3.1 Introduction

Electromagnetic radiations emitted from a cell phone, base station and Wi-Fi with high-frequency radiation can have deleterious effects on health (Gupta et al., 2018b, Moradi et al., 2016b, Jing et al., 2012, Pall, 2016a). The cell phones and their base station continuously emanate electromagnetic radiations having sufficient electric and magnetic field strength to change the orientation of atoms in molecules (Moradi et al., 2016a). These changes in orientation of atom lead to alterations in biological activity related to health (Challis, 2005a). Repeated exposure to EMR increases biological interactions between EMR, brain and liver (Kumar et al., 2010). This lead to alteration in the neuronal circuits of the brain resulting in depressive-like symptoms and enzymatic changes in liver in animals (Rad et al., 2015b, Challis, 2005a). EMR-900 (1h/3months) increased the levels of aspartate aminotransferase (AST) and alanine transaminase (ALT) in liver of experimental rats (El-Bediwi et al., 2011a). Exposure to EMR (1140-1290 nm) influences the enzyme kinetics, due to induction of liver enzymes leading to changes in the metabolism of drugs (Vojisavljevic et al., 2007b). The liver is an essential organ for metabolism and is the main organ for detoxification. Most important criteria for pathological changes of the liver cells are measured in terms of changes in the levels of AST and ALT (Parise et al., 2006). Clinical studies have reported that an increase in the levels of serum AST and ALT in liver tissue are associated with psychological distress and depression in patients (Helmchen et al., 1996). These findings reveal that both monoamines and enzymatic factors are compromised in depression which can be altered by exposure to EMR.

Depression is a common, neuropsychiatry disorder that affects approximately 20% of the worldwide population (Lépine and Briley, 2011). Alteration in

monoaminergic system is the main factor for the pathophysiology of depression. The pathophysiology of depression includes the alteration in the levels of serotonin neurotransmitter in the prefrontal cortex (Monteggia et al., 2007, Brenes and Fornaguera, 2008, Clark-Raymond and Halaris, 2013). Further, the modulation of the monoaminergic system may alter the anhedonic effect. The preclinical study suggested that chronic exposure leads to disturbances in monoamine neurotransmitters (Sinha, 2008a). (Sinha, 2008). Fluoxetine is one of the first-line treatment for the management of depression (Kupfer, 2005) because of their superior safety and tolerability profiles compared to other antidepressants (Rossi, Barraco, & Donda, 2004). FLX is more safer than Tricarboxylic acids (TCA) and Monoamine inhibitors (MOAIs) and currently most prescribed antidepressant. FLX offers several advantages over other antidepressant drug such as no anticholinergic activity and no propensity to cause seizure or arrhythmia (Kupfer, 2005).CYP_{450}2D6 (CYP2D6) is a drug metabolizing enzyme which is involved in the metabolism of FLX (Fjordside et al., 1999).

CYP2D6 is the first enzyme in the brain that causes regeneration of serotonin and conversion of tyramine into dopamine in the cortical region of the brain. It also helps in the metabolism of CNS acting drugs in the liver (Penas-Lledo et al., 2013), (Nebert et al., 2013). Dysregulation of the serotonin and dopamine in the brain is associated with the development of depressive-like symptoms in experimental rats (Grace, 2016). We have earlier shown that EMR induces depressive-like symptoms (chapter 2.2). EMR can also alter liver function (Vojisavljevic et al., 2007b). Therefore, there is a possibility of EMR to interfere with the metabolism of fluoxetine in the liver. Thus, in this study, sub-chronic treatment with FLX used for the antidepressant activity in EMR exposed rats. However, there is a lack of information on the possible

pathophysiological mechanisms involved in the induction of depressive-like symptoms by non-ionizing EMR. Particularly, the effects of the higher frequency of EMR-2450 MHz have not been evaluated in detail. Thus, based on the above studies, we presume that exposure to higher frequency EMR-2450 MHz may cause depressive-like symptoms in experimental rats. Thus, various doses of FLX were used to treat the depressive-like symptoms in experimental rats. But high frequency of EMR exposure also altered the CYP2D6 enzyme in liver leading to liver toxicity.

Therefore, in the present study, the neuroprotective activity of FLX was investigated against monoaminergic and dopaminergic dysfunction in PFC of EMR-2450 MHz exposed experimental rats. Further, CYP2D6 was assessed as a measure of FLX metabolism in the liver as well as in the PFC of EMR exposed rats. Moreover, the liver function caused due to FLX was evaluated by measuring the changes in level of AST and ALT in the presence of CYP2D6 enzymes in the above brain regions in EMR exposed rats (depicted in figure 3.1).

3.1.1 Hypothesis

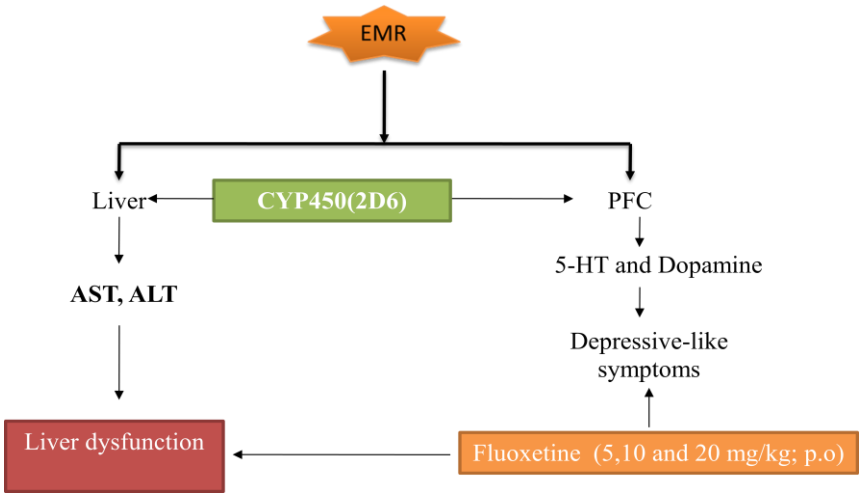


Figure 3.1 Proposed hypothesis of effect of EMR on pharmacokinetic of FLX and liver toxicity in rats.

3.2 Materials and methods

3.2.1 Animals

Inbred Charles-foster albino male rats weighing about (220±20 g) and eight weeks old were purchased from the central experimental animal facility centre, Institute of Medical Sciences, Banaras Hindu University (IMS-BHU). The animals were housed in a home cage made up of polypropylene at 25 ± 2 °C temperature and RH 44–56%, light and dark cycle of 12:12 h respectively. The entire animal acclimatized for one week before experiments. The food pellets were provided (paramount pvt.ltd.) and water was allowed *ad libitum*. All the experiments were conducted based on given guidelines (CPCSEA-2010; IMS-BHU; Approval no.: Dean/2015/CAEC/1414).

3.2.2 Chemicals

Fluoxetine and norfluoxetine were gift sample from Cadila pharmaceutical limited (Ahmedabad; India). CYP2D6 Elisa kit (Cat No. E3082Hu; Bioassay Technology Laboratory; China), SGOT kit (cat no. 120204; ERBA India), SGPT kit (cat no. 120207; ERBA; India) were purchased. All other chemicals and analytical grade reagents of high-performance liquid chromatography were procured from local supplier.

3.2.3 EMR exposure apparatus and Design (Refer Chapter 2.1 Page no. 20)

3.2.4 Measurement of Power density and specific absorption rate of abdomen and brain region

The power density was calculated by the formulae described in the earlier study (Gupta et al., 2018). The average power density was 0.1227 W/m^2. The whole body SAR values were found between the 0.070 W/kg. The value of SAR in the head region was found to be 0.131W/kg (2450 MHz) with a value of power density 0.1227 W/m^2. The SAR was calculated using the following formulae [SAR= 5.94*average length of

animals*power density/electromagnetic range in GHz*average wt. of an animal; whereas, Avg. length of animal=17 cm and Avg. wt. of animal=200g and average length head of animal = 3 cm] (Gandhi et al., 1977a).

3.2.5 Experimental design

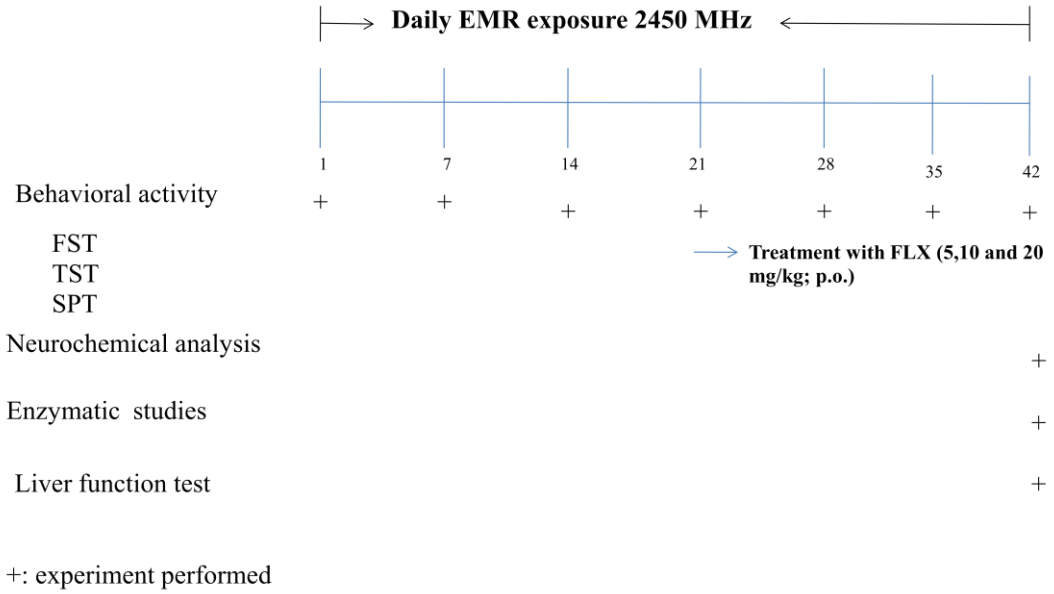


Figure 3.2 Schematic representation of the experimental design. '+' denotes experiment performed.

The experimental protocol is a treatment schedule designed to evaluate the effect of EMR on both depressive-like symptoms and the pharmacological effect of FLX. The rats were randomly divided into six groups with eight animals each through G* power analysis software. The groups were control, *perse* (control+FLX-20 mg/kg), EMR-2450, EMR-2450+FLX-5mg/kg, EMR-2450+FLX-10mg/kg and EMR-2450+FLX-20mg/kg. The groups EMR-2450 (E), E_1, E_2 and E_3 were exposed to electromagnetic radiations between 10 am to 2 pm for 1h up to D-21. After 15 min of EMR exposure on D-1 to D-21 at 7 days interval, the behavioral paradigms, i.e. Forced Swim Test (FST),

Tail Suspension Test (TST) and Sucrose Preference Test (SPT) were performed (fig 3.2). All the behavioral observations were recorded and evaluated using ANY-maze (version-3.72, USA) video tracking system. Behavioral experiments were performed at an interval of 2 h between SPT and FST, and 20 min between FST and TST. Food restriction was done at D-0, 6, 13 and 20 for 24h, and no experiment was performed during SPT. All the behavioral parameters were performed during the light period. After 24 h, we have measured percentage of sucrose intake on D-1, 7, 14 and 21. On D-21, rats have showed depressive-like behavior and thereafter treatment (n=8) was started with FLX (5mg, 10mg and 20mg; administered it orally (p.o.) from day 21 to day 42 (21-day treatment schedule). On D-42, the effective doses of FLX to treat EMR induced depressive-like behavior in rats followed by behavioral parameters were assessed. On D-42, animals were killed by decapitation and blood was collected to estimate the kinetic parameters of FLX in EMR exposed rats along with AST, ALT estimation in serum (n=8). Prefrontal cortex (PFC) was immediately microdissected and liver stored at -80^{0}C for further analysis. Out of eight from each group, cortical tissue (n=4) from four animals were used for VEGF, CYP2D6 levels by Elisa kit and other four were used for serotonin, dopamine analysis by HPLC in cortical brain tissues (n=4).

3.2.6 Evaluation of behavioral performance

3.2.6.1 Forced swimming test (FST) (Refer to chapter 2.2-page no. 60)

3.2.6.2 Tail suspension test (TST) (Refer to chapter 2.2-page no. 61)

3.2.6.3 Sucrose preference test (SPT) (Refer to chapter 2.2-page no.61)

3.2.6.4 Assessment of Serotonin and Dopamine in PFC (Refer to chapter 2.2-page no.62)

3.2.6.5 Estimation of level of CYP2D6 in prefrontal cortex and liver

CYP2D6 levels in prefrontal cortex of brain tissues (n=4/group) were measured using a commercially available kit. The cortical and liver tissues were (150 mg/ml) homogenized in phosphate buffer and centrifuged for 10 min at 10,000g at 4°C. The brain and liver extracts were then divided into 100 µl triplicate samples for the CYP2D6 determination (Singh et al., 2009).

3.2.6.6 Estimation of level of AST and ALT in blood serum

The serum samples were examined for liver (SGOT and SGPT) marker enzymes using commercial colorimetric assay kits, following the manufacturer's protocol.

3.2.6.7 Pharmacokinetics Studies

Pharmacokinetic estimation was performed after completion of twenty-one days treatment schedule of FLX. We have orally administered the standard drug i.e., FLX, dissolved in distilled water. Rats were dosed at 5, 10 and 20 mg/kg orally through oral gavage. The dose was selected based on an earlier study on the effect of FLX on stress linked neuropsychiatric disorders (Paliwal et al., 2018). On D-42 blood samples (0.25 ml) were collected at 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h after oral administration through retro-orbital plexus. Blood samples were transferred to pre-labelled heparin-coated sampling tubes at the respective time points. An equal volume of saline was replaced after each blood withdrawal. After the withdrawal of the blood sample, the blood plasma was stored at -80ºC until analysis (Paxinos & Franklin, 2019).

3.2.6.8 HPLC Analysis

HPLC analysis was performed based on the isocratic method consisting of a Kontron Dionex LC pump (LPG3400A), Kontron Dionex ultraviolet detector (UVD 340U). Experiment was performed using symmetry C18 (4.6 9 250 mm) column and maintained the temperature at 25°C. Mobile phase consisted of an ACN: water (20:90

v/v) (Rossi et al., 2004) and the flow rate was 1 ml/min. The volume of sample (20 µl) solution was prepared for each injection and absorbance being detected at 205, 226 nm (Sawyer & Howell, 2011). Retention time was 3.6 min for NFL 4.5 min for FL and 7.2 min for internal standard. Standard curves were prepared daily, spiking blank plasma and treated plasma with known concentrations of FL and NFL. The calibration graphs were all linear with acceptable correlation coefficients ($r > 0.98$).

3.2.6.9 Statistical analysis

Experimental data were expressed as mean ± standard error of mean (SEM). All the behavioral data were analyzed using the repeated measures of Two-way ANOVA followed by Bonferroni post hoc test. Other datasets were analyzed using one-way analysis of variance (ANOVA) followed by Newman-keuls post hoc test. $P<0.05$ was considered as statistically significant for all experimental data analysis. 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.

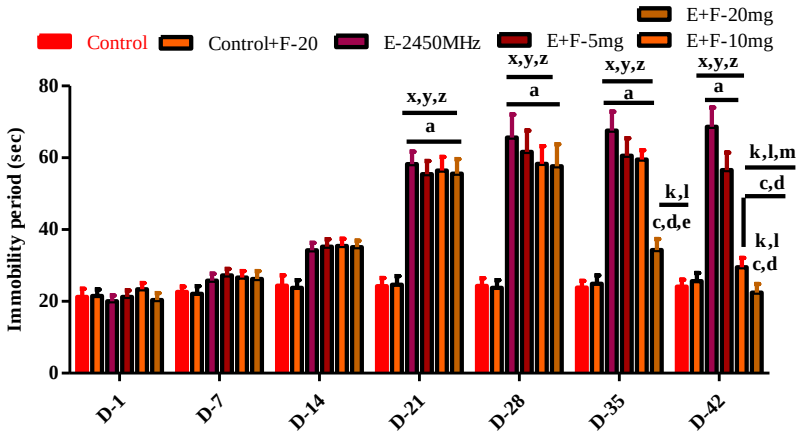
3.3 Results

3.3.1 Fluoxetine (5, 10 and 20 mg/kg) decrease immobility period during FST and TST paradigm in EMR-2450 MHz exposed rats

Fig: -3.3 shows that FLX (5, 10 and 20 mg/kg) exhibited antidepressant activity in EMR-2450 MHz exposed rats during FST and TST paradigm. Two-way ANOVA analysis depicted that there were significant differences in immobility period during FST and TST among the groups [$F (5, 294) = 77.36$; $P<0.05$] and [$F (5, 294) = 18.74$; $P<0.05$] respectively, time ([$F (6, 294) = 66.34$; $P<0.05$] and [$F (6, 294) = 17.77$; $P<0.05$] respectively, and there was significant interaction between group and time during FST [$F (30, 294) = 10.24$; $P < 0.05$] and TST [$F (30, 294) = 11.18$; $P < 0.05$]. Post hoc test demonstrated that FLX-20 mg/kg significantly reduced the immobility period but FLX-5 and 10 mg/kg did not show the significant effect from control groups

on D-35. However, the immobility period was reduced to normal level in FLX-10 and 20 mg/kg treated rats on D-42, which was insignificant to control rats.

Figure 3.3 (A)



(B)

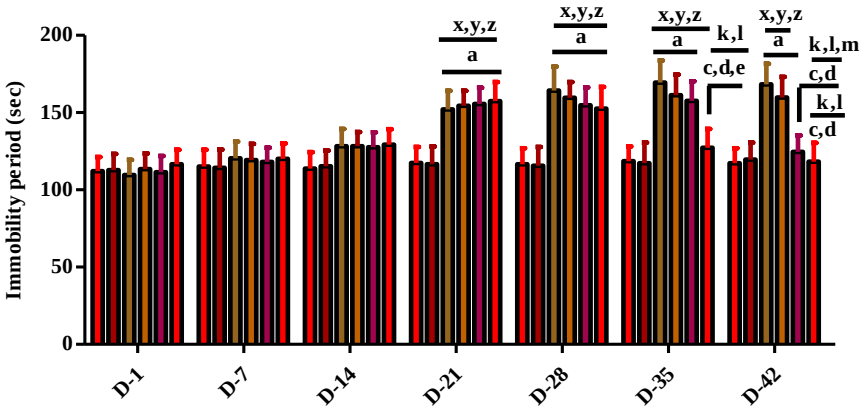


Fig 3.3 (A and B) Shows immobility period in forced swim test and (B) immobility period in tail suspension test in FLX treated rats. All values are expressed as mean±SEM. (n=8). [a]p<0.05 compared to control, [b]p<0.05 compared to EMR-2450,[c]p<0.05 compared to E+F-5mg and [d]p<0.05 compared to E+F-10mg ; [x]p<0.05 compared to D-1, [y]p<0.05 compared to D-7 and [z]p<0.05 compared to D-14 [k]p<0.05

compared to D-21, $^{l}p<0.05$ compared to D-28 and $^{m}p<0.05$ compared to D-35 [Two-way ANOVA followed by Bonferroni test].

3.3.2 Fluoxetine (5, 10 and 20mg/kg) increase sucrose consumption during sucrose preference test (SPT) in EMR exposed rats

The antidepressant activity of FLX (5, 10 and 20mg/kg) in EMR-2450 MHz exposed rats during SPT paradigmsis shown in Fig-3.4. Two-way ANOVA analysis demonstrated that there were significant differences in sucrose consumption during SPT among groups [$F(5, 294) = 18.43$; $P<0.05$], time [$F(6, 294) = 10.97$; $P<0.05$] and interaction between the group and time for sucrose intake during SPT [$F(30, 294) = 11.89$; $P < 0.05$]. In EMR-2450 MHz exposed group, we observed 7% and 18% decreased sucrose consumption on D-7 and D-14 than control, 47% decreased sucrose consumption on D-21 compared to control. However, treatment with FLX-20 mg/kg increased sucrose consumption in EMR exposed rats on D-35. FLX-5 and 10mg/kg administered group did not show any significant changes in sucrose consumption compared to EMR exposed rats on D-35. Furthermore, FLX-10 and 20 mg/kg administered group maintained the sucrose consumption on D-42. However, FLX-5 mg/kg administered group did not increase sucrose consumption upto D-42.

Figure 3.4

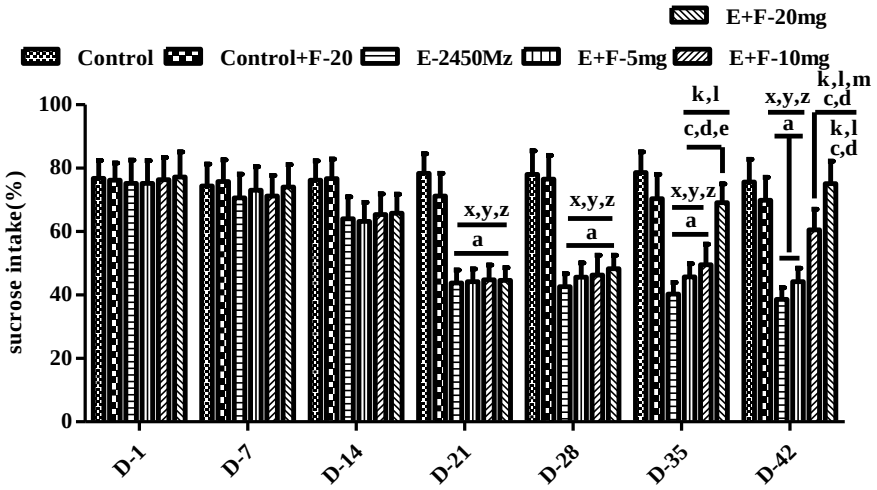


Figure 3.4 Shows percentage of sucrose intake in FLX treated rats. All values are expressed as mean±SEM. (n=8). [a]p<0.05 compared to control, [b]p<0.05 compared to EMR-2450,[c]p<0.05 compared to E+F-5mg and [d]p<0.05 compared to E+F-10mg ; [x]p<0.05 compared to D-1, [y]p<0.05 compared to D-7 and [z]p<0.05 compared to D-14 [k]p<0.05 compared to D-21, [l]p<0.05 compared to D-28 and [m]p<0.05 compared to D-35 [Two-way ANOVA followed by Bonferroni test].

3.3.3 EMR-2450 MHz alters level of serotonin and dopamine of PFC in Fluoxetine (5, 10 and 20 mg/kg) treated rats

The effect of EMR-2450 MHz on the level of 5-HT (A) and DA (B) in Fig: 3.5. One way ANOVA analysis demonstrated that there were significant difference in the levels of 5-HT [F (5, 23) = 31.65; P<0.05] and DA [F (5, 23) = 22.27; P<0.05] among the groups. Post hoc analysis depicted that FLX-10 mg/kg significantly increased levels of 5-HT and DA compared to all other treatment groups. However, FLX-5 mg/kg and 20 mg/kg did not increase 5-HT and DA on D-42.

Figure 3.5 (A) **(B)**

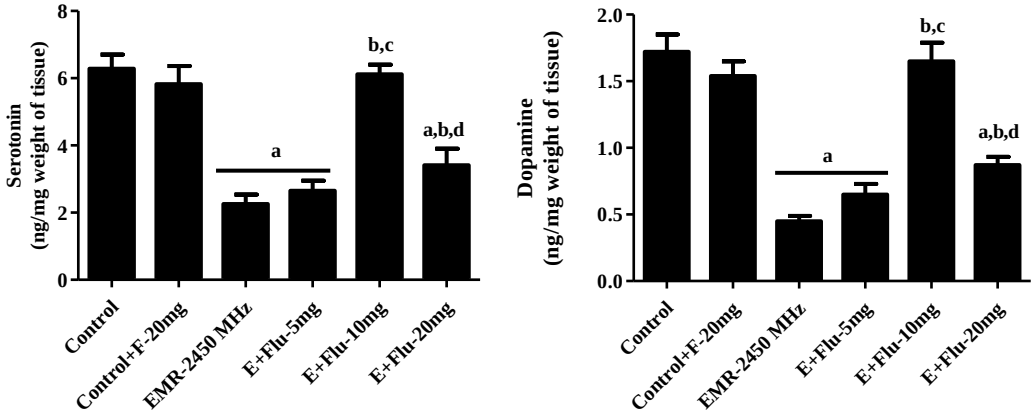


Figure 3.5 (A and B) Shows level of 5-HT and DA in prefrontal cortex. All values are expressed as mean±SEM. (n=4). [a]p<0.05 compared to control, [b]p<0.05 compared to control+F-20,[c]p<0.05 compared to EMR-2450 and [d]p<0.05 compared to E+F-5mg [One-way ANOVA followed by Student–Newman Keuls test].

3.3.4 Effect of EMR on CYP2D6 of liver and cortical region of brain in fluoxetine treated rats

CYP2D6 is primarily expressed in liver and highly expressed in the cortical region of brain. It plays an important role in the oxidative metabolism of FLX. The effect of EMR on the level of CYP2D6 in liver and PFC in FLX treated rats shown in fig:-3.6 (A and B). One way ANOVA analysis showed that there were significant difference in the levels of CYP2D6 in liver [F (5, 23) = 18.61; P<0.05] and PFC [F (5, 23) = 32.91; P<0.05] among the groups. Post hoc analysis showed that EMR and C+FLX-20 mg/kg significantly decreased level of CYP2D6 as compared to control group in rats. However, FLX-5 and 10 mg/kg treated groups show insignificant increase in CYP2D6

level in liver and brain. Further, FLX-20 mg/kg treated group shows significant decrease in CYP2D6 level in liver and brain of experimental rats on D-42.

Figure 3.6 (A) **(B)**

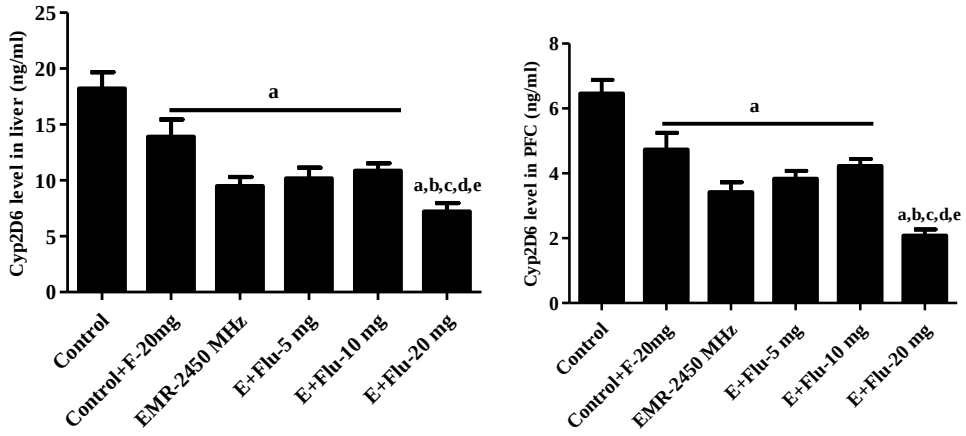


Figure 3.6 (A and B) Shows level of CYP450 2D6 in liver and PFC tissues in EMR exposed rats. All values are expressed as mean±SEM. (n=4). $^{a}p<0.05$ compared to control, $^{b}p<0.05$ compared to C+F-20, $^{c}p<0.05$ compared to EMR-2450,$^{d}p<0.05$ compared to E+F-5mg and $^{e}p<0.05$ compared to E+F-10mg [One-way ANOVA followed by Student–Newman Keuls test].

3.3.5 Effect of EMR on the pharmacokinetics of fluoxetine

Kinetic parameters of FLX and NFLX after oral doses of 5, 10 and 20 mg/kg FLX to rats are summarized in table:-3.1. FLX was absorbed relatively slowly as indicated by Tmax, which ranged from 2 to 3 h at the 5 and 10 mg/kg doses (mean 2.5 h) and more than 14 h at the highest dose (20 mg/kg) tested ($P< 0.05$ versus the 5 and 10 mg/kg doses). Further, maximum concentration C_{max} of FLX-10 mg/kg reached in blood was equivalent *to perse* group (C+F-20).

However, EMR+ F-5 mg/kg was found to be significantly decreased C_{max} compared to *perse* group indicating that it was unable to reach the drug concentration in blood plasma in EMR exposed rats. E+F-20 mg/kg significantly increased C_{max} compared to *perse* group indicating FLX concentration increased in blood plasma in EMR exposed rats. Furthermore, AUC values, normalized to the 10 mg/kg dose, were 7.01±0.54, 6.3±0.46, 7.4±0.65 and 13.50±0.8 for *perse* group, 5, 10 and 20 mg/kg, administration respectively. There were no changes in half-life of FLX ($t_{1/2}$), C+20, 5 and 10 mg/kg in EMR exposed rats. However, E+FLX-20 mg/kg significantly increased half-life $t_{1/2}$ to 17.4 h indicating decrease in elimination of drug in EMR exposed rats. Increase in Tmax of NFLX was not observed in *Perse* group, EMR+ F-5, 10 increased with the therapeutic dose of FLX (10 mg/kg). The AUC of NFLX was found to be significantly decreased in blood plasma compared to AUC of FLX in EMR exposed rats. The Cmax of NFLX shows transient saturation at the dose of 5 and 10 mg/kg. The Cmax value of NFLX was found to be significantly decreased due to long term exposure to EMR. Therefore, long term exposure to EMR-2450 MHz alters the pharmacokinetics of FLX-20 mg/kg.

Table 3.1

Parameters	Dose (mg/kg)			
	Control+20	**5**	**10**	**20**
Fluoxetine				
T_{max} (h)	5.25±0.45	2.40±0.31[a]	2.50±0.36[a]	14.42±2.52[a,b,c]
C_{max} (ng/ml)	0.58±0.026	0.40±0.02[a]	0.50±0.05	0.71±0.11[b,c]
AUC (ng/ml)	7.01±0.54	6.30±0.9	7.40±0.83	13.50±0.17[a,b,c]
$t_{1/2}$ (h)	7.40±0.88	6.50±1.4	7.20±1.41	17.42±0.84[a,b,c]
Norfluoxetine				
T_{max} (h)	8.25±0.45	4.50±0.87[a]	7.32±1.04[b]	6.83±4.93[b]
C_{max} (ng/ml)	0.22±0.026	0.20±0.05	0.21±0.03	0.13±0.12[a,b,c]
AUC NFL (ng/ml)	3.09±0.54	2.14±0.6	1.83±0.22[a]	1.22±0.05[a,b]
$t_{1/2}$ (h)	25.4±0.88	11.10±2.1[a]	13.60±5.88[a]	Nd

Table:-3.1 Kinetic parameters of Fluoxetine (FLX) and Norfluoxetine (NFLX) after oral doses of FLX hydrochloride to rats. All values are expressed as mean±SEM. (n=4). [a]$p<0.05$ compared to *Perse* group, [b]$p<0.05$ E+F-5mg and [c]$p<0.05$ compared to E+F-10mg [One-way ANOVA followed by Student–Newman Keuls test.

3.3.6 Effect of fluoxetine on ALT and AST in EMR exposed liver of experimental rats

The effect of FLX on ALT and AST levels in long term exposure of EMR in liver shown in Fig: 3.7 (A and B). One way ANOVA analysis demonstrated that there were significant difference in the levels of ALT [F (5, 23) = 51.11; P<0.05] and AST [F (5, 23) = 19.08; P<0.05] among the groups. Post hoc analysis depicted that FLX-5 and 10 mg/kg showed insignificant increase ALT and AST levels as compared to control group. However, control+FLX-20 and EMR+FLX 20 mg/kg has shown significant increase in ALT and AST levels compared to all other groups. Hence, control+FLX-20 and EMR+FLX-20 mg/kg increased ALT and AST levels in liver of experimental rats.

Figure 3.7

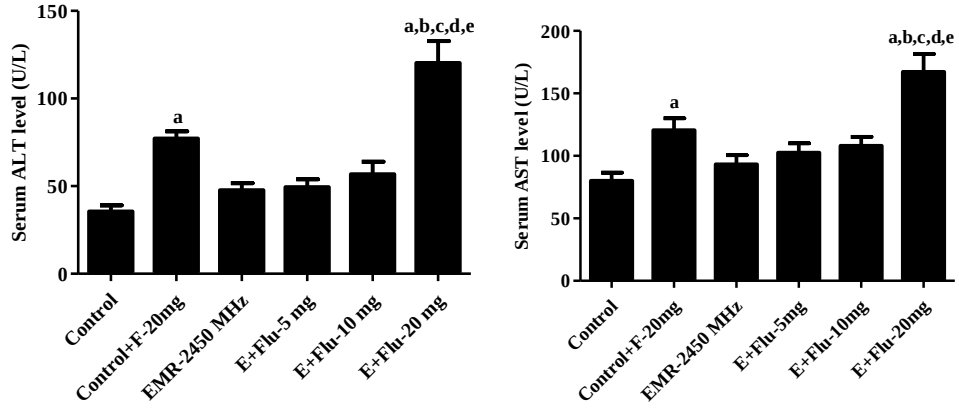


Figure 3.7 (A and **B)** Shows level of serum ALT and AST in EMR exposed FLX treated rats. All values are mean±SEM. (n=8). [a]p<0.05 compared to control, [b]p<0.05 compared to C+F-20, [c]p<0.05 compared to EMR-2450, [d]p<0.05 compared to E+F-5mg and [e]p<0.05 compared to E+F-10mg [One-way ANOVA followed by Student–Newman Keuls test].

3.4 Discussion

The salient feature of the present study is that long term exposure of EMR didnot affect the liver function. Repeated EMR-2450 MHz exposure decreased CYP2D6 thereby altered the pharmacokinetic profile of FLX. Long term exposure to EMR-2450 decreased CYP2D6 levels in PFC as well as in liver resulting in disturbed metabolism of FLX. Further, EMR-2450 MHz altered the pharmacokinetics of FLX in blood plasma suggesting altered FLX concentration in the liver. EMR subjected rats showed depressive-like behavior which was significantly attenuated by FLX at the dose of 10 and 20 mg/kg. FLX-10 mg/kg increased levels of serotonin and dopamine in PFC of the EMR-2450 MHz exposed rats. However, these effects were not observed with FLX-20 mg/kg.

In our earlier study, we have shown that repeated exposure of EMR-2450 MHz caused an increase in immobility period in FST and TST in experimental rats. Treatment with FLX-10 and 20 mg/kg significantly attenuated the EMR-induced depressive-like behavior during FST and TST, on D-35 and D-42 respectively. FLX-10 mg/kg showed delayed onset of action to produce antidepressive activity in experimental rats. This delayed onset of action may be due to delay in desensitization of autoreceptor as suggested previously that, SSRIs shows its effect after three weeks of treatment schedule due to desensitization of ($5HT_{1A}$) autoreceptors in rats (Hervás et al., 2001).

Further, the antidepresssant effect can also be assessed by sucrose preference test (SPT) by measuring anhedonic behavior (Willner et al., 1992, Moreau et al., 1995). In this study, EMR-2450 MHz exposed animals showed anhedonic response by

decreasing sucrose preference behaviour from D-21 to D-42. Treatment with FLX at the dose of 10 and 20 mg/kg alleviated the EMR-induced anhedonia on D-35 and D-42 respectively. This finding is parallel with the previous report on stress induce anhedonic behaviour is attenuated by FLX-12mg/kg (Moreau et al., 1995, Felger et al., 2013).

The neurobiology of depression involves the modulation of serotonergic and dopaminergic system (Gantz et al., 2015). A preclinical report suggested that decrease in levels of serotonin and dopamine in PFC leads to development of depressive-like symptom in rats (Venzala et al., 2013). It has been reported that short term exposure EMR-1439 MHz through time division multiple access (TDMA) field did not alter serotonin synthesis in rats (Hata et al., 2005). In the present study, long term exposure to EMR-2450 MHz decreased levels of serotonin and dopamine in PFC of experimental rats implicating the exposure of EMR at high frequency modulated the neurobiology of depression. Treatment with FLX-10 mg/kg significantly increased the levels of serotonin and dopamine in PFC in EMR exposed rats. However, FLX at the dose of 20 mg/kg did not produce any significant effect on EMR-induced decrease in 5HT and DA level. Therefore, present finding suggested that, the modulation of 5HT and DA was only attenuated by median dose of FLX (10 mg/kg). This may due to increased turnover of 5HT and DA at the highest dose of FLX. This finding is supported by previous report on, long term treatment with SSRI caused increased metabolism of 5-HT (Celada et al., 2004).

The synthesis of serotonin and dopamine is also dependent on CYP2D6 level in PFC (Mizui et al., 2016). Reports suggested that CYP2D6 helped in the metabolism of the tyramine into dopamine and regeneration of serotonin from 5-methoxytryptamine in PFC (Peñas-LLedó and LLerena, 2014).

Further, it has been reported that the role of CYP2D6 is the oxidative metabolism of SSRIs like FLX (Preskorn, 1994). Preclinical studies reported a decreased level of CYP2D6 might result in depression-like phenotypes (Smith et al., 2016). It has been proposed that the decrease in the CYP2D6 level showed decreased FLX metabolism in liver and regeneration of serotonin in the cortical region of brain (Yu et al., 2003). The level of 5-HT and DA are reduced in rat model showing depressive-like behavior (Bamji et al., 2006, Clark-Raymond and Halaris, 2013). In the present study, long term exposure to EMR-2450 decreased CYP2D6 level in liver and PFC, which indicated that there is a decrease in FLX metabolism and disturbed serotonin regeneration in experimental rats. Therefore, long term exposure to EMR-2450 MHz altered 5-HT and DA activity in PFC and CYP2D6 activity in brain and liver.

In the present study, FLX (5, 10 and 20 mg/kg) was administered from D-21 of experimental design. FLX-20 mg/kg significantly decreased CYP2D6 level in brain and liver due to disruption in the metabolism of FLX in liver on D-42. An earlier report suggested that FLX is metabolized in the liver by CYP2D6 (Assasa, 2010). Hence, long term exposure to EMR altered FLX metabolism in rats and increased the FLX concentration in the liver. However, there were significant decreases in CYP2D6 level in control+FLX-20 mg/kg compared to control in experimental rats. FLX-5 and 10 mg/kg did not increase in CYP2D6 level compared to EMR exposed rats. Therefore, a low dose of FLX did not show changes in metabolism in the EMR exposed rats. Previous study suggested that alteration in FLX metabolism leads to liver toxicity in rats (Inkielewicz-Stępniak, 2011). In the present study, repeated exposure to EMR-2450 MHz did not produce hepatotoxicity. Repeated exposure of EMR decreased CYP2D6 metabolite in liver lead to alter the pharmacokinetic parameters of fluoxetine. These Modifications lead to deposition of fluoxetine in liver causes liver dysfunction in EMR exposed rats.

However, long term exposure to EMR reduced the metabolism of FLX-20 mg/kg via CYP2D6 inhibition.

FLX is metabolized into NFLX by N-demethylation conjugation and excreted through liver (Fura, 2006). The pharmacological effect of NFLX is same as the FLX, and good correlation of concentrations between blood and brain (Pawluski et al., 2014). In the present study, long term exposure to EMR-2450 MHz altered the PK of FLX by inhibiting CYP2D6 level in liver as well as in PFC. In support of present finding, a clinical report suggested that, the changes in the metabolism of fluoxetine due to inhibition of CYP2D6 in liver lead to altered pharmacokinetics of FLX (Heikkinen et al., 2003). The PK of FLX is altered in depressed patients (Gourion et al., 2004). The extent of first-pass metabolism by the liver after oral administration of FLX was estimated from Cmax, Tmax, AUC and $t_{1/2}$ (Caccia et al., 1990). In the present study, we have performed PK study for FLX using NFLX as standard which is an active metabolite of FLX. We have compared *perse* group (FLX C+20), EMR+FLX-5, 10 and 20 mg/kg. There was significant increase in Tmax, Cmax, AUC and $t_{1/2}$ of EMR+FLX-20mg/kg as compared to *perse* group implicating the EMR exposure has inhibited the metabolism of FLX by inhibiting the CYP2D6.

The pharmacodynamic of FLX can be correlated with its PK. In present study, FLX-10 and 20 mg/kg showed antidepressant activity. The FLX-10 mg/kg induced antidepressant activity may be due to increase in serotonin and dopamine levels. However, FLX-20 mg/kg induced anti-depressant-like phenotype is independent of monoaminergic system as it did not change serotonin and dopamine levels. Interestingly, the reduced level of monoamines was also observed in *perse* group. this activity of FLX-20 mg/kg can be due to its inhibitory effect on CYP2D6 enzyme which is responsible for the regeneration of monoamines (Peñas-LLedó and LLerena, 2014).

Which was further confirmed the FLX at highest dose caused reduction in metabolism. This finding is supported by the previous report on long term (about one month) administration of FLX (24 mg/kg) leads to severe hepatic injury in rats (Inkielewicz-Stępniak, 2011). The pharmacodynamic of FLX is also affected by the liver function. In our study exposure of EMR did not modulate the ALT and AST levels. The levels of AST and ALT have also not been affected by low (5 mg/kg) and median dose (10mg/kg) of FLX. However, FLX at highest dose (20mg/kg) induced liver toxicity by increasing the AST and ALT levels. The AST and ALT levels were also increased in FLX treated control rats which further, confirm the toxicity produced by FLX-20 mg/kg. Therefore, FLX-10 mg/kg was a better therapeutic dose as an antidepressant in long term exposure to EMR induced depressive-like symptoms in rats.

3.4.1 Summary

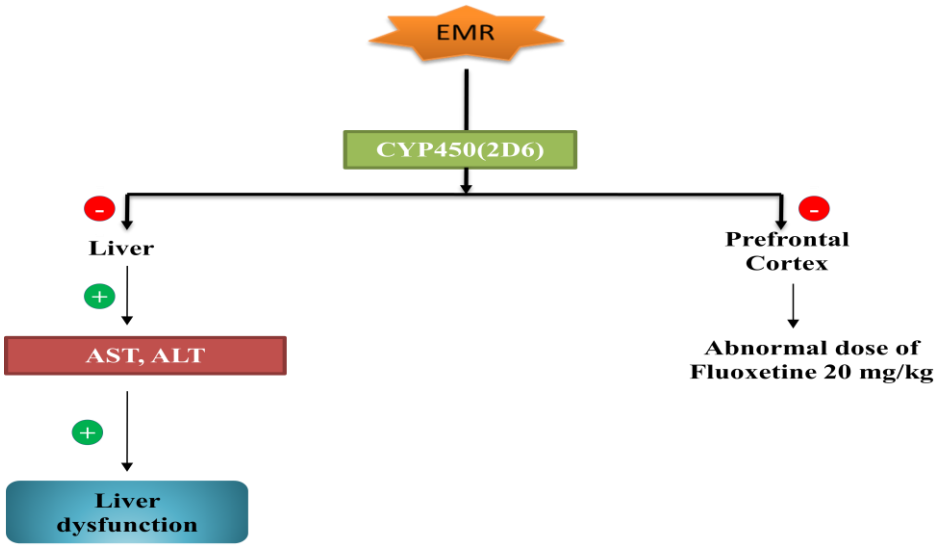


Figure 3.8 Summary of hypothesis

In figure 3.8 shows that long term exposure to EMR decreased CYP2D6 and thereby reduced metabolism of FLX. In FLX-20 mg/kg to EMR exposed control rats, there was decrease in CYP2D6 level and treatment also produced liver toxicity. Long

term exposure to EMR decreased 5-HT and DA which was only attenuated by moderate dose of FLX (10mg/kg). EMR induced depressive-like behavior was attenuated by FLX-10 and 20 mg/kg. Therefore, FLX-10 mg/kg can be prefer dose for alleviating EMR induced depressive-like symptoms without producing any toxicity in rats.