ABSTRACT

Objective: Duloxetine, a selective serotonin and noradrenaline reuptake inhibitor used in major depressive disorders, urinary incontinence and diabetic neuropathic pain. It is reported to be associated with several types of liver injuries, including hepatocellular, cholestatic and mixed hepatocellular-cholestatic patterns. The objective of this study was to assess the effect of duloxetine or its metabolites on oxidative stress-induced liver damages.

Methods: In this study, animals were divided into five groups. In the first group, only the vehicle was given orally for 21 d. The second group has been considered as a hepatotoxic control group where Erythromycin was given orally for 14 d and remaining three groups have been considered as test groups where duloxetine, fluvoxamine and duloxetine along with fluvoxamine were administered orally for 21 d. Liver GSH, oxidised lipid malonaldehyde (MDA), superoxide dismutase (SOD), catalase (CAT), protein carbonyl (PC) and plasma alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) levels were measured to determine the level of hepatotoxicity. Scanning electron microscopy (SEM) study of liver tissues was also performed to examine the liver injuries.

Results: GSH and SOD levels were found to be decreased in duloxetine-treated groups with respect to the hepatotoxic control group, whereas increased level of MDA, CAT and PC signify the damages of liver cells. Increased level of plasma ALT, AST and ALP at the same time indicated liver tissue damage. Opposite effects were observed in the case of duloxetine and fluvoxamine-treated groups. SEM of liver tissues revealed that the tissue injury occurred in Duloxetin treated groups, whereas the restoration of normal tissue architecture took place due to the administration of duloxetine and fluvoxamine-treated groups.

Conclusion: Our results collectively indicated that hydroxylated and epoxide metabolites of duloxetine might have hepatotoxic potential due to oxidative stress produced by the release of free radicals or reactive oxygen species.

Keywords: Duloxetine, CYP1A2 enzyme, Hepatotoxicity, Oxidative stress

INTRODUCTION

Duloxetine (DLX; fig. 1A) [N-methyl-γ-(1-naphthoxy)-2-thiophene-propylamine] is a selective serotonin and noradrenaline reuptake inhibitor approved by the USFDA for the treatment of major depressive disorders. In addition, it is also used in stress-induced urinary incontinence and diabetic peripheral neuropathic pain [1]. DLX is highly bind to plasma proteins (>90%) and mainly metabolised by various cytochrome (CYP) enzymes, such as CYP1A2 and CYP2D6 in humans. Although, the naphthyl ring underwent epoxidation and subsequently formed adduct with glutathione (GSH), but thiophene moiety showed inert after bioactivation [1]. The bioactivation of naphthyl ring mediated through CYP1A2 enzyme corresponds to 4-hydroxy DLX, 6-hydroxy-5-methoxy DLX and 4, 6-dihydroxy DLX [4]. Sometimes, naphthyl ring could generate reactive metabolites, i.e. epoxides which may cause hepatotoxicity [2]. The role of the CYP2D6 enzyme during bioactivation of DLX was not prominent in the case of both rats and humans.

Fig. 1: Structures of (A) DLX and (B) FLX

Recently, it has been reported that DLX was reported to be associated with several cases of hepatocellular, cholestatic and mixed hepatocellular-cholestatic patterns of liver injuries [3]. Hepatobiliary diseases were estimated to occur in about 8 per 100,000 cases, while elevation of enzyme level increased thrice the value of normal range as observed in 0.9 to 1.7% of DLX treated subjects [4, 5]. Idiosyncratic liver damages were estimated to occur about 1-2 per 100,000 cases of exposure in DLX [4]. In a pooled analysis of 17615 subjects, the incidence of serum ALT level was increased three times than normal value [6]. Hanje et al. (2006) reported and explained the cause of fulminating hepatic failure and death during DLX therapy [7]. Taking into all these considerations related to DLX inducing hepatotoxicity, the question arose whether DLX or its metabolites have any role in hepatotoxicity or not? It has been reported that DLX is metabolised by both CYP1A2 and CYP2D6. Therefore, the objective of this study was to evaluate the effect of Duloxetine or its metabolites on oxidative stress-induced hepatotoxicity. In order to further ascertain the oxidative stress-induced hepatotoxicity, docking studies were also performed accordingly.

MATERIALS AND METHODS

Materials

Duloxetine (DLX; Batch Number: DL0040713) and Fluvoxamine (FLX; fig. 1B, Batch Number: LT-OFLM/014/12-13) were received from Indian companies namely Hetero Drugs Limited, Hyderabad, and Mehta API Pvt Ltd, Mumbai, respectively. Disodium ethylenediamine tetracetic acid (EDTA), disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium citrate and
Animal grouping and experimental design

Male Wistar rats (weighing 90-140 g) were purchased from Animal Hydrogen bonds were evaluated.

their binding affinity (kcal/mol) and counting of probable previously recognised active site amino acid sequence. Finally, the best configuration. Later, grid box was set according to using DogP active site recognize where the ligand has been shown the best configuration. Later, grid box was set according to previously recognised active site amino acid sequence. Finally, their binding affinity (kcal/mol) and counting of probable hydrogen bonds were evaluated.

Docking studies

Docking studies of DLX were performed using Argus lab, Pyrx virtual screening 0.8 and Autodock 4.0 along with Autodock vina. Before the docking study, we identified the active site domain using DogP active site recognize where the ligand has been shown the best configuration. Later, grid box was set according to previously recognised active site amino acid sequence. Finally, their binding affinity (kcal/mol) and counting of probable hydrogen bonds were evaluated.

Animal grouping and experimental design

Male Wistar rats (weighing 90-140 g) were purchased from Animal House, CSIR-CDRI, Lucknow, India (Approval No. UIP/IAEC/2014/FEB./07) and were kept in polypropylene cages under standard conditions of temperature (25±1 °C) with 12 h light and dark conditions, diet and water ad libitum for 7 d. Animals were randomised and divided into following 5 groups having 6 animals in each group (n=6). Rats were kept fasted overnight before starting the experiment with free access to water. Drugs were dissolved separately in distilled water as per the following treatment schedule. The first group served as normal control where animals were given only vehicle orally (1 ml/kg BW) for 21 d. The second group has been considered as hepatotoxic control, where ERY was given orally (100 mg/kg BW, served as hepatotoxic control) for 14 d [8]. The remaining three groups were considered as test groups where DLX, FLX and DLX+FLX where of analytical grades with 99% purity and in-house distilled water was used throughout the experiment.

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Plasma ALT, AST and ALP

ALT level was slightly higher in DLX (~ 57 U/ml) treated group than normal control group (~ 40 U/ml) as shown in table 2. ALT (~ 37 and 32 U/ml) level in plasma was found to be decreased in both FLX and DLX+FLX treated groups. ERY acted as a hepatotoxic control, which demonstrated the higher concentration of ALT in plasma.

There was a significant increase in plasma AST level in both ERY (~182 U/ml) and DLX treated groups (~174 U/ml). AST level (~150 U/ml) was closer to normal control after FLX and DLX+FLX treated groups. A similar result of plasma concentration of ALP was higher in both ERY and DLX treated groups. FLX and DLX+FLX treated groups also showed the similar higher concentration of plasma ALP in compared to normal control (table 2).

Table 2: Various enzyme concentrations in plasma

| Groups   | ALT (U/ml) | AST (U/ml) | ALP (U/ml) |
|----------|------------|------------|------------|
| Control  | 40.1±2.71  | 150.0±7.62 | 830.5±8.98 |
| ERY      | 56.3±1.42  | 182.3±8.01 | 956.9±4.55 |
| DLX      | 57.2±2.39  | 174.2±7.82 | 914.6±6.88 |
| FLX      | 37.5±1.98  | 143.5±7.95 | 820.3±9.53 |
| DLX+FLX  | 32.6±2.82  | 147.8±8.06 | 780.9±1.18 |

Data were presented as mean±SD (n=6). Statistically, significant differences were observed between control and test groups [one way-ANOVA followed by Bonferroni multiple comparison test; *(p<0.001, ^p<0.01)]

Biochemical estimations in liver tissues

In order to understand the mechanism of hepatotoxicity of DLX in Wistar rats, various oxidative stress-related parameters like GSH, MDA, SOD, CAT and PC in liver tissues were measured. As depicted in table 3, it was observed that GSH level was found to be decreased in DLX treated group. DLX and DLX+FLX treated groups also showed no toxic effect because of similar concentrations of biochemical parameters as in normal control. The level of MDA was higher in both ERY and DLX treated groups (~ 2.0 nM/mg of protein) as shown in table 3. This concentration again normalised for FLX and DLX+FLX treated groups. Similar trends were also observed for PC assay where the formation of PC was higher in ERY and DLX treated groups (table 3). In order to determine the oxidative stress-based hepatotoxicity, CAT and SOD enzyme level was measured separately. CAT is most abundant in the liver, which is mainly responsible for the catalytic decomposition of H$_2$O$_2$. Increase in concentration of H$_2$O$_2$ in DLX treated group depicted that there was less amount of CAT enzyme available in the tissues to decompose H$_2$O$_2$ (table 3). It was observed that SOD enzyme level was also decreased in DLX treated group in compared to normal control, FLX and DLX+FLX treated groups.

Table 3: Various oxidative stress parameters in liver

| Groups   | SOD (U/mg of Protein) | CAT (nM H$_2$O$_2$ decomposed/min/mg of protein) | GSH (μM/mg of Protein) | TBARS (nM of MDA/mg of protein) | PC (μg/mg of protein) |
|----------|-----------------------|-----------------------------------------------|------------------------|--------------------------------|----------------------|
| Control  | 2.3±0.13              | 75.20±4.51                                   | 60.67±3.64             | 1.79±0.29                      | 0.41±0.03            |
| ERY      | 1.1±0.06              | 50.33±5.50                                   | 42.15±2.52             | 5.36±0.22                      | 0.69±0.04            |
| DLX      | 1.17±0.07             | 97.88±3.97                                   | 37.80±2.96             | 5.12±0.20                      | 0.65±0.05            |
| FLX      | 2.17±0.18             | 70.87±5.39                                   | 63.01±2.68             | 1.41±0.20                      | 0.41±0.03            |
| DLX+FLX  | 1.80±0.10             | 34.2±4.05                                    | 60.46±4.82             | 2.43±0.14                      | 0.42±0.02            |

Data were presented as mean±SD (n=6). Statistically, significant differences were observed between control and test groups [one way-ANOVA followed by Bonferroni multiple comparison test; *p<0.001]

SEM analysis of liver

SEM analysis showed the presence of lesions in DLX treated group, whereas, it was absent in both FLX and DLX+FLX treated groups (fig. 3).

DISCUSSION

Most of the hepatotoxic drugs are generally involved in increasing levels of liver enzymes and GSH level due to oxidative stress induced by some specific drugs, whereas some other drugs may take part in the alteration of liver mitochondrial functions and expression of liver genes [17-19]. Mechanism of oxidative stress also plays an important role in causing hepatotoxicity produced by some drugs like acetaminophen [20]. It is assumed that some orally administered drugs are involved in the generation of free radicals which may further elevate the tissue TBARS, PC and may reduce the CAT, GSH and SOD levels in liver, and increased level of plasma ALT, AST and ALP were collectively responsible for liver damages.

Orally active DLX was metabolised by CYP1A2 to corresponding hydroxylated and epoxide metabolites which had a tendency to bind with GSH [1, 2]. This practical data was also confirmed by docking studies where we found that CYP1A2 was the major enzyme for DLX metabolism (fig. 2 and table 1). Taking into consideration of above observations, it was questionable whether parent DLX is hepatotoxic or its metabolites. To get the actual answer, metabolism of DLX using FLX (CYP1A2 inhibitor) was conducted, and various biochemical parameters were evaluated.

Our results collectively suggested that all the enzyme levels were higher for DLX treated groups than control group whereas this level is slightly lower for both FLX and DLX+FLX treated groups as shown in table 2. Both AST and ALT are the liver enzymes which is responsible for transamination of amino acids whereas ALP is responsible for catalysis of organic phosphate esters. These are the key enzymes of
liver, which are extracted out during liver damages [16, 21]. The increase of these enzymes in plasma during DLX treatment indicated the hepatic damage, which was further improved by FLX treatment. This was an indirect indication of liver damage, but it could not be explained how DLX produced toxicity in the liver after oral administration.

The DLX treated rats depleted more reduced glutathione (GSH) than FLX and DLX+FLX treated groups (table 3). GSH is a tripeptide, which is most abundant in all tissues including liver. GSH has a major role in oxidation-reduction process, resulting in the formation of disulfide glutathione (GSSG) [22] during oxidative damage. Reduction of GSH level by DLX is an indication of oxidative stress-induced liver damage, which was improved during FLX treatment. The decrease in the levels of GSH in DLX treated rats represented the higher utility of GSH during oxidative damage which was completely restored during a single dose of FLX and combined dose of FLX with DLX.

To prove the oxidative stress induced hepatotoxicity, the tissue MDA levels were also performed. Oxidation of lipids is another important parameter to measure the oxidative stress in living body [17]. From the result; it was observed that tissue MDA level was higher for DLX treated group, which was again restored to a normal level during FLX, and DLX+FLX treated rats (table 3). The Higher amount of MDA formation during DLX treatment was directly represented the tissue MDA level for DLX is an indication of oxidative stress-induced liver damage, oxidisation-reduction process, resulting in the formation of disulfide glutathione (GSSG) [22] during oxidative damage. Reduction of GSH level by DLX is an indication of oxidative stress-induced liver damage, which was improved during FLX treatment. The decrease in the levels of GSH in DLX treated rats represented the higher utility of GSH during oxidative damage which was completely restored during a single dose of FLX and combined dose of FLX with DLX.

In order to understand the relationship between oxidative stress and hepatotoxicity, PC assay was performed where a higher amount of PC was formed than DLX treated groups (table 3). This assay indirectly indicated that oral administration of mechanisms in drug-induced hepatotoxicity. Curr Med Chem 2008;3:143-53.

CONCLUSION
The hepatotoxicity of DLX might be due to oxidative stress produced by hydroxylated and epoxide metabolites, which may release free radicals or reactive oxygen species. Further studies in this context, such as preparation of hydroxylated and epoxide metabolites and measurement of their hepatotoxic potential are beyond the scope of this study.

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CONFLICT OF INTERESTS
Authors declare that they have no conflict of interest.

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