were scattered in between the normal cells in the seminiferous tubule.
The serum testosterone level significantly decreased in group II (p<0.001) and group III (p<0.001) as compared to the group I and also the level of testosterone decreased in group III (p<0.001) as compared to the group II. After the completion of the exposure duration of 6 months, there was a significant increase in the body weight within each group (p<0.05 in group I, p<0.001 in group II and p<0.0001 in group III) as compared to their respective baseline body weights, and there was also a significant difference between the body weight of group I and group III (p<0.05), while there was no significant difference (p>0.05) in the body weight between group I and group II as well as group II and group III.

These are important findings indicating dose response relationship. It can be clearly concluded that the extent of damage in the sperm cells and decline in various normal testicular structures and functions were directly proportion to the exposure duration. Maximum degenerative changes were observed in the group which was exposed for maximum duration. Here it is important to note that the most of the previous studies were conducted by exposing the animals for a few days, weeks or months, but we applied long duration exposure in the experiment.

The majority of reviewed studies were conducted in laboratories. This fact cannot represent the realistic situation of cell phone communication. On the other hand, the in vivo and simultaneously in situ studies are very scarce. That is because this kind of experiment is very difficult to carry out, and interaction with other exogenous factors could change the results. One particular deficiency in most studies is that they describe experiments with acute or short-term exposure of animals on EMF. Experiments are needed to perform long term exposure in order to demonstrate the chronic impact of EMF. More studies, however, are needed to identify precisely the mechanism involved in the reduction of sperm quality and testicular structure.

The safety concerns being raised against telecommunication technology, however, require that more studies are needed to reassure the general populace about their safety. While it is recognized that scientific inquiries may not be able to prove that any technology is absolutely safe, the bundle of information gathered will enable...
the regulators to incorporate adequate margin of safety in RF guidelines, such that in 
ev events that guidelines are exceeded, severe ill effects will not occur.