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EFFECT OF EMF RADIATION GENERATED BY CELL PHONES ON HISTOLOGICAL ASPECTS OF LIVER OF CHICK EMBRYO

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ABSTRACT

The possible hazardous effects of radiations emitted from mobile phone have put major public concern today. Some studies concluded that these radiations caused damage to DNA, hormonal, metabolic changes, developmental delay, increased mortality rate, free radical production, and immunological effects while other studies have controversial effects and showed that electromagnetic field radiations had some beneficial effects also. This study was designed to observe the effects of exposure of electromagnetic radiations from mobile phones on histology of chick embryo liver. Freshly laid fertile hen eggs of '*Rhode Island Red*' species were divided into Control group (Group A= 6 eggs) and exposed group (Group B=6 eggs). Both the groups were kept in separate incubator in 37°C temperature and 50-55% humidity was maintained. An exposed group had been kept with an active mobile device which was daily rung four times 15 minutes each during the period of incubation. The control as well as exposed embryos was extracted on day 14 of incubation. All the embryos were observed for mortality, if any, live embryos chilled to death and liver got dissected. The liver tissues were further processed for micro-technique and slides had been prepared from liver tissue. All the slides had been stained by double staining procedure and observed under light microscope. In radiation exposed liver sections, there were less no. of sinusoids, decreased hepatic cords, heavy bleeding in central and portal veins, dilated hepatic veins, and portal area congested with hepatocytes while control embryos showed normal structure of liver. There was an adverse effect of electromagnetic field exposure on chick embryo mortality. Also hazardous effects had been observed due to electromagnetic radiations emitted from mobile phones in chick embryo liver.

KEYWORDS

Chick embryo, EMF, Exposure, Liver and Mobile phone.

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INTRODUCTON

With advancement of technology from the last two decades, use of electronic gadgets had been increased. Its constant use is also leading into increase in continuous exposure to the electromagnetic radiations. We are continuously floating in the pool of electromagnetic field due to wireless telecommunications. As people get constantly exposed every day to electromagnetic fields, it is the matter of great debate whether they can be hazardous to human health. Today, mobile phone being an essential part of

our lifestyle it is the general topic of concern due to potential risk associated with it. The use of mobile phones makes telecommunications easier, economical and convenient. These phones operate on electromagnetic field radiations. EMF radiations are broadly categorized into ionizing (X-ray and cosmic rays) and non-ionizing (radiofrequency or extremely low frequency or power frequency) radiations. The radiations can cause thermal as well as non-thermal effects on living being. These radiations have a capacity to heat up cells and tissues and can possibly cause damage to DNA. Every living system has its own internal electrochemical environment and radiations from external sources caused disturbances in their electrochemical activities. Various studies have been performed which proved that excess use of mobile phones increased anxiety, headache, fatigue, deafness, tremors, depressions and sleeping disorders in humans[1]. Scientific studies concluded that, EMF radiations from mobile phones affect living organisms[2]. Some finding also suggested that higher usage of mobile phones could increase risk of brain tumor[3]. The ultrahigh frequency EMF radiations from 2G cell phones had an effect on lens development and DNA damage in chick embryo[4]. [5]Found that there were decrease in number of house sparrows and decline in their population due to electromagnetic radiations from mobile phone base stations. EMF exposure was found to alter gonadal functions, reproductive endocrine hormones, pregnancy, embryonic and fetal development in various in vitro and in vivo studies[6]. There is lot of evidences indicating EMF exposure had an effect on hormonal, metabolic and stress-like behaviors in male wistar Rats[7], caused developmental delay[8], detrimental effects[9] in early period of incubation in chick embryos and increased mortality in chick embryos[10,11]. Some studies on EMF reviews the exposure of EMF from phone on wildlife and concluded that EMF as a potential cause for decline in animal population[12]. Had an effect on development and reproduction in most of the vertebrate animals[13]; and can influence several biological functions at cellular and tissue level, modulating intracellular reactive oxygen species and cell cycle progression[14]. In an epidemiological study, it has been concluded that, mobile phone use adversely affected the quality of semen by decreasing viability, motility, sperm count and morphology of sperm in human[15]. A study on chick embryo revealed that there was significant increase in whole body temperature due to different intensities of EMF radiations[16]. Another study on radiofrequency field exposure on human leukocytes cells revealed that there were significant changes in leukocytes behavior including very rapid changes in shape of leukocytes[17].

Conversely no other biological effects were reported by others. There were neither increase in the incidence of chromosomal aberrations nor exchanges in sister chromatid occurred in cultured human lymphocyte and no significant genotoxic effects were found in human lymphocytes in vitro study[18,19]. A study indicated that EMF exposure act via activation of voltage gated calcium channels to produce therapeutic effects such as bone growth stimulation through differentiation and maturation of osteoblasts by involving elevation/nitric oxide/protein kinase G pathway and produced adverse effects such as single stranded DNA break by involvement of Ca^{2+}/elevation/nitric oxide/per-oxy-nitrite/free radical (oxidative stress) pathway[20]. There were no changes observed in peripheral blood parameters on exposure of radiofrequency radiations[21]. One study on electromagnetic field based on phenomenon of resonance revealed that, there were some evidences from in vitro and in vivo experimental studies resulting in apoptosis of tumor cells and concluded that EMF frequency may have beneficial or therapeutic effects on cancer treatment[22].

Considering all these reviews, the present study was designed to demonstrate whether EMF field could be the factor for histological changes in liver of chick embryo after exposure with EMF radiations emitted from mobile phones. For this, chick embryo was used as an experimental animal. As Chick has an *in-vivo* development, it hatches after a brief period of 21 days of incubation and any maternal factors could not interfere with it. Various workers demonstrated chick embryo as a model for various studies[23,24]. Histological study of liver had been carried out to observe changes, if any, in the development of liver due to exposure of EMF radiations in chick embryo. Liver being the

largest organ in the body, it performs major functions such as hematopoiesis in embryonic period, detoxifying compounds from blood, produces bile, regulates glucose levels through glycogen storage and performs essential exocrine, endocrine and metabolic functions in the body.

MATERIALS AND METHODS

Freshly laid fertile chicken eggs of RIR (*Rhode Island Red*) species were procured from Govt. Poultry Farm, Camp Road, Amravati and were incubated in two batches. Each batch comprise of 12 eggs out of which 6 eggs were kept in standard egg incubator at 37±5° C and 55%humidity. Those were treated as control group. Remaining 6 eggs were treated as exposed group and kept in the separate incubator under similar condition of temperature and humidity and had an arrangement for mounting mobile phone at the upper center of eggs. A popular brand mobile phone having bandwidth 900-1900 MHz with SAR value 1.11 W/kg in head and 0.22 W/kg in body measured by FCC was used for exposure. The SAR value is the Specific Absorption Rate at which human body absorbs energy when exposed to radiofrequency or electromagnetic field. Mobile phone was kept on standby mode for all over 21 days of incubation period and had been rung in a silent mode for 4 times in a day for 15 min. each. All the eggs were rotated manually for 3 to 4 times a day. The eggs were routinely observed from 3rd day of incubation onwards by candling method. Egg candling is the process of observing the appearance, growth and development of chick embryo without breaking of egg shell.

After completion of 14 day of incubation, 6 eggs were sacrificed each from both control as well as exposed group. All the embryos were examined for viability, external morphology and body weight and length had been noted. The photographs of embryos were taken with Canon Powers hot G11 Camera. All the live embryos were chilled to death, liver had been dissected out and fixed in Carnoy's fixative (Glacial acetic acid = 25 ml; Absolute alcohol = 75ml) to avoid post-mortem changes followed by dehydration with series of alcohol grades and cleared in xylene, embedded in paraffin wax (58-60° C). Blocks were made by using soft paraffin wax and transverse sections of liver having 3-5 µ thickness were cut from rotary microtome and taken on slide coated with Mayer's albumen (2ml egg albumin+2ml glycerol + Thymol crystals). Single slide contain 3 to 4 sections. Sections were taken from dissected livers of about8 embryos each from control and exposed group. The sections were spread properly on slide by using spreader and later stained with H & E by double staining method and studied under light microscope (Carl Zeiss, Germany). Images were taken with Axiocam Erc5s camera (Carl Zeiss, Germany) attached with software Zen 2012.

RESULTS AND DISCUSSION

In the present study, it had been observed that there were high mortality rate and abnormalities were seen in exposed group on 14th day of incubation. While dissecting embryos, internal bleeding had been seen in exposed embryos. All the histological sections were observed under microscope. The changes in histological structure were observed in exposed liver sections.

Microscopic examinations of histology of control liver showed rows of hepatocytes arranged in sheets with prominent nuclei and these hepatocytes extended up to central vein. Also there are number of blood sinusoids in between hepatic cords. Hepatic cords are the sheets of hepatocytes extended up to central vein and separated by blood sinusoids. Portal vein is prominently seen and in normal shape with hepatic artery and bile duct. Hepatic vein is in normal shape. The central vein and portal area is surrounded with connective tissues. The venous duct is continued with intermediate portion.

In radiation exposed liver sections, the number of sinusoids had been decreased so that the hepatocytes are getting congested and less number of hepatic cords are seen. The blood sinusoid is the area for entering plasma from blood in hepatocytes in which metabolites and toxins from plasma get absorbed into nearby hepatocytes. The less number of blood sinusoids either could not performed the process of detoxification and it might not be absorbed metabolites from blood due to less number of sinusoids in exposed embryo liver. In

exposed liver sections, the clusters of blood cells have been seen in central vein and portal vein which indicates that either there may be heavy bleeding or infiltration of lymphocytes. The portal veins appeared congested with hepatocytes and blood cells. The venous duct and intermediate portion get fused hence there might be complications in transport of blood in the exposed liver. Hepatic veins are dilated and portal area is somewhat expanded in exposed liver.

The hazardous or beneficial effect of electromagnetic field on humans as well as animals is a topic of most of the earlier researches[10]. Now-a-days mobile phone culture is spreading rapidly and became an essential part of our lifestyle. Not only adults but children also frequently use mobile phones today.

In this study, the exposure of chick embryo to electromagnetic radiations having wavelength 900-1800 MHz caused abnormalities in chick embryo, increased mortality rate, subcutaneous bleeding and significant changes in liver which includes lack of blood sinusoids, degeneration of hepatocytes, disruption of architecture, heavy bleeding in portal veins and dilated central veins surrounded with congested hepatocytes. A study by[25] on rat hepatocytes concluded that there was a significant increase in hepatocytes injury after long term exposure of mobile phone which supports results obtained in the present study.

One histo-pathological study on albino mice liver stated that, there were highly significant changes in the glutamic oxaloacetic transaminases (GOT), glutamic pyruvic transaminases (GPT), and total protein (TP) on exposure of electric field as well as on microscopic level, there were some alterations in histology of liver including hepatic tissues with two portal tracts, dilated central vein engorged with blood cells and necrotic changes in hepatocytes and hepatic tissues[26].

This finding also supports current results. An electromagnetic field exposure study on guinea pig liver concluded that there were highly cytoplasmic vacuolation of glycogen in exposed liver[27]. [28]Noticed apoptosis in hepatocytes, hypertrophied binuclei, focal necrosis, dilated hepatic sinusoids and destruction of bile ductules while studying the effects of non-ionising electromagnetic field on liver of male mice. An enzymatic study had been taken by[29] on male rats showed increase in concentration of all liver enzymes including Bilirubin, Aspartate Aminotransferase, Alanine Aminotransferase, Albumin, Total Protein, Lipid Peroxidase and Glutathione conc. above the normal concentration.

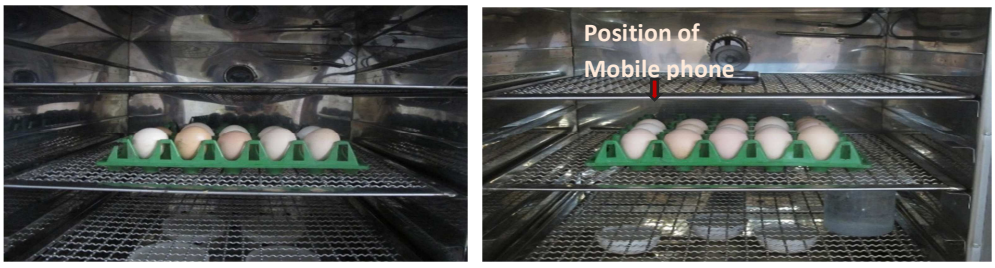


Figure No.1: Arrangement of eggs for control and exposed groups respectively provided with normal incubating temperature and humidity

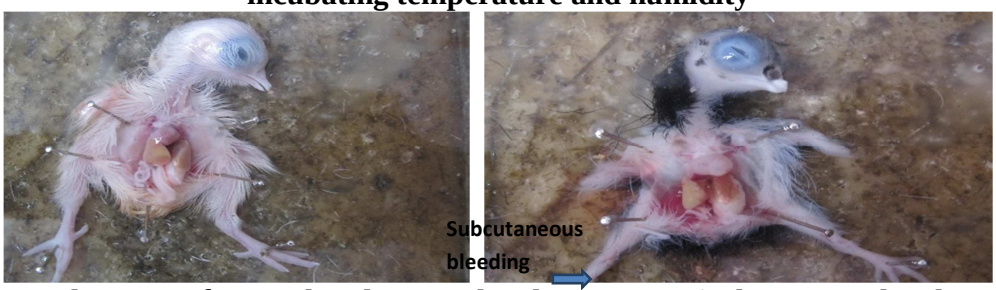


Figure No.2: Dissected organs of control and exposed embryo respectively. Exposed embryo showed internal bleeding

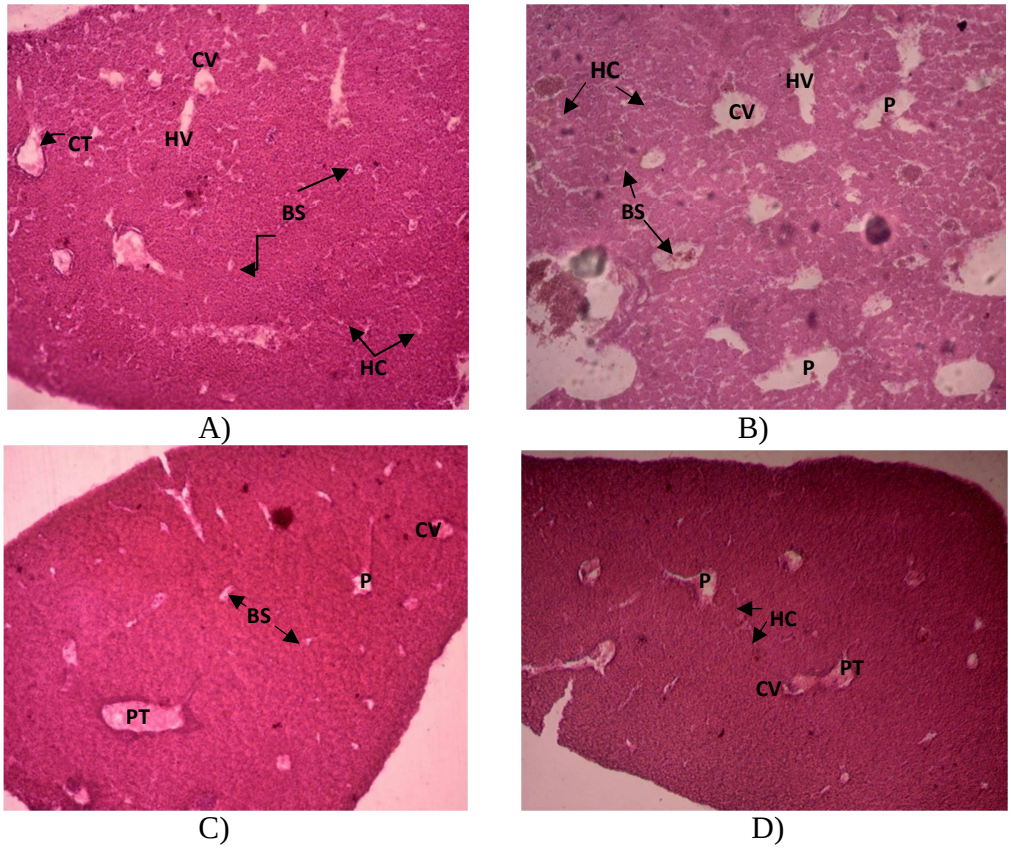


Figure No.3: Showing photomicrograph in 10x objective indicating hepatic cords and blood sinusoids in A) and B) control group liver sections while less no. of hepatic cords and blood sinusoids in C) exposed sections. In exposed sections, connective tissues surrounding central vein and portal area have been disappeared. D) central vein get fused with portal traid

Abbreviations: P- portal area, BS- blood sinusoids, HV- hepatic vein, HC- hepatic cords, CT- connective tissue, CV- central vein

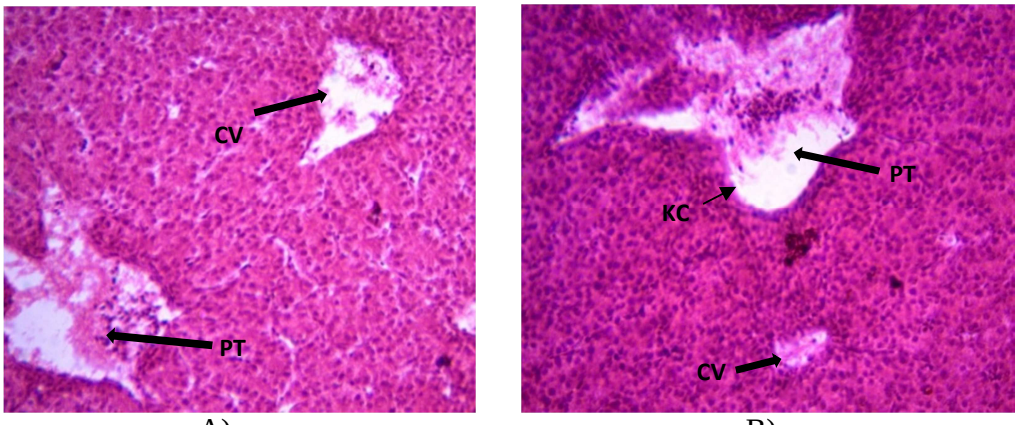
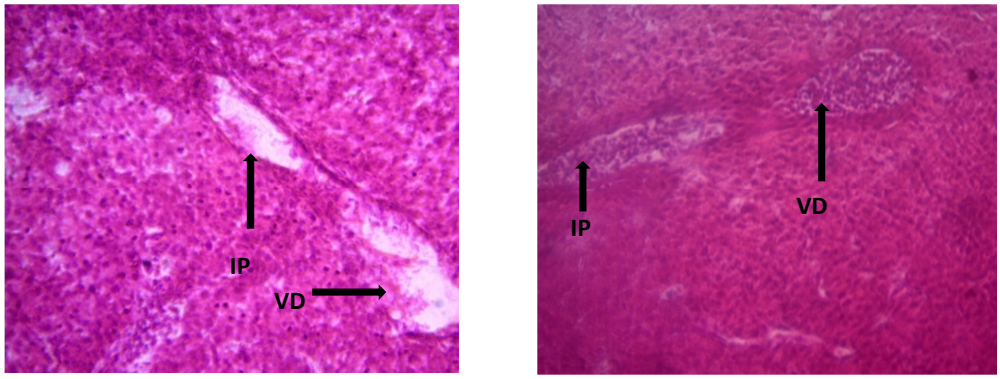


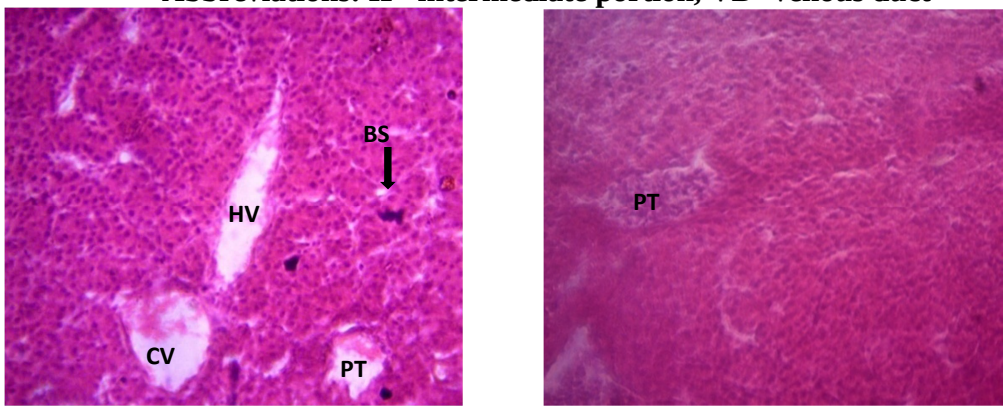
Figure No.4: Showing photomicrograph in 40x objective indicating upper central vein and lower portal triad in A) control liver sections and upper portal triad with accumulation of Kupffer cells in periphery and lower central vein with mild degenerative effects and destruction of cellular architectures in B) exposed sections

Abbreviations: PT-portal triad, CV- central vein, KC-kupffer cells



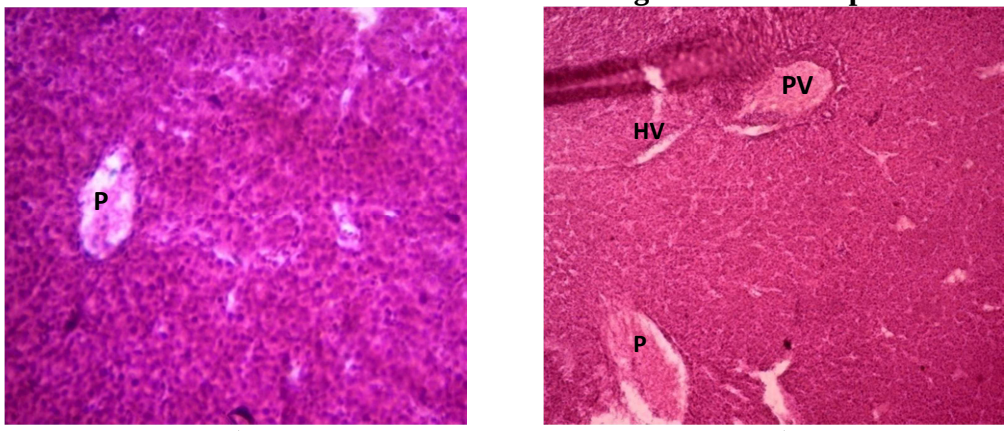
A) B)

Figure No.5: Showing photomicrograph in 40x objective visualizing venous ducts and intermediate portion in A) control and B) exposed sections of liver. Venous duct filled with blood cells in exposed liver sections Abbreviations: IP- intermediate portion, VD- venous duct



A) B)

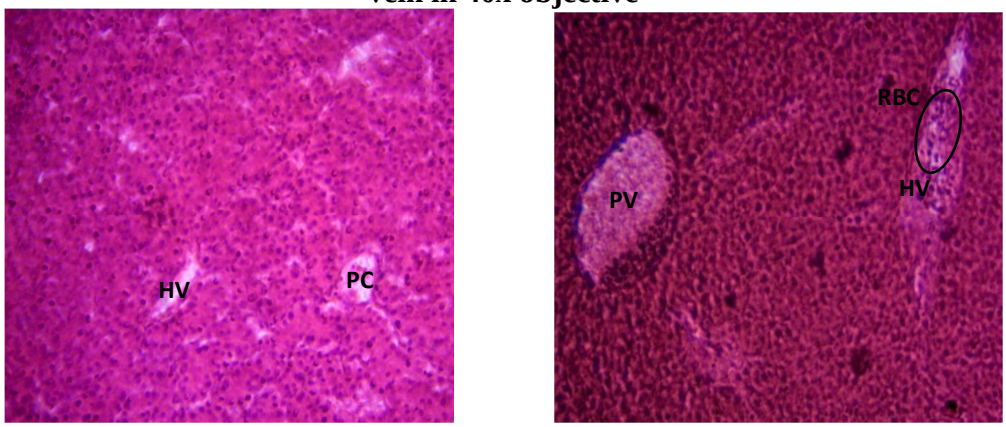
Figure No.6: Showing photomicrograph in 40x magnification indicating central vein, portal area, portal triad and hepatic vein in A) Control group having normal architecture and B) exposed liver sections showing portal triad filled with blood cells and mild degeneration of hepatic cells



A) B)

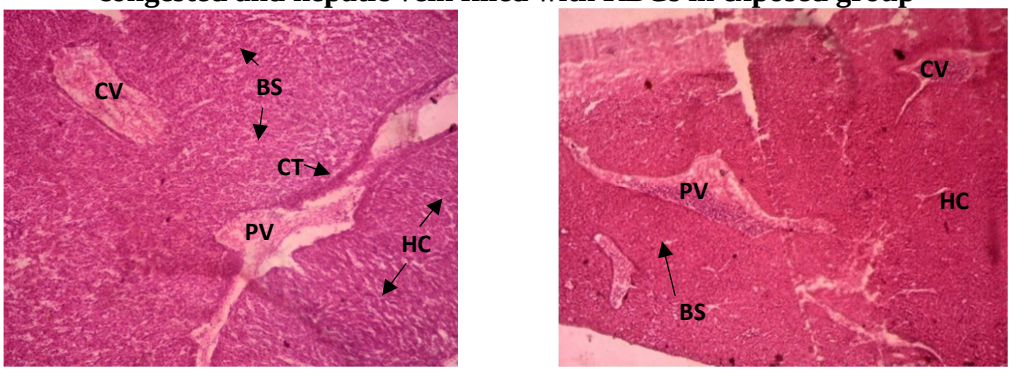
C)

Figure No.7: showing photomicrograph indicating A) portal area in control sections in 40x objective, B) portal vein in control sections in 10x objective and C) exposed group sections showing infiltration of cells in portal vein in 40x objective



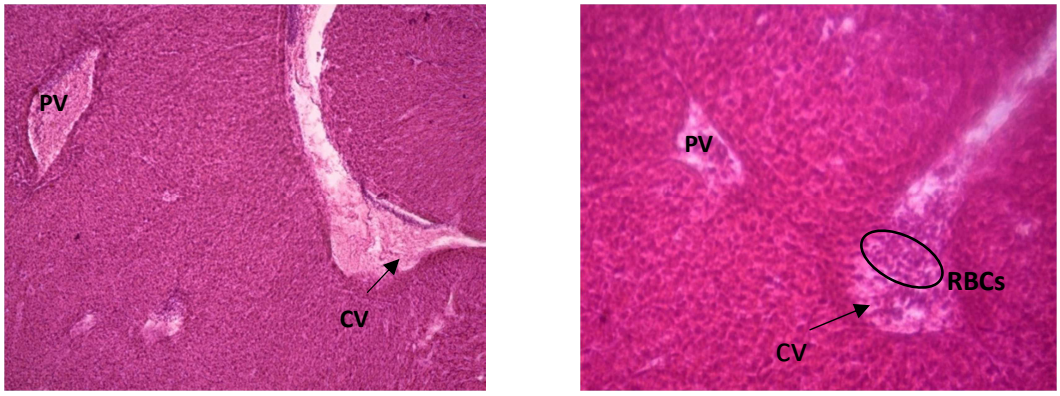
A) B)

Figure No.8: A) showing hepatic vein and portal canal in control sections of liver while B) portal vein is very congested and hepatic vein filled with RBCs in exposed group



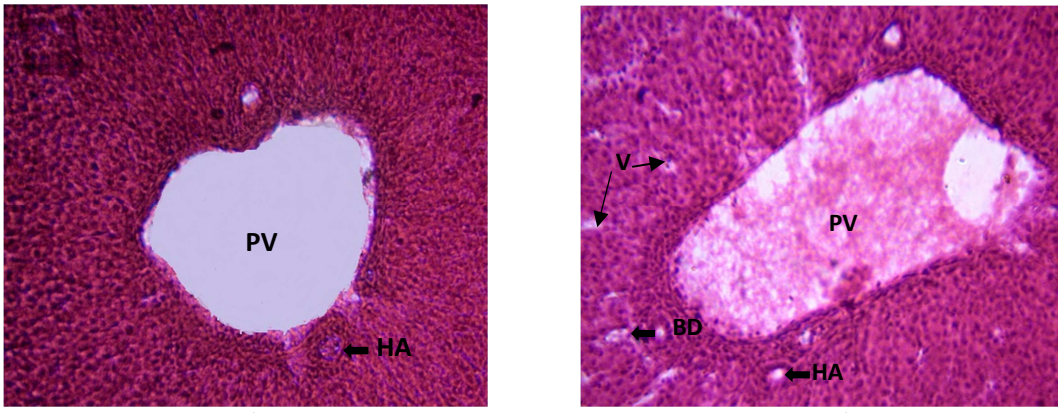
A) B)

Figure No.9: Photomicrograph in 10x objective showing A) control liver sections with more no. of blood sinusoids, hepatic cords and extended portal vein lined with connective tissues B) exposed liver sections showing very less no. of blood sinusoids and hepatic cords and portal vein not surrounded by connective tissues and filled with RBCs which indicates heavy bleeding.



A) B)

Figure No.10: A) control liver sections showing extended central vein and portal vein in 10x and B) exposed liver sections in 40x objective showing heavy bleeding in central vein filled with RBC and destruction of portal vein



A) B)

Figure No.11: Photomicrograph in 40x objective showing chick embryo liver sections indicating portal vein in A) control liver and B) exposed liver with dilation of portal vein and vacuolation in the sections.

CONCLUSION

From this histological study, it had been concluded that the electromagnetic radiations from mobile phones might be one of the major factor for bringing significant changes in exposed liver of chick embryos. Further study might be required including exposure of chick embryo liver to varying intensities of electromagnetic field as well as exposure of various vital organs of chick embryos to EMF radiations. Similar studies is needed using mammalian experimental model or using mammalian liver cell line for establishing results in mammals.

From the above results, it should be suggested that, there should be very low usage of mobile phones during pregnancy, as the electromagnetic radiations from it might be harmful for developing foetus.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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