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In vivo investigation on the chronic hepatotoxicity induced by sertraline

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ABSTRACT

Although sertraline is widely prescribed as relatively safe antidepressant drug, hepatic toxicity was reported in some patients with sertraline treatment. The present study was conducted to investigate the morphometric, hepatotoxicity, and change in gene expression of drug metabolizing enzymes. Male healthy adult rabbits (*Oryctolagus cuniculus*) ranging from 1050 to 1100 g were exposed to oral daily doses of sertraline (0, 1, 2, 4, 8 mg/kg) for 9 weeks. The animals were subjected to morphometric, hepatohistological, histochemical and quantitative real-time polymerase chain reaction analyses. Sertraline chronic exposure induced morphometric changes and provoked histological and histochemical alterations including: hepatocytes hydropic degeneration, necrosis, nuclear alteration, sinusoidal dilation, bile duct hyperplasia, inflammatory cells infiltration, portal vessel congestion, Kupffer cells hyperplasia, portal fibrosis and glycogen depletion. In addition, the gene expression of drug and arachidonic acid metabolizing enzymes were reduced significantly (p value < 0.05). The most affected genes were *cyp4a12*, *ephx2*, *cyp2d9* and *cyp1a2,* demonstrating 5 folds or more down-regulation. These findings suggest that chronic sertraline treatment induced toxic histological alterations in the hepatic tissues and reduced the gene expression of drug metabolizing enzymes. Patients on chronic sertraline treatment may be on risk of hepatotoxicity with reduced capacity to metabolize drugs and fatty acids.

1. Introduction

Sertraline, a selective serotonin reuptake inhibitor, is used in treatment of depression, obsessive–compulsive, panic, and social anxiety disorders (Borkowska et al., 2002). In addition, this drug is used in alleviation symptoms of premenstrual dysphoric disorder, in treatment of premature ejaculation and vascular headache (Hoehn-Saric et al., 2000). Sertraline is the most prescribed antidepressant in US market (Grohol, 2014). Reports showed that the efficacy of sertraline for depression is similar to other classical tricyclic antidepressants, while its side effects are much less pronounced (Schramm et al., 2007). Studies showed that sertraline was capable of causing developmental toxicity and lowered feeding rates of the tadpoles of the African clawed frog (*Xenopus laevis*) at environmentally relevant concentrations via effects on the neuroendocrine system (Conners et al., 2009). Moreover, chronic exposure to sertraline caused prognosis breathlessness related to eosinophilic pneumonia and diffuse pulmonary fibrosis (Thornton et al., 2009). In addition, it was reported that sertraline administration induced ventricular tachycardia, bleeding and bilateral maculopathy (Patel et al., 2013; Eslami Shahrbabki and Eslami Shahrbabaki, 2014; Ewe et al., 2014).

The hepatic side effects of sertraline were reported in multiple clinical studies (Collados et al., 2010; Park and Ishino, 2013; Suen et al., 2013). It was reported that sertraline exhibited severe hepatitis, cholestasis and bile duct paucity among psychiatric patients (Suen et al., 2013; Conrad et al., 2016). Also, it was reported that sertraline increased the gene expression of apoptosis and endoplasmic stress biomarkers in hepatic human hepG2 cell line and induced mitochondrial dysfunction in rat hepatic tissues (Chen et al., 2014a, b; Li et al., 2012). Some studies showed that sertraline induced the gene expression of stress biomarkers, such as PERK, IRE1α, and CHOP, in the endoplasmic reticulum of the human hepatic cell line HepG2 (Chen et al., 2014a). Furthermore, mRNA level of genes involved in endogenous and xenobiotic compounds metabolism was widely changed after larval zebra fish were exposed to sertraline (Park et al., 2012).

Limited studies were carried out on the influence of sertraline in the liver, with lack of studies conducted on the histomorpholometric influence of this drug and on the mRNA levels of drug metabolizing enzymes in the liver. With these objectives, the present study aimed to investigate the hepatic histomorphological changes and gene expression of phase I, II and arachidonic-cytochrome P450 (CYP450) enzymes in the hepatic tissues of healthy male rabbits after chronic sertraline exposure.

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2. Material and methods

2.1. Experimental animals

Forty male rabbits (*Oryctolagus cuniculus*), weighing 1050–1100 g and of similar age (7 months old) were obtained from the animal house of Jerash University. The animals were housed at 23 ± 1 °C, and 12 h light-12 h dark cycle. Animals were divided into a control group and four test groups of 8 rabbits each and all animals were separately caged and maintained on standard laboratory animal diet pellets ad libitum.

2.2. Drugs and chemical

Sertraline powder and sodium chloride were obtained from Sigma-Aldrich (St. Louis, USA).

2.3. Experimental protocol

Following a period of stabilization (7 days), members of the treated groups were exposed to oral feeding with a daily dose of sertraline (1, 2, 4, 8 mg/kg body weight respectively) for 9 weeks. Sertraline solutions with 0.45% sodium chloride were prepared so that the necessary dose could be administered orally in a volume of one ml. The daily doses were 0, 1, 2, 4 or 8 mg/kg for consecutive 9 weeks.

The experimental protocol was approved by Jerash University Bioethical Committee and the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

2.4. Physical observation

Daily observation throughout the study was made for mortality, general well being and behavior patterns in the sertraline-treated rabbits and the control animals.

2.4.1. Body weight monitoring

The rabbit's body weight was monitored at the beginning of treatment and after 9 weeks of treatment.

2.4.2. Percentage absolute liver weight and liver index

Rabbits were euthanized and the liver was taken from each rabbits immediately for the determination of the percentage absolute liver weight. The grade of change in the liver index (L_x) induced by sertraline doses was calculated according to the following equation:

$$\text{Liver index} = \frac{\text{Average weight of the liver/Average weight of the experimental rabbit}}{\text{Weight of the control liver/weight of the control rabbits}}$$

2.5. Histological processing

Fresh portions of the median lobe of the liver from each rabbit of all groups were cut out rapidly, fixed in 10% neutral buffered formalin and then dehydrated with ascending grades of ethanol (70, 80, 90, 95 and 100%). Dehydration was then followed by clearing the tissue samples in 2 changes of chloroform before being impregnated with 2 changes of melted paraffin wax, embedded and blocked out. Sections (4–5 μm) were stained according to Jarrar and Taib (2008), with the following conventional histological and histochemical stains: hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), Best's carmine stain, Mallory trichrome and Prussian blue reaction. Stained sections of control and treated rabbits were examined for alterations in the architecture, portal triads, hepatocytes, sinusoids and for the presence of necrosis, fatty change, portal fibrosis and cirrhosis.

2.6. RNA extraction and cDNA synthesis

The control animals and those subjected to 4 mg/kg/day of sertraline for 9 weeks were investigated in this part of the current study. The invested dose is equivalent to the daily dose for humans, based on the surface area of the animal body (Nair and Jacob, 2016). Liver biopsy was taken from each animal for RNA extraction and purification using Trizol RNA extraction solution (Thermo Fisher Scientific, Waltham, Massachusetts, USA), isopropyl alcohol and 75% ethanol, according to the manufacturer's instructions. After that, RNA was converted to cDNA using cDNA Synthesis Kit® (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Briefly, 3 mg of total RNA was added to a reaction mixture containing 100 pmol oligo (dT), 2.5 mM dNTP, 0.1 M DTT, first strand buffer and 100 unit of M-MLV reverse transcriptase and incubated at 42 °C for 50 min. The RNA and cDNA concentrations were measured using the Nanodrop Instrument Quawell DNA/Protein Analyzer (Sunnyvale, CA, USA).

2.7. Gene expression analysis

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using Bio-Rad CFX96 machine (Bio-Rad, California, USA). Briefly, 1 ng of cDNA was added to a reaction mixture containing SYBR™ Green PCR Master Mix (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and selective primers for cDNA sequence of drug- and arachidonic acid-metabolizing enzymes. The PCR conditions used were as follows: denaturation at 95 °C for 3 min followed by 40 cycles of denaturation at 95 °C for 10 s and annealing at 53 °C for 30 s. The gene expression of the target genes was normalized to the expression of the house keeping gene *gapdh*, as described previously (Livak and Schmittgen, 2001). The results were presented in fold change of gene expression of the sertraline-treated group to control group.

2.8. Statistical analyses

All continuous results in this study were expressed by the average ± standard deviation. Two-tailed student *t*-test (p-value < 0.05) was used as a statistical tool for comparison the effect of different sertraline doses.

3. Results

No mortality occurred in any of the experimental groups of the present investigation. In comparison with the control group, sertraline treated animals (8 mg/kg) demonstrated rapid respiration and oozing of pinkish fluid droplets from the nose.

3.1. Morphometric alterations

After 9 weeks of sertraline administration, variation of the body weight during sertraline exposure is given in Table 1. It is observed that sertraline administration prevented the significant increase in the total body weight as seen in the control group after 9 weeks exposure.

The liver average weight and the percentage absolute liver weight to control group together with the change in the liver index are shown on Table 2. It is noticed that there is a mild but non-significant (p value > 0.05) decrease in the percentage absolute liver weight of the subjects exposed to 2–8 kg/ml sertraline doses.

3.2. Gross examination

Inspection of the internal organs of each dissected animal with the bare eye and dissecting lenses revealed the following macroscopic abnormalities:

Table 1
Change on the average body weight **(g)** of rabbits subjected to different doses of sertraline for 9 weeks.

Dose	Starting Weight	Weight after 9 weeks	P-value
Control group (Received 0.45% sodium chloride)	1079 ± 105	1211 ± 116	0.035*
Received sertraline (1 mg /kg body weight)	1095 ± 115	1208 ± 118	0.068
Received sertraline (2 mg /kg body weight)	1087 ± 126	1099 ± 117	0.85
Received sertraline (4 mg /kg body weight)	1206 ± 119	1210 ± 124	0.95
Received sertraline (8 mg /kg body weight)	1093 ± 128	1085 ± 126	0.87

* Represents statistically significant (p-value < 0.05) using student *t*-test.

3.2.1. Pulmonary hemorrhage and congestion

The lungs of sertaline-treated rabbits showed congested pulmonary parenchyma. This alteration was more prominent in rabbits exposed to 8 mg/kg of sertraline (Fig. 1a & b).

3.2.2. Urinary bladder congestion

Mucosal congestion of the urinary bladder was observed in all animals exposed to 4 mg/kg and more for 9 weeks (Fig. 1c & d). Moreover, mesentery and the intestine of rabbits exposed to 4 mg/kg and more of sertraline demonstrated moderate congestion.

3.3. Histological alterations

The lobular architecture in the liver of all control rabbits was preserved and kept intact (Fig. 2a & b). On the other hand, exposure to sertraline for 9 weeks has produced the following hepatic histological and histochemical alterations:

3.3.1. Hepatocytes hydropic degeneration

Hepatocytes of sertraline-treated rabbits demonstrated swelling vacuolated cytoplasm. This alteration associated with necrosis and was more prominent in the liver of rabbits received 8 mg/kg sertraline and to lesse extent in the liver of animals received 4 mg/kg sertraline (Fig. 3a & b).

3.3.2. Hepatocytes necrosis

Massive necrosis was noticed in most hepatocytes of sertraline-treated rabbits. This alteration was more pronounced within the pericentral zone hepatocytes where some of these cells demonstrated brown pigmentation (Fig. 4a).

3.3.3. Hepatocytes nuclear alterations

Nuclear karyopyknosis, karyorrhexis, karyolysis, vesiculation, anisonucleosis and marked binucleation, were observed in the hepatocytes of sertraline-treated rabbits. Nuclear vesiculation appeared in the hepatocytes of rabbits received 4 or 8 mg/kg sertraline. This alteration was less frequent in rabbits received 2 mg/kg or less of sertraline. Anisonucleosis, binucleation, karyorrhexis and karyolysis were seen in the hepatocytes of all treated rabbits received 4 mg/kg sertraline and more (Fig. 4b–d). Some hepatocytes of sertraline treated rabbits showed occasional pyknosis specially the necrotic ones and those exhibited hydropic changes.

3.3.4. Inflammatory cell infiltration

Infiltration of inflammatory cells in the lobular and periportal hepatic spaces was seen. The infiltrate cells were mainly lymphocytes and plasma cells (Fig. 4e).This alteration was mainly demonstrated in the liver of rabbits received 8 mg/kg.

3.3.5. Sinusoidal dilatation

This hepatic lesion was seen over the entire hepatic lobules with predominance in the central area (Fig. 5a). The widening of the blood sinusoids was more prominent in the liver of rabbits exposed to 8 mg/kg sertraline and to lesser extent in the liver of those received 4 mg/kg of the drug.

3.3.6. Bile duct hyperplasia

Dilatation of the bile duct was seen together with proliferation of the lining epithelium in sertraline-treated rabbits in comparison with the control ones. This abnormality was noticed in rabbits received 4 mg/kg or more sertraline.

3.3.7. Kupffer cells hyperplasia

Kupffer cells enlargement and increased in number was seen due to sertraline exposure (Fig. 5b). This alteration appeared in all rabbits exposed to sertraline but was more prominent in those exposed to 4 mg/kg and above.

3.3.8. Congestion of hepatic vessels

Portal vessels and central veins congestion was observed mainly in the hepatic tissues of rabbits received 4 mg/kg and more (Fig. 5c).

3.3.9. Portal space fibrosis

Fibrotic alteration was observed in the hepatic portal triads (Fig. 5d). This change was seen mainly in the hepatic tissues of rabbits received 8 mg/kg sertraline.

3.3.10. Mucosal urinary bladder congestion

In comparison with the control animals, the submucosa of the

Table 2
Amount of change on the relative ratio of liver weight to body weight and the liver index of rabbits subjected to overdoses of sertraline for 9 weeks.

Dose	Average liver weight (g)	Average body Weight (g)	The percentage absolute liver weight	Liver index (L_x)
Control group (Received 0.45% sodium chloride)	59.9 ± 2.6	1194 ± 6.5	5.0 ± 0.004	–
Received sertraline (1 mg /kg body weight)	61.1 ± 3.3	1208 ± 7.8	5.1 ± 0.004	1.02
Received sertraline (2 mg/kg body weight)	54.5 ± 2.2	1099 ± 5.7	4.9 ± 0.004	0.98
Received sertraline (4 mg/kg body weight)	53.7 ± 2.1	1210 ± 3.4	4.4 ± 0.006	0.88
Received sertraline (8 mg/kg body weight)	51.8 ± 1.4	1085 ± 4.6	4.7 ± 0.003	0.94

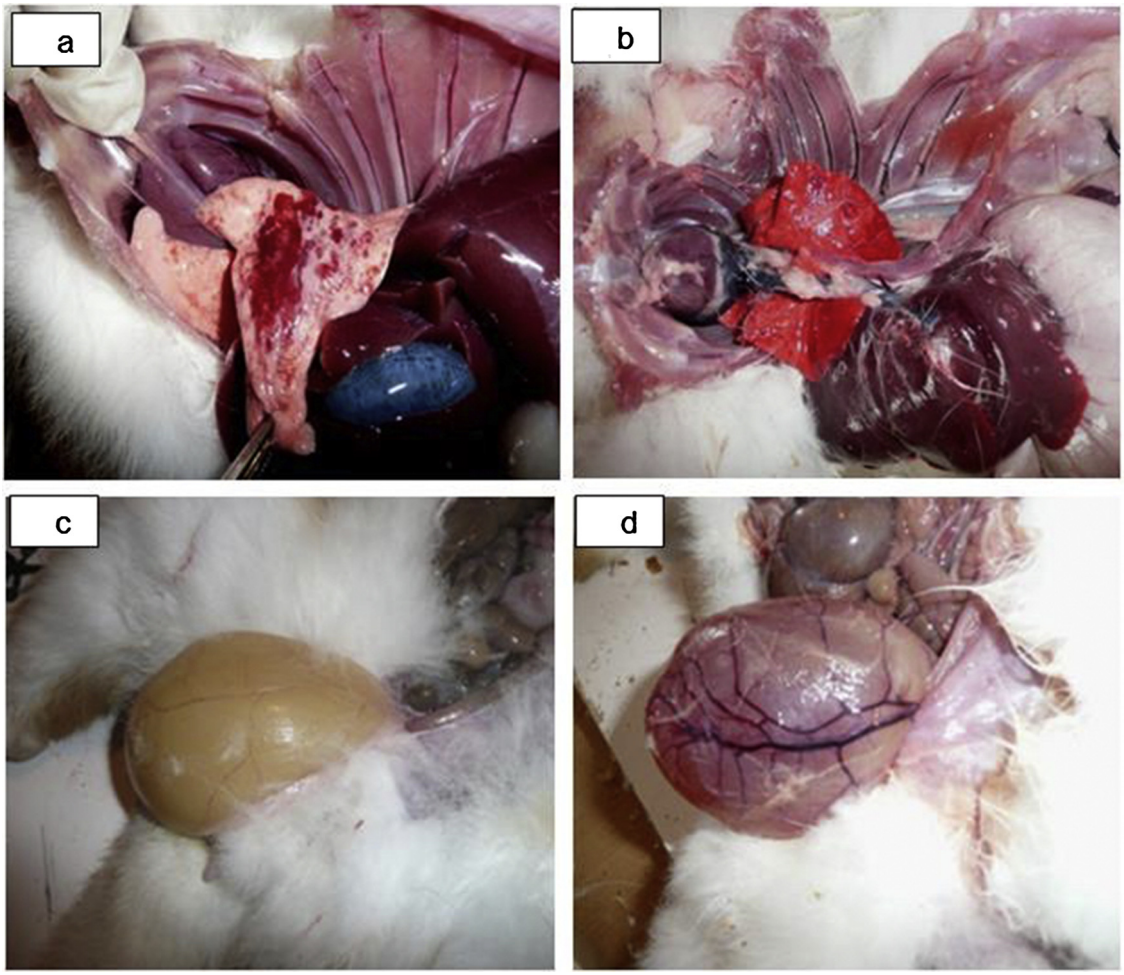

Fig. 1. (a–d). Light photographs demonstrating: (a) Lung of sertaline-treated rabbit demonstrating congested pulmonary parenchyma subjected to 4 mg/kg of sertraline, (b) Lung of sertraline-treated rabbit demonstrating congested pulmonary parenchyma subjected to 8 mg/kg of sertraline, (c) Urinary bladder of control rabbit, (d) Urinary bladder of sertraline-treated rabbit (4 mg/kg) demonstrating mucosal congestion.

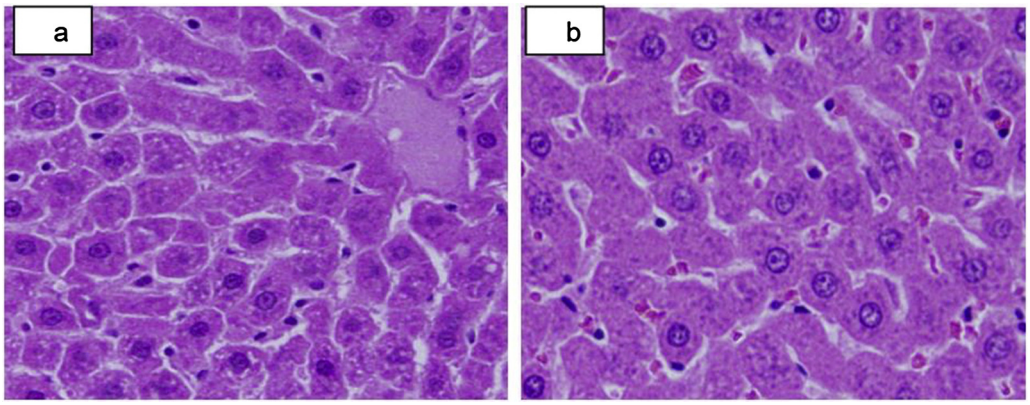

Fig. 2. (a–b). Light micrograph in the liver of control rabbits demonstrating: (a) Well-intact hepatic lobular architecture, (b) Normal hepatocytes with normal Kupffer cells distribution together with blood sinusoidal dilatation. H&E stain.

urinary bladder demonstrated passive congestion while the adventitia layer showed active congestion (Fig. 5e & f).

3.3.11. Glycogen depletion

Hepatocytes of sertraline treated rabbits showed presence of glycogen depletion in comparison with those of control rabbits. This depletion was more prominent in the liver of rabbits exposed to 8 mg/kg sertraline for 9 weeks (Fig. 6a & b).

However, Table 3 demonstrates simple scoring system tabulating variations between the hepatic histological alterations between groups.

3.4. Gene expression alterations

The effect of 4 mg/kg/day sertraline administration on phase I, II and arachidonic acid-CYP P450 gene expression is summarized in Table 4. Sertraline administration significantly (p value < 0.05, *t*-test)

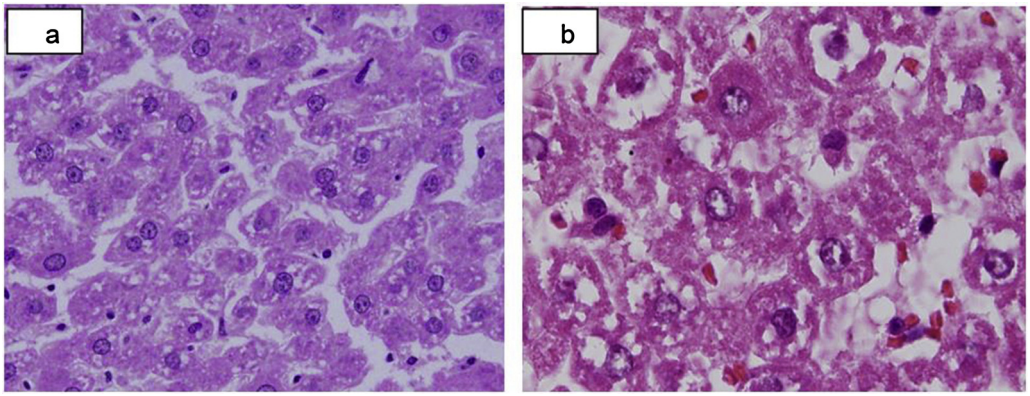

Fig. 3. (a–b). Light micrograph in the liver of sertraline-treated rabbits demonstrating: (a) Hydropic degeneration (4 mg/kg, 9 weeks), (b) Necrotic hepatocytes with swelling vacuolated cytoplasm (8 mg/kg, 9 weeks). H&E stain.

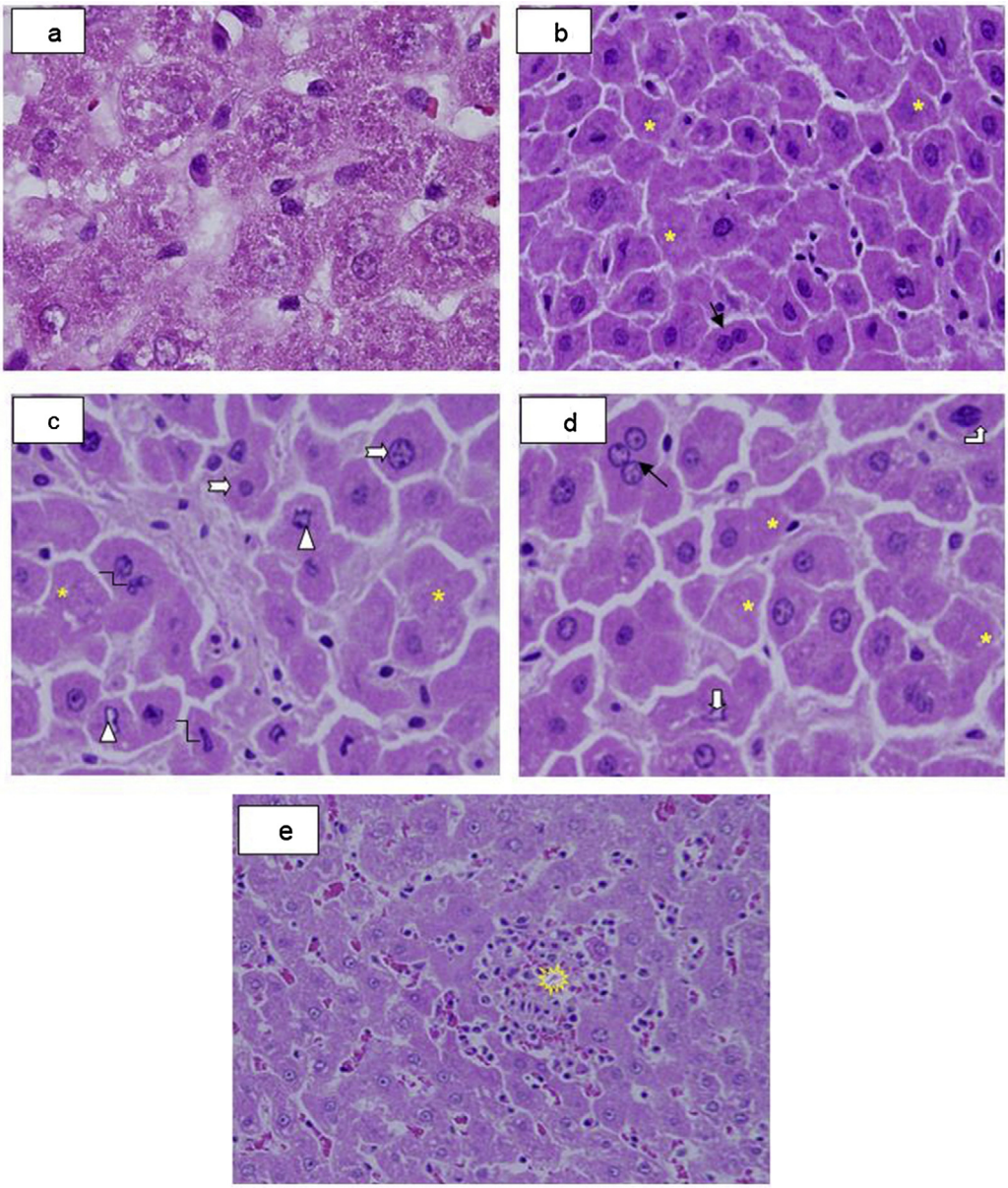

Fig. 4. (a–e). Light micrograph in the liver of sertraline (4 mg/kg)-treated rabbits demonstrating: (a) Necrotic hepatocytes. Note the brown pigmentation in the cytoplasm of the necrotic hepatocytes. H&E stain, (b) karyorrhexis, karyolysis (stars), anisonucleosis and binucleation (arrow) (c) Karyorrhexis (elbow connector), karyolysis (star), anisonucleosis (notched arrow) and nuclear membrane irregularity (triangles) (d) Nuclear karyopyknosis (bent-up arrow), karyorrhexis (down arrow), karyolysis (stars), and marked multinucleation (arrow). H&E stain, (e) Aggregate of inflammatory cells (explosion), (8 mg/kg). H&E stain.

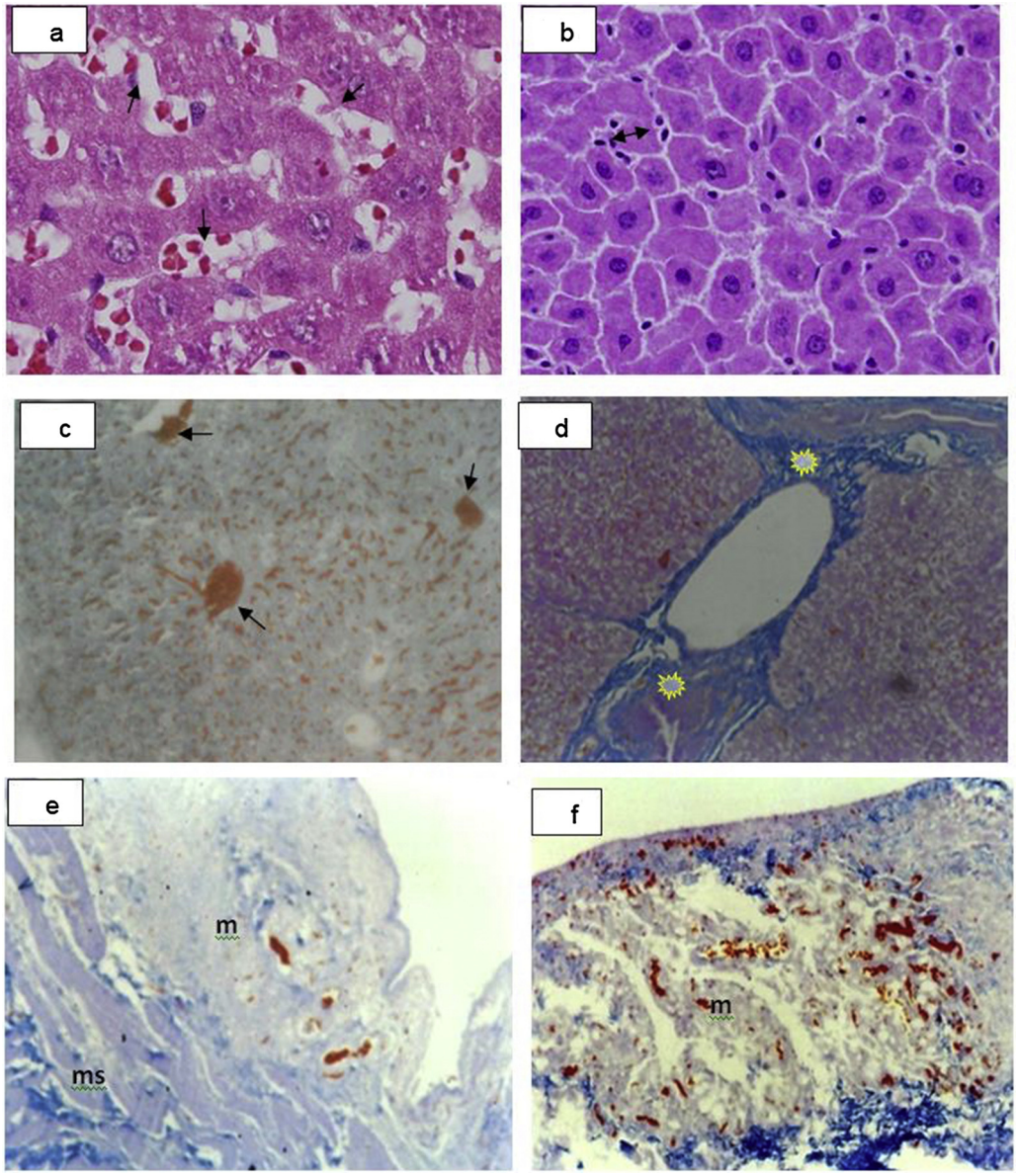

Fig. 5. (a–f). Light micrograph in the liver of sertraline-treated rabbit demonstrating: (a) sinusoidal dilatation (arrows). H&E stain, (8 mg/kg) (b) Kupffer cells hyperplasia (double arrow) (4 mg/kg). H&E stain, (c) Portal vessel and central veins congestion (arrows). H&E stain, (d) Hepatic portal fibrosis (explosions). Mallory trichrome stain, (e) Section in the urinary bladder of control rabbit, m, mucosa; ms, muscularis. Mallory trichrome stain, (f) Congested mucocal urinary bladder of setraline-treated rabbit received 8 mg/kg for 9 weeks. Mallory trichrome stain.

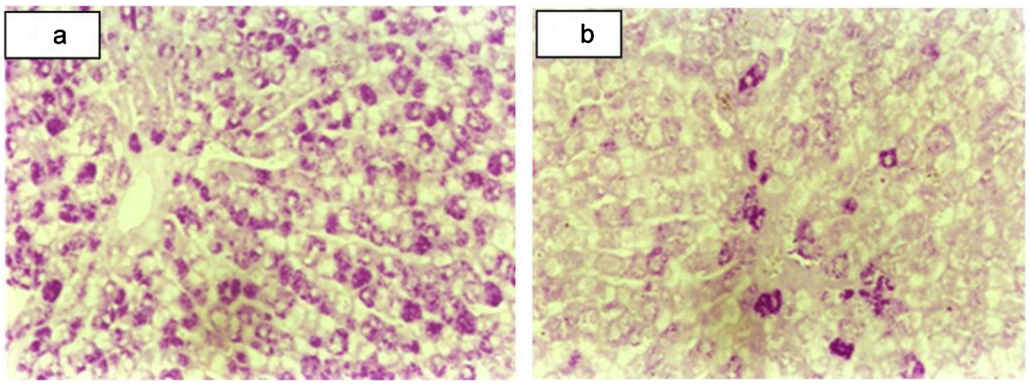

Fig. 6. (a–b). Light micrographs demonstrating: (a) Glycogen in the liver section of control rabbit. PAS stain, (b) Depletion of glycogen in the liver section of setraline-treated rabbit received 8 mg/kg for 9 weeks. PAS stain.

Table 3
Histological alterations induced in the liver of rabbits exposed to sertraline for 9 weeks.

Dose	Hydropic degeneration	Hepatocytes necrosis	Hepatocytes nuclear alterations	Inflammatory cell infiltration	Sinusoidal dilatation	Bile duct Hyperplasia	Kopffer cells hyperplasia	Hepatic vessels congestion	Portal space fibrosis	Glycogen depletion
Control group (Received 0.45% sodium chloride)	−	−	−	−	−	−	−	−	−	−
Received sertraline (1 mg/kg body weight)	−	−	−	−	−	−	±	−	−	−
Received sertraline (2 mg/kg body weight)	±	+	±	−	±	−	+	±	−	±
Received sertraline (4 mg/kg body weight)	+	+ ±	+	±	+	+	+ +	+	+	+
Received sertraline (8 mg/kg body weight)	+ +	+ +	+	+	+ +	+	+ +	+ +	+ +	+ +

Key: (−) absent, (±) less frequent, (+) moderate, (+ +) prominent.

down-regulated the mRNA levels of phase I drug metabolizing enzymes *cyp1a2, 2e6, 2c22, 2d9 and 3a11*. The most reduction in mRNA level of phase I drug-metabolizing enzymes was seen for *cyp2d9* gene with −8.1 fold change. The gene expression of phase II drug-metabolizing enzymes was also reduced significantly (p value < 0.05, *t*-test). *Nat2* gene was the most down-regulated phase II drug-metabolizing enzyme with 5.6 fold reduction in gene expression. Although the gene expression of *tpmt* was down-regulated by 1.7 folds in sertraline treated group, this reduction didn't reach the statistical significance level (*p* value > 0.05, *t*- test).

The gene expression of arachidonic acid-CYP P450 metabolizing enzymes was markedly decreased. The mRNA levels of arachidonic acid hydroxylases enzymes *cyp4a12* and *ephx2* were highly reduced to 10.3 and 11.3 folds, respectively, while the mRNA level of arachidonic acid expoxygenase enzyme *cyp2j5* was reduced by 4.5 folds. Therefore, the ratio of arachidonic acid hydroxylation to epxoygenation gene expression was reduced in the liver of sertraline-treated animals. Lastly, a mild reduction (2.6 folds) in the gene expression of drug transporter gene *slc2a1* was observed in the liver tissues of sertraline-treated group.

4. Discussion

Sertraline is potent anti-depressant drug with less side effects in comparison with other classical antidepressants. However, hepatic toxicity was reported with sertraline treatment (Collados et al., 2010; Park and Ishino, 2013; Suen et al., 2013). The present study showed that chronic exposure to sertraline could cause multiple morphometric histological and histochemical alterations and influenced the mRNA levels of drug and fatty acid metabolizing enzymes in the liver tissues. Accordingly, patients on chronic sertraline treatment may be at risk of hepatotoxicity with reduced capacity to metabolize drugs and fatty acids.

Sertraline undergoes first-pass metabolism in the liver that plays an important role in determination the bioavailability of this drug (De Vane et al., 2002). Sertraline is metabolized mainly by multiple CYP450 enzymes, such as CYP2C9, CYP3A4, and CYP2D6 isoforms (Obach et al., 2005). It has been reported that CYP450 s mediate activation of drugs to toxic metabolites which induce hepatotoxicity (Villeneuve and Pichette, 2004). Therefore, the hepatotoxicity observed with chronic sertraline exposure, in this study, might be due to sertraline-CYP450 oxidized metabolites formed in the hepatic tissues or due to sertraline itself.

The observed histoligical alterations associated with sertraline administration are in line with the previous studies showed that sertraline induced toxic hepatic alterations in the liver (Chen et al., 2014a, b; Li et al., 2012; Abdel-Salam et al., 2013). Sertraline was reported to induce endoplasmic reticulum stress and apoptosis in human hepatic HepG2 cells (Chen et al., 2014a, b). In mitochondria isolated from rat liver, sertraline exhibited mitochondrial dysfunction through adenosine triphosphate depletion, induction of mitochondrial permeability transition, inhibition of mitochondrial respiration complexes, and uncoupling oxidative phosphorylation (Li et al., 2012). Furthermore, sertraline treatment to mice caused liver stress by decreasing of paraoxonase 1 and increasing malondialdehyde and nitric oxide levels in the liver (Abdel-Salam et al., 2013).

Prominent alterations were demonstrated in the hepatocytes nucleoli of setraline treated animals including anisokaryosis, binucleation, karyorrhexis, karyopyknosis and karyolysis. Anisonucleosis was found to be associated with hepatic dysplasia and carcinomatous lesion (Zusman et al., 1991). Anisokaryosis represents a consequence of cell injury usually observed in regenerating cells and might be related to increased cellular activity and nuclear interruption in the mechanism of detoxification (Gerlyngl et al., 2008). Karyopyknosis is an irreversible alteration characterized by condensation of chromatin in the nuclei of cells undergoing necrosis or apoptosis while karyorrhexis and karyolysis are destructive fragmentation and complete dissolution of the

Table 4
Gene expression of phase I, II and arachidonic acid-CYP450 genes in the hepatic tissues after chronic sertraline administration.

Gene	Protein name	The effect of sertraline on gene expression	Fold Change	*p* value
Phase I: drug metabolizing enzyme gene				
cyp1a2	Cytochrome 1a2	Down-regulation	−5.8	< 0.05
cyp2e1	Cytochrome 2e1	Down-regulation	−2.5	< 0.05
cyp2c29	Cytochrome 2c29	Down-regulation	−5.0	< 0.05
cyp2d9	Cytochrome 2d9	Down-regulation	−8.1	< 0.05
cyp3a11	Cytochrome 3a11	Down-regulation	−2.1	< 0.05
Phase II: drug metabolizing enzyme gene				
ugt2b1	UDP-glucuronosyltransferase 2b1	Down-regulation	−3.8	< 0.05
ugt1a1	UDP-glucuronosyltransferase 1a1	Down-regulation	−3.5	< 0.05
nat2	N-acetyltransferase	Down-regulation	−5.6	< 0.05
tpmt	Thiopurine methyltransferase	Down-regulation	−1.7	> 0.05
dhpd	Dihydropyrimidine dehydrogenase	Down-regulation	−4.5	< 0.05
Drug transporter				
slc22a1	Organic cation transporter 1	Down-regulation	−2.6	< 0.05
Arachidonic acid metabolizing enzyme gene				
cyp2j5	Cytochrome 2j5	Down-regulation	−4.5	< 0.05
cyp4a12	Cytochrome 4a12	Down-regulation	−10.3	< 0.05

chromatin matter of a necrotic or dying cell (Pandey et al., 2008). Moreover, the demonstrated hepatic necrosis might indicate oxidative stress by glutathione depletion as a result of sertraline toxicity.

Inflammatory cell infiltration was seen in the hepatic tissue of animals exposed to sertraline, indicating that this drug interacts with liver tissues. This could also suggest an interference of sertraline with the antioxidant defense mechanism which may lead to reactive oxygen species generation and induction of inflammatory response (Johar et al., 2004).

The observed hydropic degeneration due to sertraline treatment might indicate liver injury induced by this drug. Hydropic degeneration is a result of ion and fluid homeostasis disturbance that lead to an increase of intracellular water (Schrand et al., 2010). This alteration is usually accompanied by lysosomal hydrolytic enzyme leakages that lead to cellular degeneration (Del Monte, 2005). The induced hydropic degeneration by sertraline exposure might be resulted from disturbances of hepatocytes membrane function due to the drug toxicity that lead to massive influx of water and Na^+ due to sertraline stress. Moreover, the drug under study induced bile duct hyperplasia. This might suggest that this drug and/or its metabolites are excreted via bile secretion with an irritation effect on the bile duct epithelium.

The findings of the current work demonstrated that sertraline activated the phagocytic activity of Kupffer cells. These phagocytic cells are considered as a defense mechanism of detoxification (Neyrinck, 2004). This alteration is related autophagy throughout the hepatic tissues to help in removing the accumulated chemicals, drugs and poisons and may indicate hepatic oxidative stress related with the amount of injurious to the hepatic tissues induced by sertraline intoxication. On the other hand, the induced congestion in the hepatic blood vessels due to sertraline exposure could be resulted from the vasodilator effect of sertraline (Van Melle et al., 2004). In addition, sertraline induced partial depletion of glycogen in the hepatocytes of animals subjected to sertraline. This finding may indicate an effect of sertraline on glucose absorption or on the mechanism of glycogenesis or/and glycolysis.

The current study demonstrated hepatotoxic histological alterations, after sertraline exposure. These alterations were coupled with down regulation of drug and arachidonic acid-metabolizing enzyme genes. Our findings are in line with Farrell, 1999 and Verbeeck, 2008 studies where the gene expression and the capacity of drug metabolizing enzymes decreased in patients with hepatic dysfunctions. Moreover, the findings of the present study are in agreement with those indicated in the corrected report of Davies and Klowe (1998) where they identified the liver as the target organ of sertraline. They reported that sertraline exposure could demonstrate hepatomegaly, hepatocellular hypertrophy, fatty change, and elevation of serum tramsaminases.

The alteration in the gene expression of drug metabolizing enzymes may influence the clearance and hence the pharmacokinetics and clinical responses of other co-administrated drugs with sertraline. It was reported that sertraline decreased the clearance of the antipsycotic olanzapine (Davies et al., 2016) and the antiviral eavirenz (Melis et al., 2015) and increased the side effects of the anticancer imatinib (Osorio et al., 2017). This drug-drug interaction between sertraline and other drugs might be due, at least in part, to the down-regulation of drug metabolizing enzymes in the livers exhibited by sertraline administration.

The alteration of gene expression of drug and arachidonic acid-metabolizing enzymes was observed in the hepatotoxicity of some drugs. For instance, the anti-tuberculosis rifampin induced hepatotoxicity in mice which was coupled with down-regulation of gene expression of arachedonic acid-cyp450s (Kim et al., 2017). In addition, it is reported that the gene expression of drug metabolizing enzymes was reduced in hepatic injury and diseases, such as cirrhosis and sepsis (Higuchi et al., 2007). Furthermore, the inflammatory mediator interleukin, which is released during hepatic injury (Elbekai et al., 2004), decreased the gene expression of phase I, II drug metabolizing enzymes and drug transporters (Klein et al., 2015). In this study, we found that sertraline administration induced inflammation in the liver tissues, as shown in the histological examinations, which might down-regulate the gene expression of drug and arachidonic-P450 metabolizing enzymes.

One may conclude from the findings of current work that chronic exposure to sertraline produces considerable morphometric and hepatic histological alterations that may affect the liver functions. In addition, the findings suggest that sertraline may reduce the gene expression of drug and arachidonic acid-P450 metabolizing enzymes. Moreover, the results of the present study indicate a need to investigate the effect of sertraline among patients with hepatic disorders and to test the safety of using sertraline among people on risk of liver dysfunctions.

Competing interest

The authors declare that they have no conflict of interests of any type.

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References

Abdel-Salam, O., Youness, E., Khadrawy, Y., Sleem, A., 2013. Brain and liver oxidative stress after sertraline and haloperidol treatment in mice. J. Basic Clin. Physiol. Pharmacol. 24 (2), 115–123.

Borkowska, A., Pilaczyńska, E., Araszkiewicz, A., Rybakowski, J., 2002. The effect of sertraline on cognitive functions in patients with obsessive-compulsive disorder. Psychiatr. Pol. 36 (6 Suppl), 289–295.

Chen, S., Xuan, J., Couch, L., Iyer, A., Wu, Y., Li, Q.Z., Guo, L., 2014a. Sertraline induces endoplasmic reticulum stress in hepatic cells. Toxicology 1 (322), 78–88.

Chen, S., Xuan, J., Wan, L., Lin, H., Couch, L., Mei, N., Dobrovolsky, V., Guo, L., 2014b. Sertraline, an antidepressant, induces apoptosis in hepatic cells through the mitogen-activated protein kinase pathway. Toxicol. Sci. 137 (2), 404–415.

Collados, V., Hallal, H., Andrade, R., 2010. Sertraline hepatotoxicity: report of a case and review of the literature. Dig. Dis. Sci. 55 (6), 1806–1807.

Conners, D., Rogers, E., Armbrust, K., Kwon, J., Black, M., 2009. Growth and development of tadpoles (Xenopus laevis) exposed to selective serotonin reuptake inhibitors, fluoxetine and sertraline, throughout metamorphosis. Environ. Toxicol. Chem. 28 (12), 2671–2676.

Conrad, M., Cui, J., Lin, H., 2016. Sertraline-associated cholestasis and ductopenia consistent with vanishing bile duct syndrome. J. Pediatr. 169, 313–315 e1.

Davies, S., Klowe, M., 1998. Preclinical toxicological evaluation of sertraline hydrochloride. Drug Chem. Toxicol. 21 (4), 521–537.

Davies, S., Mulsant, B., Flint, A., Meyers, B., Rothschild, A., Whyte, E., Kirshner, M., Sorisio, D., Pollock, B., Bies, R., STOP-PD study group, 2016. SSRI-antipsychotic combination in psychotic depression: sertraline pharmacokinetics in the presence of olanzapine, a brief report from the STOP-PD study. Hum. Psychopharmacol. 31 (3), 252–255.

De Vane, C., Liston, H., Markowitz, J., 2002. Clinical pharmacokinetics of sertraline. Clin. Pharmacokinet. 41 (15), 1247–1266.

Del Monte, U., 2005. Swelling of hepatocytes injured by oxidative stress suggests pathological changes related to macromolecular crowding. Med. Hypothesis 64 (4), 818–825.

Elbekai, R., Korashy, H., El-Kadi, A., 2004. The effect of liver cirrhosis on the regulation and expression of drug metabolizing enzymes. Curr. Drug Metab. 5 (2), 157–167.

Eslami Shahrbabki, M., Eslami Shahrbabaki, A., 2014. Sertraline-related bleeding tendency: could it be dose-dependent? Iran J. Psychiatry Behav. Sci. 8 (3), 81–83.

Ewe, S., Abell, R., Vote, B., 2014. Bilateral maculopathy associated with sertraline. Australas. Psychiatry 22 (6), 573–575.

Farrell, G., 1999. Effects of disease on expression and regulation on CYPs. Mol. Aspects Med. 20 (2), 55–70.

Gerlyngl, P., Åbyholm, A., Grotmol, T., Erikstein, B., Huitfeldt, H., Stokke, T., Seglen, P., 2008. Binucleation and polyploidization patterns in developmental and regenerative rat liver growth. Cell Prolif. 26 (6), 557–565.

Grohol, J., 2014. Top 25 Psychiatric Medication Prescriptions for 2016. World of Psychology/CEO Psych Central. https://psychcentral.com/blog/top-25-psychiatric-medications-for-2016/.

Higuchi, S., Wu, R., Zhou, M., Ravikumar, T., Wang, P., 2007. Downregulation of hepatic cytochrome P-450 isoforms and PPAR-gamma: their role in hepatic injury and proinflammatory responses in a double-hit model of hemorrhage and sepsis. J. Surg. Res. 137 (1), 46–52.

Hoehn-Saric, R., Ninan, P., Black, D., Stahl, S., Greist, J., Lydiard, B., McElroy, S., Zajecka, J., Chapman, D., Clary, C., Harrison, W., 2000. Multicenter double-blind comparison of sertraline and desipramine for concurrent obsessive-compulsive and major depressive disorders. Arch. Gen. Psychiatry 57 (1), 76–82.

Jarrar, B., Taib, N., 2008. Histochemistry: Techniques and Horizon. King Saud University Press.

Johar, D., Roth, J., Bay, G., Walke, R.J., Kroczak, T., Los, M., 2004. Inflammatory response, reactive oxygen species, programmed (necrotic-like and apoptotic) cell death and cancer. Rocznik Akademii Medycznej w Bialymstoku 49, 31–39.

Kim, J., Nam, W., Kim, S., Kwon, O., Seung, E., Jo, J., Shresha, R., Lee, T., Jeon, T., Ki, S., Lee, H., Lee, S., 2017. Mechanism investigation of rifampicin-induced liver injury using comparative toxicoproteomics in mice. Int. J. Mol. Sci. 18 (7).

Klein, M., Thomas, M., Hofmann, U., Seehofer, D., Damm, G., Zanger, U., 2015. A systematic comparison of the impact of inflammatory signaling on absorption, distribution, metabolism, and excretion gene expression and activity in primary human hepatocytes and HepaRG cells. Drug. Metab. Dispos. 43 (2), 273–283.

Li, Y., Couch, L., Higuchi, M., Fang, J., Guo, L., 2012. Mitochondrial dysfunction induced by sertraline, an antidepressant agent. Toxicol. Sci. 127 (2), 582–591.

Livak, K., Schmittgen, T., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta C(T)) method. Methods 25, 402–408.

Melis, V., Usach, I., Gandía, P., Peris, J., 2015. Inhibition of efavirenz metabolism by sertraline and nortriptyline and their effect on efavirenz plasma concentrations. Antimicrob. Agents Chemother. 60 (2), 1022–1028.

Nair, A., Jacob, S., 2016. A simple practice guide for dose conversion between animals and human. J. Basic. Clin. Pharm. 7 (2), 27–31.

Neyrinck, A., 2004. Modulation of Kupffer cell activity: physio-pathological consequences on hepatic metabolism. Bull Mem Acad Roy de Méd Belgique 159 (5-6), 358–366.

Obach, R., Cox, L., Tremaine, L., 2005. Sertraline is metabolized by multiple cytochrome P450 enzymes, monoamine oxidases, and glucuronyl transferases in human: an in vitro study. Drug Metab. Dispos. 33 (2), 262–270.

Osorio, S., Escudero-Vilaplana, V., Reguilón-Gallego, L., Gómez-Centurión, I., Díez, J., Ferrer-Marín, F., 2017. Severe liver toxicity in a chronic myeloid leukemia patient probably induced by a drug interaction between imatinib and sertraline. J. Oncol. Pharm. Pract. 23, 1078155217735689.

Pandey, G., Srivastava, D., Madhuri, S., 2008. A standard hepatotoxic model produced by paracetamol in rat. Toxicol. Int. 15 (1), 69–70.

Park, S., Ishino, R., 2013. Liver injury associated with antidepressants. Curr. Drug. Saf. 8 (3), 207–223.

Park, J., Heah, T., Gouffon, J., Henry, T., Sayler, G., 2012. Global gene expression in larval zebrafish (Danio rerio) exposed to selective serotonin reuptake inhibitors (fluoxetine and sertraline) reveals unique expression profiles and potential biomarkers of exposure. Environ. Pollut. 167, 163–170.

Patel, N., Golwala, H., Stavrakis, S., Schechter, E., 2013. Sertraline-induced ventricular tachycardia. Am J. Ther. 20 (6), e720–2.

Schramm, E., van Calker, D., Dykierek, P., Lieb, K., Kech, S., Zobel, I., Leonhart, R., Berger, M., 2007. An intensive treatment program of interpersonal psychotherapy plus pharmacotherapy for depressed inpatients: acute and long-term results. Am J. Psychiatry 164 (5), 768–777.

Schrand, A., Rahman, M., Hussain, S., Schlager, J., David, A., Smith, D., Syed, A., 2010. Metal-based nanoparticles and their toxicity assessment. WIREs Nanomed. Nanobiotechnol. 2, 544–568.

Suen, C., Boyapati, R., Simpson, I., Dev, A., 2013. Acute liver injury secondary to sertraline. BMJ Case Rep pii: bcr2013201022.

Thornton, C., Maher, T., Hansell, D., Nicholson, A., Wells, A., 2009. Pulmonary fibrosis associated with psychotropic drug therapy: a case report. Med. Case Rep. 3, 126.

van Melle, J., Buikema, H., van den Berg, M., van Buiten, A., van Veldhuisen, D., Boonstra, P., van Gilst, W., 2004. Sertraline causes strong coronary vasodilation: possible relevance for cardioprotection by selective serotonin reuptake inhibitors. Cardiovasc. Drugs Ther. 18 (6), 441–447.

Verbeeck, R., 2008. Pharmacokinetics and dosage adjustment in patients with hepatic dysfunction. Eur. J. Clin. Pharmacol. 64 (12), 1147–1161.

Villeneuve, J., Pichette, V., 2004. Cytochrome P450 and liver diseases. Curr. Drug Metab. 5 (3), 273–282.

Zusman, I., Kozlenko, M., Zimber, A., 1991. Nuclear polymorphism and nuclear size in precarcinomatous and carcinomatous lesions in rat colon and liver. Cytometry 12 (4), 302–307.