4.1 Introduction

There is an exponential growth in the use of cell phones and Wi-Fi. Though cell phones are an essential part of our day to day communication, exposure from the electromagnetic radiation emanating from them can lead to various disorders. Further, set up of mobile phone towers in cities close to residential areas in order to amplify the rate and connectivity has further increased the exposure to electromagnetic radiation. These high frequency radiation (2450 MHz) can have serious harmful effects on human health (Gupta et al., 2018b, Moradi et al., 2016b, Gupta et al., 2018c); (Moradi et al., 2016a). Radiation on these frequency, have non-ionizing, non-thermal and adequate energy to convey alter the normal body physiology (Sinha, 2008a). Apart from this, the exposure to these radiofrequencies leads to alter the mechanical and rheological properties of blood (Challis, 2005a, Rad et al., 2015b). These changes in the flow dynamics of blood can lead to alterations in the mucus membrane properties, an important defensive factor in our body especially in the gastrointestinal system (Sørbye and Svanes, 1994). Clinical reports suggest that long term use of cell phone radiation showed reduction in regional cerebral blood flow (Aalto et al., 2006a). Further, preclinical reports suggested that chronic exposure to EMR alters blood physiology due to an increase in viscosity of blood (Aalto et al., 2006a, Moradi et al., 2016b) resulting in diminished angiogenic activity which in turn plays a significant role in the pathophysiology of gastric ulcer. Preclinical as well as clinical studies suggest that vascular endothelial growth factor (VEGF) is activates angiogenesis and its decrease causes gastric ulcer in experimental rats (Kermani et al., 2005). Electromagnetic radiation not only affects the peripheral functions but can have severe adverse central effects too (Sinha, 2008a), (Gupta et al., 2018c). Repeated EMR exposure has been

linked to the generation of stress, depression, memory deficits, and neuroinflammation (Zhang et al., 2014, Kahya et al., 2014, Deshmukh et al., 2015). However, clinical report suggest that use of mobile phone can lead to headache, sleep anomalies and depression (Hossmann et al., 2003, Levin, 2015). Furthermore, researchers have reported that subjects who were exposed chronically to EMR have decreased serotonin levels (Ezz et al., 2013). Serotonin, an endogenous enterogastrone inhibits gastric acid secretion, and promotes the release of gastric and colonic mucosa (Ezz et al., 2013). Therefore, alterations in the serotonergic system following EMR exposure can potentially lead to acid peptic disorders (Ormsbee III and Fondacaro, 1985). Mahdavi et al. reported that sub-chronic exposure to cell phone radiation causes increase the level of stress hormones in the body (Mahdavi et al., 2014b). Previous report have showed that long term secretion of corticosterone amplifies gastric acidity and pepsin release with subsequent peptic ulceration, perforation, or hemorrhage (Gray, 1951). Therefore, repeated exposure to EMR can potentially lead to disorders of the gastrointestinal system. Short term exposure with ionizing radiation at a dose level of 6 gray (gray is an unit of ionizing radiation, which is equivalent to one joule radiation energy absorbed/kg of organ or tissue weight) causes gastric ulcer in rats (El-Ghazaly et al., 2011). This type of radiation is used as therapy for treatment of cancer (Layer et al., 1986). However, there are no experimental studies on the effect of non-ionizing EMR-2450 on pathological effects on gastric tract. Therefore, the present study was conducted to study the adverse effects of long term exposure with cell phone radiation on gastrointestinal system. To understand the pathophysiological mechanisms, we also investigated the inflammatory and hemodynamic changes in the gastrointestinal tract (GIT) of rats following chronic exposure to EMR-2450 MHz frequencies emanating

through cell phones and Wi-Fi. In addition, we also evaluated the pharmacology of anti-ulcer drug in the treatment of EMR-induced ulcer.

The mechanism of gastric ulcer is based on disproportion of two main factors such as protective and aggressive factors (Rademaker and Hunt, 1991). Omeprazole (OMZ) is the first line treatment of gastric ulcer (Salas, Ward, & Caro, 2002). OMZ is the agent which inhibit the H^+/K^+ATPase enzyme only when acid gastric secretion is more in abdomen (Jackson et al., 2016). OMZ inhibits gastric acid secretion through parietal cell by specific suppression of H^+/K^+-ATPase. OMZ irreversibly blocks the final step of acid production at the secretory surface of the gastric mucosa (Shin & Sachs, 2008) and increase the gastric blood flow and micro vessel in the granulation tissues in subjected rodents (Mattsson & Larsson, 1987). In earlier preclinical ulcer models, OMZ (20mg/kg) has shown significant improvement in healing of gastric tissues (Watanabe et al., 2001). Further, chronic exposure to EMR enhance oxidative stress and inflammatory markers (TNF-α and IL-6) in the brain of experimental rats (Megha et al., 2012). Thus, we hypothesize that the EMR exposed animals can have deranged gastric function with increased levels of inflammatory cytokines. Furthermore, OMZ can decrease the expression of proinflammatory cytokines related to gastric ulcer in rodents such as TNF-α and IL-6 (Chanchal et al., 2016). The genesis of gastric ulcer occurs due to a disproportion between offensive factors like acid secretion and defensive factors like mucus secretion, blood flow etc. Gastric ulcer was estimated by determining the ulcer index based on the lesion area. Further, blood flow, preliminary estimation of gastric ulcer (pH, gastric volume) and evaluation of H^+/K^+ATPase enzyme activity was examined. Furthermore, estimation of oxidative stress, assessment of pro and anti-inflammatory markers in gastric tissues was done to understand the process of

ulcerogenesis. Morphological changes of gastric tissues were done to histopathology of EMR exposed rats. OMZ is FDA approved drug and exhibits its therapeutic effects via antagonizing proton pump activity. Therefore, we assessed the dose dependent effect of OMZ against EMR-induced gastric ulcerations. This report may also give approach for the pathophysiological and pharmacological mechanisms leading to gastric ulcer following sub-chronic exposure to electromagnetic radiation (depicted in figure 4.1).

4.1.1 Hypothesis

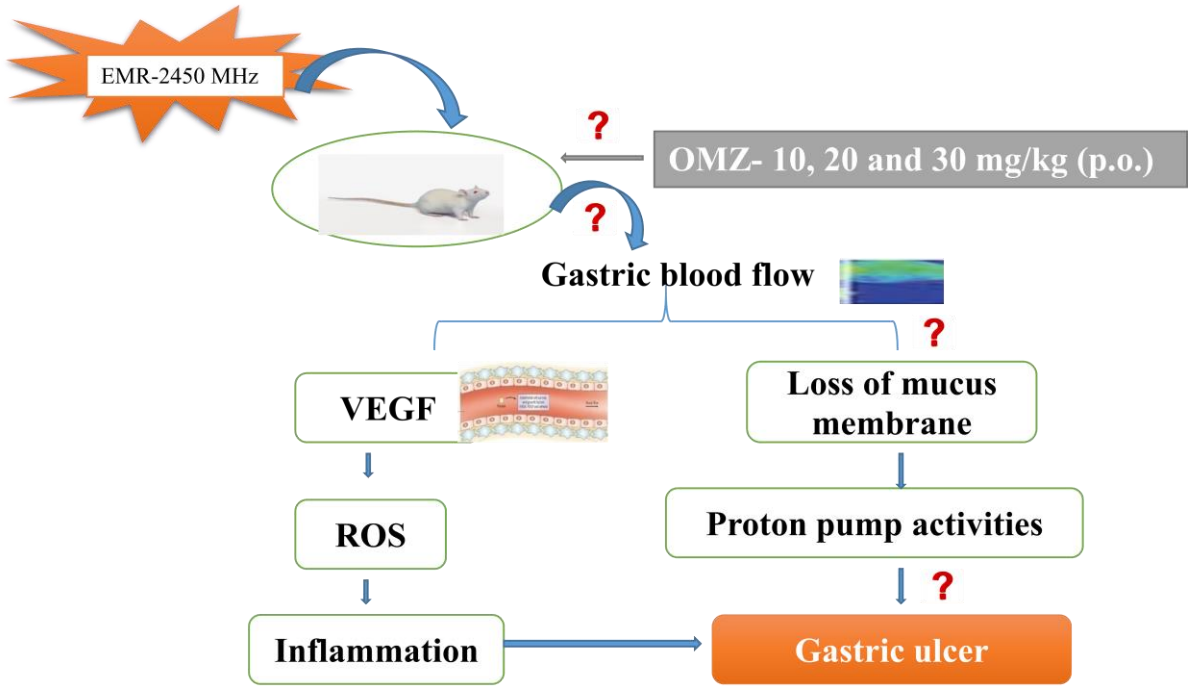

Figure 4.1 Proposed hypothesis of effect of EMR on gastric integrity and pharmacology in rats.

4.2 Materials and methods

4.2.1 Animals

Albino Wistar male rats (220±20 g) were purchased from Institute of Medical Sciences, Banaras Hindu University and were housed at 26 ± 2 °C, relative humidity 44–56% and light: dark cycle of 12:12 h (Approval No.: Dean/2015/CAEC/1414). Animals were provided with standard rodent pellet diet (Paramount Pvt. Ltd.) and water *ad libitum*. The experiment was conducted in accordance with the principles of laboratory animal care (National Research Council US Committee for the Update of the Guide for the Care and Use of Laboratory Animals 2011) guidelines.

4.2.2 Drugs and chemicals

Omeprazole was obtained from Cipla Ltd. Goa, India. The commercial ELISA kits for pro and anti-inflammatory markers purchased from Cayman chemicals (India). All other chemicals and reagents of high performance liquid chromatography (HPLC) and analytical grade were procured from local suppliers.

4.2.3 Electromagnetic Radiation Exposure System and Design (Refer chapter 2.1 page no. 20)

4.2.4 Calculation of Power density and specific absorption rate of gastric region

The power density was obtained by using the formula described in our recent report (Gupta et al., 2018). The power density was found to be 0.12 W/m^2. While the overall value of whole body SAR was found to be approximately 0.0275W/kg for 2450 MHz while the value of SAR in gastric region was found to be 0.0575W/kg.

4.2.5 Experimental design

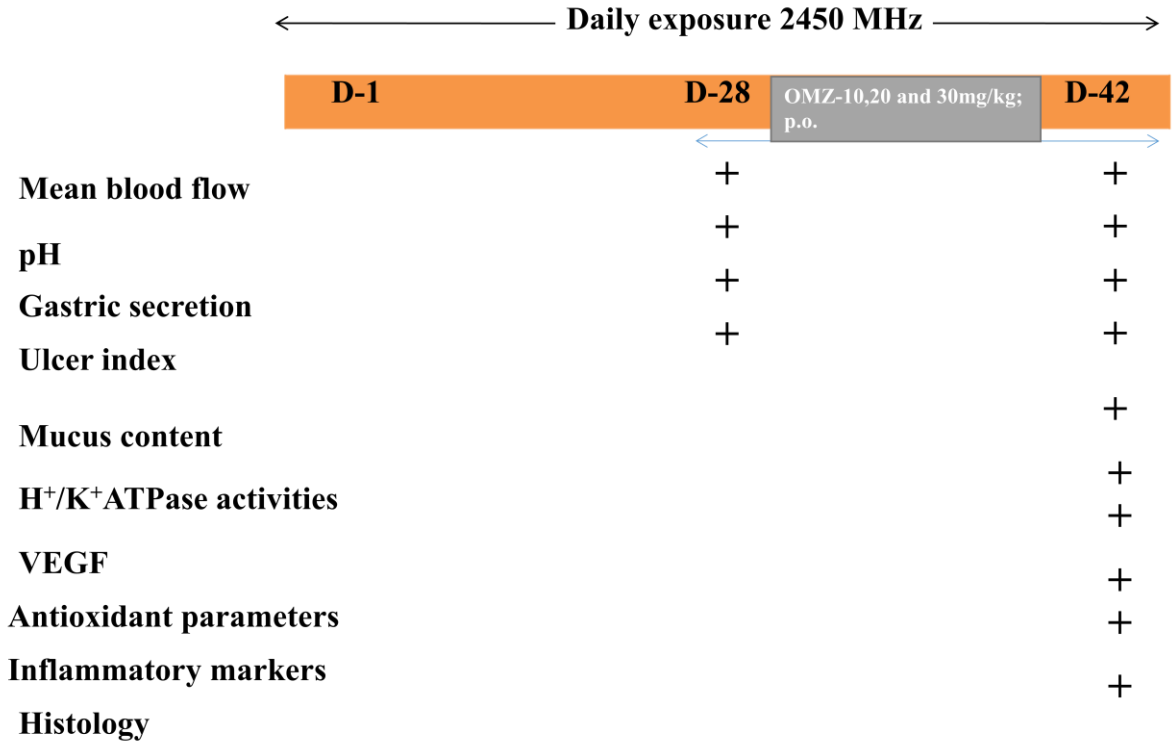

Figure 4.2 Schematic representation of the experimental design. '+' denotes experiment performed

The experimental protocol is a treatment schedule designed to evaluate effect of EMR on both gastric ulcers and pharmacological effect of omeprazole. The rats were randomly divided into five groups with twelve animals each through G* power analysis software. The groups were control, EMR-2450, EMR-2450 (1), EMR-2450 (2) and EMR-2450 (3). Four animals in each group were killed on day 28 to verify incidence of gastric ulcer and thereafter treatment (n=8) was started with omeprazole from day 28 to day 42 (14-day treatment schedule) illustrated in figure 4.2. The control rats were kept in anechoic chamber without any exposure to EMR.

On the day-1 (D-1) all the animals except from the control group were exposed to EMR without interruption for 28 days or 42 days for 1 hr. EMR exposure was between 9:00

am to 1:00 pm daily. To verify development of ulcer, on D-28, rats (n=4) were anaesthetized by pentobarbitone (35 mg/kg, i.p.) followed by assessment of stomach blood flow via laser speckle blood flow imager (Omegazone OZ-2; Omegawave, Tokyo, Japan). The animals were killed and the ulcer score, volume of gastric contents, pH, total acidity, free acidity were quantified in control and EMR exposed animals. In the present study, we have taken omeprazole to establish the pharmacological efficacy of drugs in EMR induced gastric ulcers. Previous study suggested that omeprazole alone treatment would not influence the parietal cells in the healthy rats/control groups (Hakan Blom, 1981). So, we have not added control + omeprazole group in this study. After verification of gastric ulcer on D-28, treatment on the remaining animals in EMR-1, EMR-2 and EMR-3 groups was started with OMZ-10, 20 and 30 mg/kg daily from D-29 to D-42. On D-42, rats were anaesthetized by pentobarbitone (35 mg/kg, i.p.) followed by exploration of stomach blood flow. After mean blood flow measurement, all rats were killed and immediately stomach was micro-dissected and the gastric contents were collected to estimate pH, volume, total acidity, free acidity and ulcer score. Thereafter, gastric tissues were stored at -80^0 C for further analysis. The mucus concentration and H+K+-ATPase activity were quantified in all the animals. Moreover, pro- and anti-inflammatory, oxidative stress markers and histopathology of gastric tissues were done. Out of eight rats from each group, gastric tissue from four animals (n=4) were used for H^+/K^+ATPase inhibitory activity, antioxidant parameters, VEGF, pro and anti-inflammatory marker estimations. The remaining animals (n=4) were used for histology.

4.2.6 Measurement of gastric blood flow

Gastric blood flow was estimated with laser speckle blood flow imager (Omegazone OZ-2; Omegawave, Tokyo, Japan) as reported earlier (Paliwal et al., 2018).

(LSIv3.3.1 and LIAv3.3.0). The unit of mean blood flow was an arbitrary unit (AU).

4.2.7 Estimation of pH, gastric volume, free and total acidity

The gastric contents were examined for gastric pH (Hanna Instruments HI 98107) and free and total acidity were measured according to (Gupta et al., 2015).

4.2.8 Quantification of ulcer index

To determine the degree of gross mucosal damage, stomach was cut along the greater curvature followed by washing in ice cold saline and photographed using a digital camera. The sum of the area of all lesions in the glandular portion of the stomach of each rat was calculated with Image J software program and used as the gross damage index (Helander, 1983).

4.2.9 Estimation of Mucus content

After killing, the glandular portion of stomach was collected, weighed and immersed in the alcian blue dye. The gastric mucus content was estimated by the method of (Corne, 1974). Results were expressed as µg alcian Blue/g tissue.

4.2.10 Determination of H^+K^+-ATPase inhibitory activity

Evaluation of H^+K^+-ATPase activity was done in EMR exposed gastric tissues of rats (Dorababu et al., 2006). The total protein content was estimated as per described in the methods of (Box, 1983, Lowry et al., 1951), whereas the H^+K^+-ATPase inhibitory activity was assayed by the methods of (Reyes-Chilpa et al., 2006). The amount of inorganic phosphate (Pi) liberated from the ATP was measured spectrophotometrically at 640 nm following the methods of (Griswold et al., 1951). Results of the final solution were expressed as mM Pi liberated/mg protein/h.

4.2.11 Antioxidant and free radical determination

Glandular region of stomach was obtained from EMR exposed ulcerated rats for the estimation of LPO and antioxidant enzymes (SOD, catalase and GPx). The stomach was homogenized in 0.9% ice cold saline for 30 seconds which was then centrifuged at 800 × g for 10 min and 12,000 × g for 15 min (Goel, 2001). The supernatant containing the mitochondrial fraction was used for following estimation.

4.2.12 Estimation of LPO, SOD, Catalase and Glutathione

LPO was estimated as per the method of (Ohkawa et al., 1979). The absorbance of sample was measured spectrophotometrically at 532 nm and results were expressed as nmol MDA/mg of protein. SOD was estimated by the method of (Kakkar et al., 1984). The results were expressed as units [U] of SOD activity/mg of protein. Decomposition of H_2O_2 in the presence of catalase was determined at 240 nm by given method (Beers and Sizer, 1952). The GPx activity was assayed spectrophotometrically by using method (Wendel, 1981), the method involves the glutathione (GSH) NADPH/glutathione reductase system and the dismutation of H_2O_2 at 340 nm. In this assay, the enzyme activity is measured indirectly by means of NADPH decay. The enzymatic activity was expressed in µmol NADPH/min/mg protein.

4.2.13 Measurement of VEGF levels in the gastric tissues

The gastric tissue samples were homogenized for 30 s in ice-cold PBS (10 mM, pH 7.4) and centrifuged at 20,000 g for 20 min at 4°C. VEGF level in the supernatant was measured by an ELISA kit. Protein concentration of the supernatant was determined. The final values were represented as picograms per millilitre of protein (Marsano et al., 2013).

4.2.14 Estimation of pro and anti-inflammatory markers in gastric tissues

Pro and anti–inflammatory estimation was assessed in the gastric tissues through evaluation of TNF-α, IL-6 and IL-10 using ELISA kits (Kumar et al., 2016).

4.2.15 Histopathological studies

The glandular portions of the stomach were obtained from EMR-2450 MHz exposed ulcerated rats. Tissues were dipped in 10% formalin, thereafter embedded in paraffin blocks for sectioning. The obtained sections (1-3 µm) were stained with haematoxylin and eosin dye and finally monitored and photographed using a digital camera system Leica DFC 290 (Leica Microsystems Ltd., Wetzlar, Germany) on an Intel® Pentium® D computer (Model dx2280 MT, Hp Compaq, California, USA) at 10 × magnification (Khinchi et al., 2014). The glandular portions of the stomach were obtained from EMR-2450 exposed ulcerated rats. Tissues were dipped in 10% formalin, thereafter embedded in paraffin blocks for sectioning. The obtained sections (1-3 µm) were stained with haematoxylin and eosin dye and finally monitored and photographed using a digital camera system Leica DFC 290 (Leica Microsystems Ltd., Wetzlar, Germany) on an Intel® Pentium® D computer (Model dx2280 MT, Hp Compaq, California, USA) at 10 × magnification (Khinchi et al., 2014).

4.2.16 Statistical analysis

Experimental data were expressed as mean ± standard error of mean (SEM). All the datasets were analyzed using one-way analysis of variance (ANOVA) followed by Newman-keuls post hoc test. The level of statistical significance is often expressed as a p-value between 0 and 1. In this study $p < 0.05$ were considered as statistically significant for all experimental data analysis. The lower the p-value, the greater the chances for rejection of the null hypothesis. Therefore, in our research hypothesis, there is less than a 5% probability of the null hypothesis to be correct. So there are 95% chances that our research hypothesis is true.

4.3 Results

4.3.1 Repeated exposure of EMR-2450 MHz decreased gastric blood flow in rats

In Table 4.1 (A and B) illustrates that repeated exposure of EMR-2450 MHz causes significantly reduced the blood flow (58%) compared with control rats. We observed that repeated exposure of EMR-2450 MHz significantly diminished the gastric mean blood flow in among groups [F (4, 15) =57.61; P<0.05]. OMZ-30 mg/kg at highest dose for fourteen consecutive days ameliorated the gastric blood flow in repeated exposure of EMR of rats (93%). OMZ-10 and 20 mg/kg treated EMR exposed animals (48%) showed significant difference in mean blood flow compared to control animals. One-way ANOVA revealed that OMZ-30 mg/kg restored the gastric blood flow to normal level among groups [F (4, 35) =37.67; P<0.05].

Table 4.1 (A) Repeated exposure of EMR-2450 MHz decreased gastric blood flow in rats

Mean Blood Flow	**Control**	**EMR-2450 MHz (E)**	**EMR-2450 MHz (E1)**	**EMR-2450 MHz (E2)**	**EMR-2450 MHz (E3)**
D-28	46.5±1.98	19.80±1.56[a]	19.50±1.78[a]	20.2±1.27[a]	19.10±1.18[a]

(B) Effect of Omeprazole on gastric blood flow in EMR-2450 MHz exposed rats.

Mean Blood Flow	**Control**	**EMR-2450 MHz (E)**	**E1+ O-10**	**E2+ O-20**	**E3+ O-30**
D-42	47.2±2.42	17.40±1.56[a]	25.15±1.87[a,b]	36.60±1.74[a,b,c]	45.85±2.75[b,c,d]

Table: 4.1 (A) & (B) All values are expressed as mean±SEM. [a]p<0.05 compared to control, [b]p<0.05 compared to EMR-2450MHz, [c]p<0.05 compared to OMZ-10mg/kg and [d]p<0.05 compared to OMZ-20mg/kg [One-way ANOVA followed by Student–Newman Keuls test].

4.3.2 EMR-2450 MHz causes gastric ulcerations in experimental rats

Table: -4.2 (A and B) shows the repeated exposure of EMR-2450 MHz causes change the preliminary function (gastric pH, activity of gastric acid secretion and gastric ulcer index) of stomach in experimental rats. On D-28 EMR-2450 MHz exposed rats have shown decrease in gastric pH (40%), increase in volume of gastric content (83%), Total acidity (82%), free acidity (71%) and ulcer index (70%) compared with control rats demonstrated in Table- 4.2. OMZ-30 mg/kg treated EMR exposed animal shows significantly increase gastric pH (103%), reduce the activity of gastric secretion (vol. of gastric content 98%, total acidity 94%, free acidity 97% and ulcer index ensuing complete healing of gastric tissues compared with untreated rats. We observed that OMZ-10 and 20 mg/kg treated EMR exposed animal have shown no significant changes compared to EMR exposed animals. One-way ANOVA analysis shown that there were significant changes in preliminary function of (shown in figure. 4.3 (A) & (B) among groups [F (4, 35) =52.36; P<0.05], [F (4, 35) = 69.41; P<0.05], [F (4, 35) =33.63; P<0.05], [F (4, 35) =; P<0.05] and [F (4, 35) = 411; P<0.05] respectively.

Table 4.2 (A) Effect of EMR-2450 MHz on pH, volume of gastric tissues, Total acidity and free acidity of gastric tissue on D-28 in rats.

D-28	**Control**	**EMR-2450 MHz (E)**	**EMR-2450 MHz (E1)**	**EMR-2450 MHz (E2)**	**EMR-2450 MHz (E3)**
pH	3.72±0.08	2.29±0.07[a]	2.10±0.08[a]	2.10±0.05[a]	2.12±0.0**9**[a]
Volume of gastric juices (ml/100ml)	2.24±0.06	4.10±0.14[a]	3.95±0.15[a]	4.18±0.08[a]	4.05±0.06[a]
Total acidity (meq/l)	28.25±1.48	51.48±3.12[a]	48.50±3.56[a]	49.20±2.86[a]	52.65±4.15[a]
Free acidity (meq/l)	22.48±1.15	38.38±1.26[a]	36.15±1.31[a]	38.33±1.02[a]	37.56±1.37[a]
Ulcer Index (mm^2)	00.00±00	72.19±4.96[a]	69.59±4.68[a]	70.42±5.14[a]	74.60±7.60[a]

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(B) Effect of Omeprazole on gastric tissue of EMR-2450 MHz exposed rats.

D-42	**Control**	**EMR-2450 MHz (E)**	**E1+ O-10**	**E2+ O-20**	**E3+ O-30**
pH	3.64±0.07	2.32±0.09[a]	2.65±0.08[a]	3.10±0.07[a,b,c]	3.75±0.11[b,c,d]
Volume of gastric juices (ml/100ml)	2.30±0.07	4.35±0.11[a]	4.10±0.14[a]	3.20±0.15[a,b,c]	2.25±0.10[b,c,d]
Total acidity (meq/l)	28.32±1.48	68.71±4.22[a]	46.08±3.12[a,b]	37.94±2.56[a,b,c]	26.71±2.66[b,c,d]
Free acidity (meq/l)	22.52±1.34	48.80±2.33[a]	36.38±1.26[a,b]	30.15±1.31[a,b,c]	21.80±2.33[b,c,d]
Ulcer Index (mm^2)	00.00±00	74.90±2.33[a]	48.25±2.22[a,b]	23.15±1.31[a,b,c]	3.80±0.20[b,c,d]

Table 4.2 (A) & (B) All values are expressed as mean±SEM. [a]$p<0.05$ compared to control, [b]$p<0.05$ compared to EMR-2450 MHz, [c]$p<0.05$ compared to OMZ-10mg/kg and [d]$p<0.05$ compared to OMZ-20 mg/kg [One-way ANOVA followed by Student–Newman Keuls test].

4.3.3 Effect of EMR-2450 MHz on gastric ulcer in rats on D-28 and treated rats on D-42

Figure 4.3

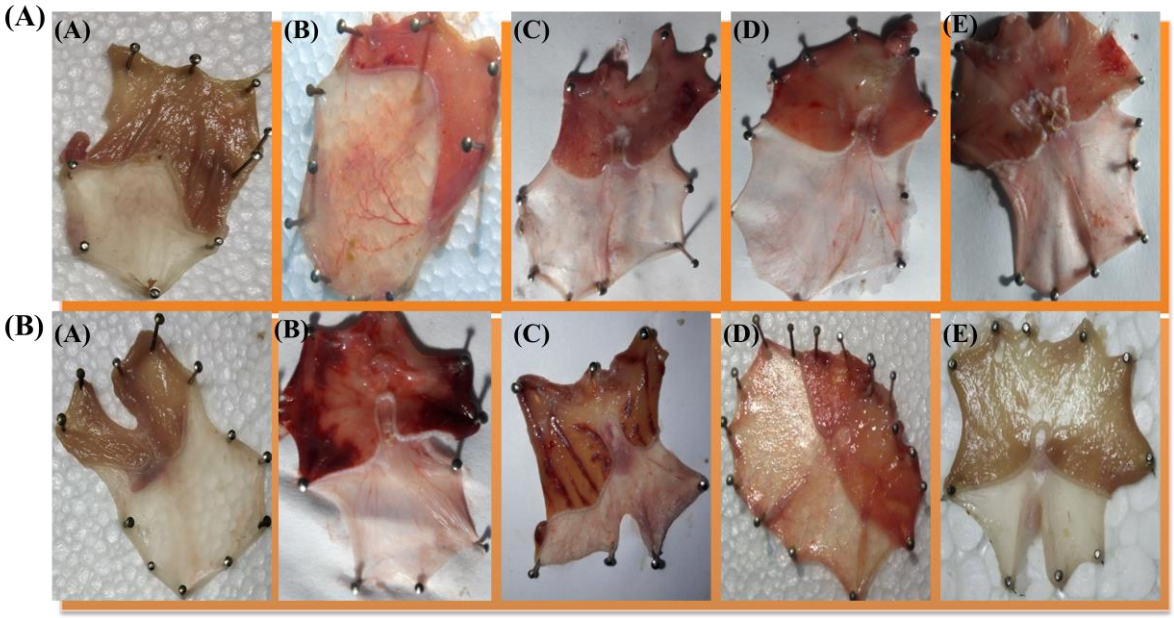

Figure: 4.3 (A) Shows effect of repeated exposure of EMR-2450 MHz on ulcer index in rat stomach (A) Control (B) EMR-2450MHz (E) (C) EMR-2450 MHz (E1) (D) EMR-2450MHz (E2) (E) EMR-2450MHz (E3) **(B)** shows changes in ulcer index after long term exposure of EMR in rat stomach. (A) Control (B) EMR-2450MHz (E) (C) E1+OMZ-10 mg (D) E2+OMZ-20 mg (E) E3+OMZ-30 mg

4.3.4 Repeated exposure to EMR-2450 MHz decreased Mucus content in rats

Mucus secretion is play an important role in the protective function of gastric mucosa in repeated exposure of EMR in rats depicted in Figure 4.4. Repeated exposure of EMR-2450 MHz caused significant modulations in the gastric mucosa compared to control rats. There was a significant difference among groups [$F(4, 15) = 95.25$; $P<0.05$]. Post hoc analysis observed that OMZ-30mg/kg significantly increased the mucus content which is comparable with control rats.

Figure 4.4

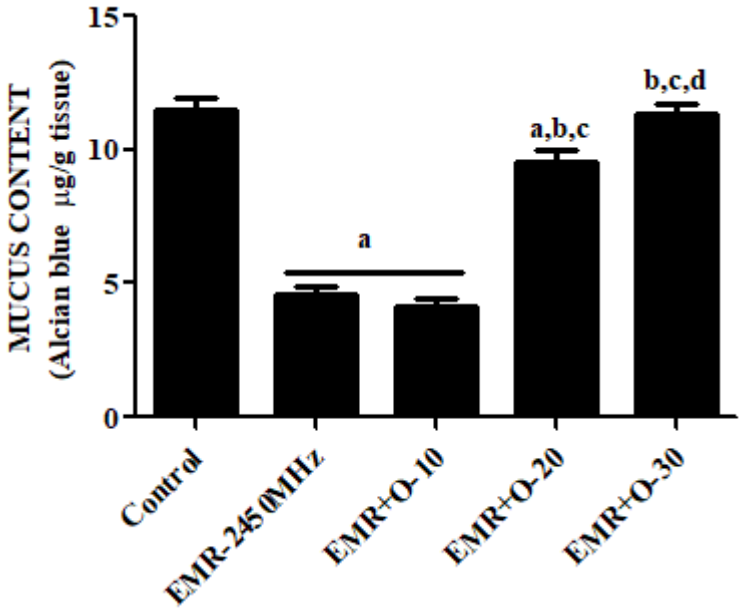

Figure 4.4 the effect of EMR-2450 MHz on mucus content in OMZ treated gastric ulcer in experimental rodents. All values are expressed as mean±SEM, (n=3). [a]p<0.05 compared to control, [b]p<0.05 compared to EMR-2450MHz, [c]p<0.05 compared to OMZ-10mg/kg and [d]p<0.05 compared to OMZ-20mg/kg [One-way ANOVA followed by Student–Newman Keuls test].

4.3.5 Repeated exposure to EMR-2450 MHz increased H^+/K^+ ATPase activity in experimental rats

Repeated exposure of EMR-2450 MHz exhibited significant increase in H^+/K^+ ATPase activity in rodents. There were significant differences in H^+/K^+ ATPase activity among groups [$F(4, 15) = 98.15$; $P<0.05$]. Moreover, highest dose of OMZ showed significant decrease in H^+/K^+ ATPase activity (In figure 4.5).

Figure 4.5

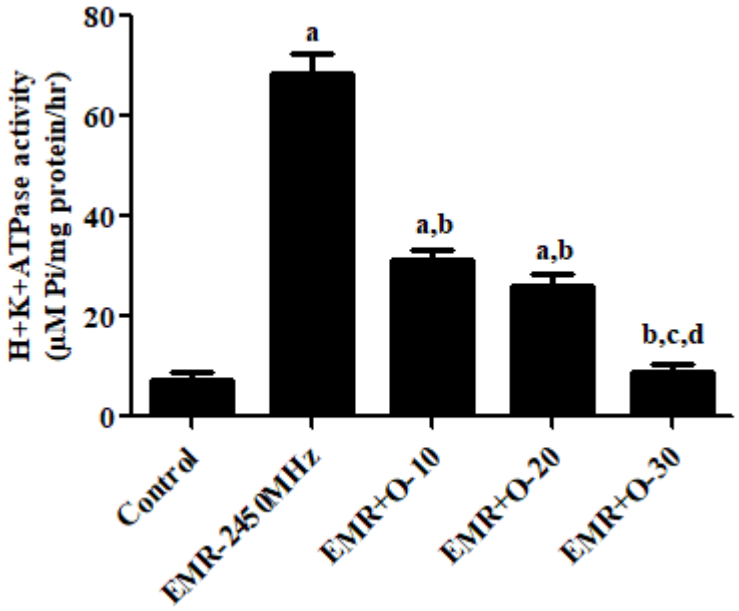

Figure 4.5 All values are expressed as mean±SEM, (n=3). [a]p<0.05 compared to control, [b]p<0.05 compared to EMR-2450MHz, [c]p<0.05 compared to OMZ-10mg/kg and [d]p<0.05 compared to OMZ-20mg/kg [One-way ANOVA followed by Student–Newman Keuls test].

4.3.6 EMR-2450 MHz modulated stress markers in animals

Table: 4.3 illustrate that EMR-2450 MHz exposure group induced the level of MDA and reduced the level of GPx, SOD and catalase in gastric tissue of rats. One way ANOVA analysis shows significant alterations in LPO, SOD, catalase and GPx among groups [F (4, 15) = 189.8; P<0.05], [F (4, 15) = 61.0; P<0.05], [F (4, 15) = 32.03; P<0.05] and [F (4, 15) = 91.89; P<0.05] respectively. Though, OMZ-30 at highest dose showed significant beneficial effects in terms of reactive oxygen species compared with median and lowest dose of OMZ.

Table 4.3 Effect of EMR-2450 MHz on oxidative stress markers of gastric tissue in rats.

S.No.	Groups	LPO (nM MDA/mg protein)	SOD (Units/min/mg protein)	Catalase (µM H_2O_2/min/mg protein)	Glutathione (nM NADPH/min/mg protein)
1	Control	**3.85±0.16**	**12.48±0.55**	**112.32±4.14**	**0.062±0.002**
2	EMR (E)	**9.26±0.25[a]**	**3.16±0.29[a]**	**47.10±3.35[a]**	**0.024±0.002[a]**
3	E+ O-10	**6.19±0.0.13[a,b]**	**6.67±0.35[a,b]**	**73.51±3.64[a,b]**	**0.043±0.002[a,b]**
4	E+ O-20	4.89±0.12[a ,b,c]	9.46±0.68[a,b,c]	90.25±4.62[a,b,c]	0.051±0.001[a,b,c]
5	E+ O-30	3.76±0.13[b,c,d]	12.83±0.62[b,c,d]	110.76±7.33[b,c,d]	0.063±0.001[b,c,d]

Table 4.3 All values are expressed as mean±SEM, (n=3). [a]p<0.05 compared to control, [b]p<0.05 compared to EMR-2450MHz, [c]p<0.05 compared to OMZ-10mg/kg and [d]p<0.05 compared to OMZ-20mg/kg respectively [One-way ANOVA followed by Student–Newman Keuls test].

4.3.7 Repeated exposure to EMR-2450 MHz decreased VEGF level in animals

Fig: -4.6 show the effect of EMR on VEGF. Statistical analysis by one-way ANOVA showed that there were significant differences among groups of VEGF [F (4, 15) = 20.20; P<0.05. Post hoc analysis showed that at EMR decreased VEGF in gastric tissues. Though, OMZ-30mg/kg treated animal showed significantly reversed the inhibition of angiogenesis by EMR-2450 MHz.

Figure 4.6

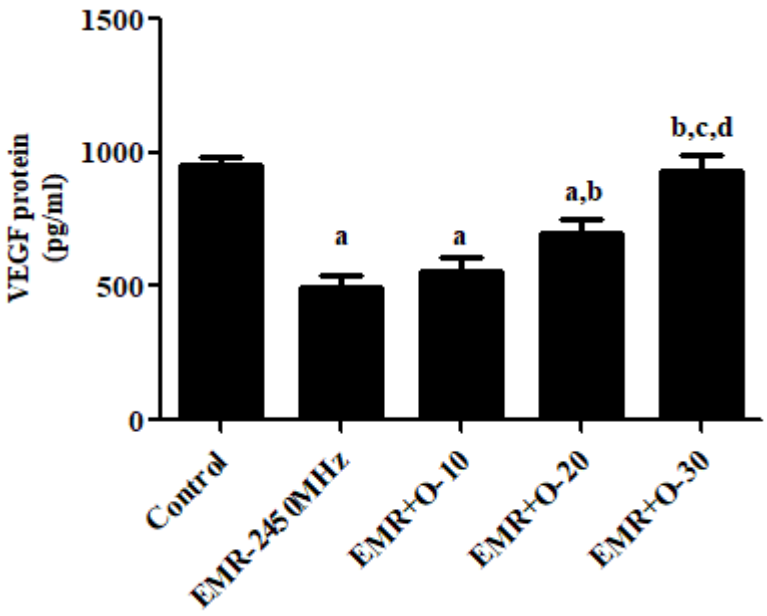

Figure 4.6 All values are expressed as mean±SEM, (n=3). [a]p<0.05 compared to control, [b]p<0.05 compared to EMR-2450MHz, [c]p<0.05 compared to OMZ-10mg/kg and [d]p<0.05 compared to OMZ-20mg/kg [One-way ANOVA followed by Student–Newman Keuls test].

4.3.8 Repeated exposure to EMR-2450 MHz modulated TNF-α, IL-6 and IL-10 in experimental rats

There was significant difference between among groups of TNF-α [F (4, 15) = 81.62; P<0.05], IL-6 [F (4, 15) = 73.20; P<0.05] and IL-10 [F (4, 15) = 121; P<0.05] respectively. Post hoc analysis showed that repeated exposure of EMR-2450 MHz caused significant increase in the levels of pro-inflammatory cytokines TNF-α and IL-6 while it exhibited decrease in anti-inflammatory cytokines IL-10 in rats. OMZ-30 treated EMR exposed rats caused significant diminish in TNF-α and IL-6 and enhance

in IL-10 level compared to EMR exposed rodents as illustrated in fig: 4.7 (A, B and C) respectively.

Figure 4.7

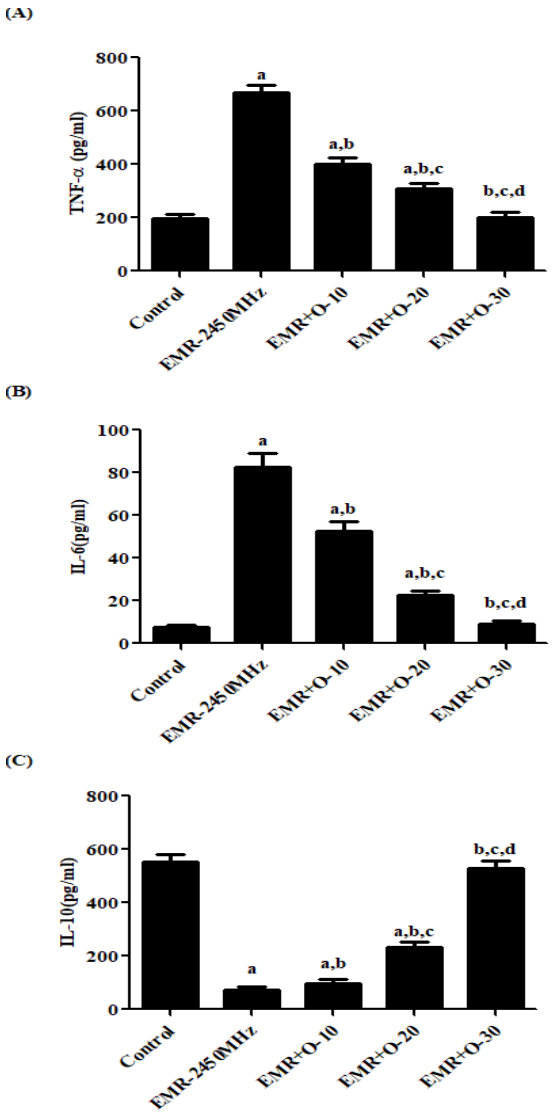

Figure: 4.7 (A,B,C) All values are expressed as mean±SEM, (n=3). [a]p<0.05 compared to control, [b]p<0.05 compared to EMR-2450MHz, [c]p<0.05 compared to OMZ-10mg/kg and [d]p<0.05 compared to OMZ-20mg/kg [One-way ANOVA followed by Student–Newman Keuls test].

4.3.9 Repeated exposure to EMR-2450 MHz changed histopathology in rats

Fig: -4.8 shows that repeated exposure of EMR showed complete thrashing of outer layer of gastric mucosa leads to necrosis and hemorrhage in experimental rats. However, OMZ-30 attenuated the EMR-induced in gastric tissues in terms of necrosis and hemorrhage (n=4).

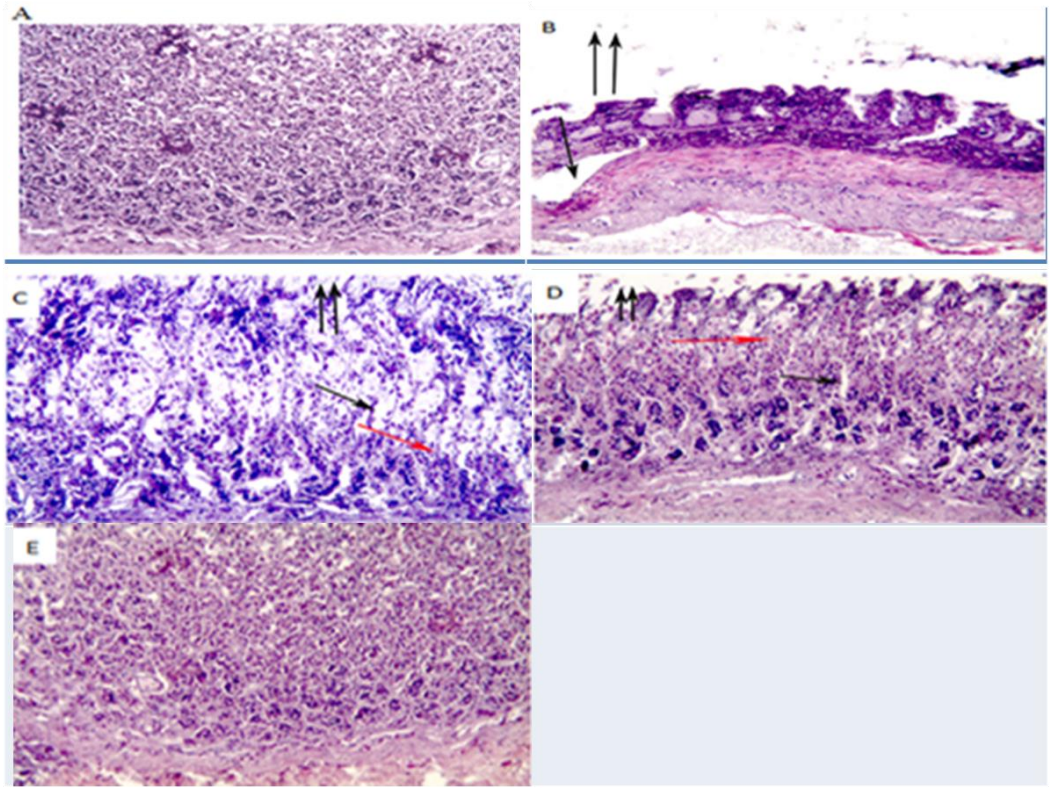

Figure 4.8 Shows changes in histopathology of Gastric tissues. (a) Control (b) EMR-2450MHz (c) E+OMZ-10mg/kg (d) E+OMZ-20mg/kg (e) E+OMZ-30mg/kg (n=3). Greater curvature of the glandular stomach and necrosis of mucous membrane, partial destruction of muscularis mucosa, congestion, bleeding and edema in submucosa of stomach in EMR exposed rats. There is significant relation between control, OMZ-10 mg/kg and OMZ-20 mg/kg groups. Treatment with OMZ-30mg/kg has shown completely maintain the gastric cytostructure of EMR subjected rats. \

4.4 Discussion

One of the salient features of this study is that the rats developed gastric ulcers upon repeated exposure to EMR-2450 MHz. On day-28, EMR-2450 MHz exposure significantly reduced gastric blood flow and pH with increased ulcer index. Long term exposure of EMR-2450 MHz produces gastric ulcer in rats due to imbalance between gastric offensive (acid secretion) and defensive factors (mucus secretion and gastric blood flow). These gastric ulcers were healed with supra-therapeutic doses of OMZ. However, normal therapeutic dose of OMZ did not show significant improvement against EMR-2450 MHz induced gastric ulcer in rats.

Rats developed gastric ulcer upon exposure to EMR-2450 MHz. The genesis of gastric ulcer may be due to the misbalance of offensive and defensive factors (Lakshmi et al., 2010). Among the defensive aspects considered important for healing of gastric ulcer is the blood flow in the gastric mucosa. The function of blood circulation in the gastric mucosal surface is to protect the gastric mucosa by removing the waste materials and maintaining the neutral pH condition of abdomen (Kawano & Tsuji, 2000). Thus, gastric mucosal circulation has been a subject of great interest because of its main function to heal the gastric ulcer (Matuszyk et al., 2016). Sub-chronic exposure to EMR-2450 MHz significantly reduced the gastric blood flow. The changes in the pattern of blood component to each other leads to sticking together and forms a stacking of coins called as rouleau formation (Havas, 2013). VEGF is a fundamental regulator of vascular permeability system which maintain the oxygen supply to tissues when blood supply is insufficient (Ashina et al., 2015). VEGF is mainly considered as a angiogenic factor which plays an important role in the pathogenesis of gastric ulcer (Kotan et al., 2012). Sub-chronic exposure to 2450 MHz

radiation decreased gastric blood flow. This decrease in gastric blood flow may be due to decrease in the level of VEGF in EMR exposed rats.

Gastric mucus is the most important protecting layer to inhibit the gastric ulcer formation (Yandrapu and Sarosiek, 2015). In this study, long term exposure to EMR-2450 MHz decreased in mucus concentration in stomach indicating it fails to maintain the protecting layer for healing. Preliminary function of abdomen altered in terms of decreased gastric pH, increase volume of gastric content, free and total acidity in the abdomen causes breakdown of protective mucosal layer (Govindarajan et al., 2006). This decrease in gastric pH might be due to the increase in hydrogen ions indicating gastric hyperacidity due to accumulation of gastric juice by H^+K^+-ATPase enzyme of the parietal cells (Shin et al., 2006). This increase in hydrogen ions is an important factor for the production of gastric acid in stomach. H^+K^+-ATPase is a membrane bound enzyme which catalyzes H^+ transport channel at the energy release by ATP hydrolysis (Yamamoto et al., 2019). This H^+ ion reacts with Cl^- ion and forms HCl resulting in hyperacidity in the stomach. Increase in HCl secretion results in decreased protection of mucosal barrier due to autodigetion of mucosa leads to increase in gastric content in stomach (Shin et al., 2006).

Long term exposure to EMR-2450 MHz caused gastric lesions which are multifactorial, which initiates with the lessening of mucus layer of gastric wall. These lessening of mucus layer are often involved due to excessive generation of oxidative stress causing damage to the cell and cellular membrane. Previous report suggested that oxidative stress is a main agent for gastric ulcer in experimental rats (Zhu and Kaunitz, 2008). In this study, long term exposure to EMR-2450 MHz increased oxidative stress leads to gastric tissue injury. Furthermore, oxidative stress plays an essential role in the activation of cytokine mediators and cause release of pro inflammatory markers such as

TNF-α and IL-6 lead to gastric tissue injury (Amirshahrokhi & Khalili, 2016). In the present study long term exposure to EMR-2450 MHz increased the levels of TNF-α and IL-6 and decreased the level of anti-inflammatory IL-10 in gastric tissues. Therefore, long term exposure to EMR-2450 caused gastric ulcer due to activation of inflammatory mediators and inhibition of anti-inflammatory cytokines.

In the recent years, PPIs are commonly used in the treatment for gastric (Patel et al., 2014). In this study, OMZ-30 mg/kg significantly inhibits the H^+/K^{+-}ATPase activity indicating decrease in H^+ secretion in the stomach. Preclinical studies suggested that OMZ-20 mg/kg improved the gastric lesions in experimental animal models (Paliwal et al., 2018b). However, OMZ-10 and 20 mg/kg was not effective in EMR exposed rats. Therefore, rats exposed to EMR-2450 MHz required certain high dose of OMZ for healing of gastric lesions.

Mucosal blood circulation is an important factor in the pathogenesis and healing of gastric ulcer (Matuszyk et al., 2016). In this study, long term exposure to EMR-2450 MHz has shown decrease in gastric blood flow. OMZ-30 mg/kg significantly recovered gastric blood flow leading to rapid healing of gastric ulcer. Therefore, continuous exposure to cell phone radiation leads to acid peptic disorders which are resistant to low dosage of OMZ. Earlier study suggested that VEGF is an angiogenic factor that restores the oxygen supply to tissues when blood circulation is inadequate (Ashina et al., 2015). OMZ-30 mg/kg increase in blood flow may be due to increase in the level of VEGF as it participates in vascular hyper-permeability and increases blood flow in gastric tissues (Ashina et al., 2015). Increase in gastric blood flow helps to maintain the mucus secretion and sustain the mucosal barrier (Kumar et al., 2016b). OMZ-30 mg/kg

increased mucus secretion in gastric ulcer, which might be accountable for generation of protection layer for gastric healing.

Increase in the gastric juice, free and total protons in stomach leads to lessening of the protective mucosal barrier due to auto digestion of the mucosa (Govindarajan et al., 2006). In this study, we observed that OMZ-30 mg/kg restored mucus membrane in gastric ulcer, indicating generation of protective layer for gastric healing. Further, treatment with OMZ-30 mg/kg healed the gastric ulcers through restored pH, gastric volume, total acidity and free acidity.

OMZ-30mg/kg reinstates the cellular components due to increased antioxidant levels similar to increased MDA level, significantly increased glutathione level to insulate the tissues from peroxide attack. SOD and catalase play a major defensive role against the reactive oxygen species (Odabasoglu et al., 2006). Treatment with OMZ-30 mg/kg in EMR exposed rats significantly increased SOD and catalase levels in gastric tissues. We further examined the role of pro and anti-inflammatory cytokines in gastric tissues. TNF-α, an earliest and primary endogenous pro-inflammatory cytokine, associated with systemic inflammation reaction and IL-6 is a pro-inflammatory cytokine responsible for immunity (Kunz et al., 2011, Natale et al., 2004). An increased level of TNF-α andIL-6 level was observed after EMR exposed rats. Treatment with OMZ-30 mg/kg decreased the levels of TNF-α and IL-6 in EMR exposed gastric tissues. IL-10 is an anti-inflammatory cytokine which can limit tissue damage caused by inflammation (Gogos et al., 2000). In this study, EMR-2450 MHz decreased IL-10 level in gastric tissues of experimental rats. However, Treatment with OMZ-30mg/kg increased anti-inflammatory cytokine (IL-10) in gastric tissues of rats. When observed histopathologically, long term exposure to EMR-2450 MHz causes gastric ulcers in the greater curvature of the glandular stomach

and necrosis of mucus membrane, partial destruction of muscularis mucosa, congestion, bleeding and edema in submucosa of stomach. Treatment with OMZ-30 mg/kg maintained the gastro architecture and protected the glandular part of the stomach in EMR exposed rats.

4.4.1 Summary

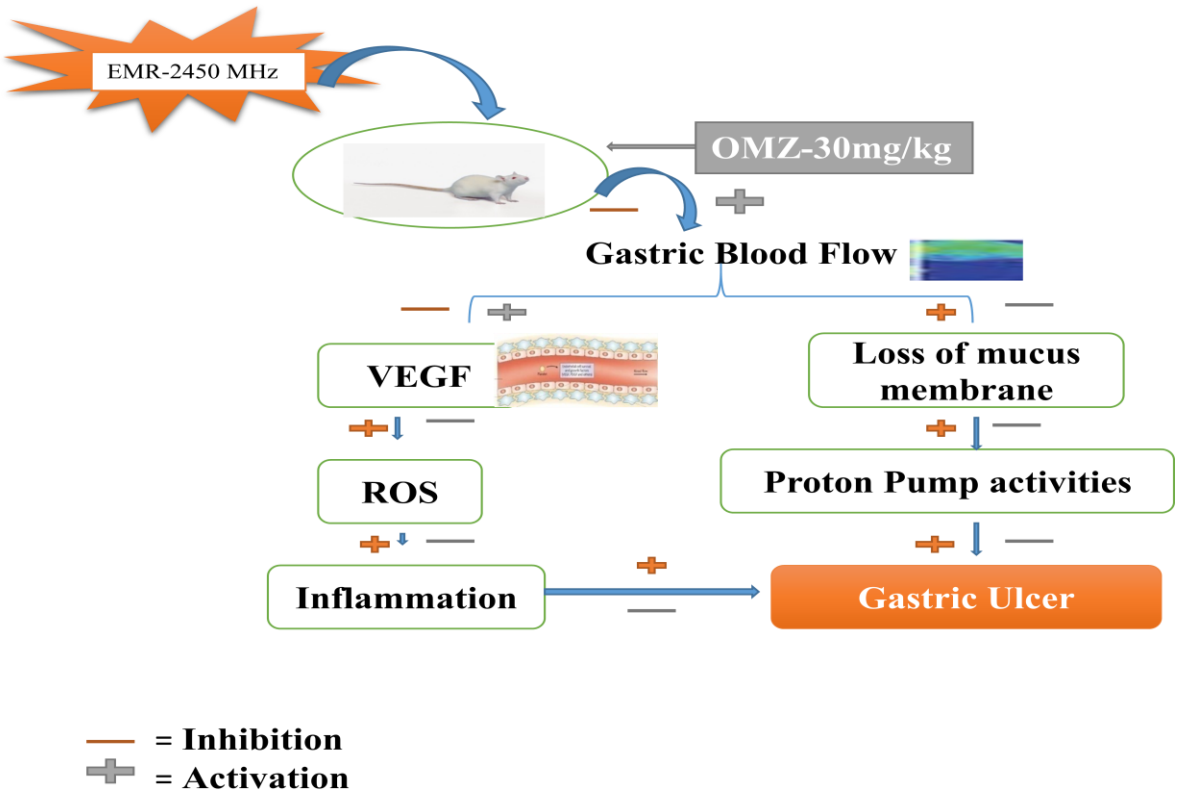

Figure 4.9 Summary of hypothesis

Figure 4.9 shows that EMR at 2450 MHz caused gastric ulcer and associated pathophysiological changes in rats. Long term exposure to EMR-2450 MHz decreased gastric blood flow and mucus secretion suggesting a direct effect on gastric mucosal membrane and increases the gastric ulcer index. EMR 2450 MHz altered preliminary function of stomach in terms of gastric pH, gastric secretion, proton pump activities and oxidative stress in gastric tissues indicating activation of ulcerogenic factors. Moreover,

EMR altered cytokines levels like TNF-α, IL-6 and IL-10 indicating that there are changes in the balance of pro- and anti- inflammatory markers in gastric tissues. Treatment with OMZ-30mg/kg restored the function of stomach in terms of gastric pH, gastric secretion, mucus secretion, proton pump activities, gastric ulcer index, oxidative stress enzymatic activities, and cytokines level and completely maintain the gastric cytoarchitecture observed from histology study in experimental rats. Therefore, higher dose of OMZ may show therapeutic effects against gastric ulcer due to chronic exposure of EMR-2450 MHz in experimental animals.