RESEARCH ARTICLE

Effect of maternal desvenlafaxine administration on the liver of albino rat fetuses

Sarah H. Ahmed[1], Abd El Wahab El Ghareeb[2, *], Ali H. Abu Almaaty[1], Heba Ali Abd El-Rahman[2]

[1]Department of Zoology, Faculty of Science, Port Said University, Port Said, Egypt. [2]Department of Zoology, Faculty of Science, Cairo University, Giza 12613, Egypt

Received: June 16, 2022; **accepted:** July 8, 2022.

Desvenlafaxine is a serotonin norepinephrine reuptake inhibitor (SNRI) that is taken orally, and has several recorded clinical trials established its effectiveness for depression therapy and selected for being the antidepressant of choice for pregnant women who suffer from anxiety or depression. It has been investigated that desvenlafaxine caused a decline in fetal weight in pregnant rats and rabbits. Concerns about its developmental safety have been raised. There isn't a lot of information about desvenlafaxine's safety during pregnancy. The present study aims to investigate the possible histopathological, biochemical, and immunohistochemical effects on fetal livers of Wistar rats during the gestation period through maternal administration of desvenlafaxine. Thirty pregnant rats (*Rattus norvegicus*) weighing (180-200 g) were divided into three groups, ten rats in each group. The control group was administered an oral dose of distilled water and the treated groups were administered two oral doses of desvenlafaxine (5.14 mg/kg and 10.28 mg/kg) daily from the 5th day to the 19th day of gestation. At the end of the gestation period, the animals were sacrificed. Examination of the fetal livers from pregnant rats treated with desvenlafaxine showed several histopathological changes in the fetal liver. Oxidative injury was noticed in the fetal liver as the level of lipid peroxidation malondialdehyde (MDA) was remarkably elevated, while a severe reduction in glutathione (GSH), enzymatic activities of catalase (CAT), and superoxide dismutase (SOD) were found. Immunohistochemical analysis of fetal rat livers showed that groups treated with desvenlafaxine demonstrated a significant increase of BAX and caspase-3 protein immunoreactivity comparing to that in the control group. It has been found that gestational usage of desvenlafaxine may cause fetal risk to the liver. Desvenlafaxine should be used by pregnant women only if its benefits are more than the potential risks.

Keywords: antidepressant; pregnancy; histopathology; immunohistochemistry; oxidative stress.

[*]**Corresponding author:** Abd El Wahab El Ghareeb, Department of Zoology, Faculty of Science, Cairo University, Giza 12613, Egypt. Phone: 0020 100 566 6552. Email: drelghareeb@yahoo.com.

Introduction

Around 10% of pregnant women suffer from depression, while up to 20% of women experience depressive symptoms during their pregnancy [1]. The pregnancy period extends a woman's wellbeing considerations beyond herself to involve her unborn child. Just recently, there is a growing awareness in both mental health services and maternity of the concern of estimating and treatment of antenatal depression. This in part arises out of the realization that antenatal depression, anxiety, and other mood disorders are not only influencing the mother's health but also influencing the offspring [2].

Desvenlafaxine (Pristique™), a serotonin-norepinephrine reuptake inhibitor (SNRI), was manufactured as a sustained-release tablet, which had several recorded clinical trials established its effectiveness and safety for depression therapy. These trials resulted in approval from the US Food and Drug Administration (FDA) in February 2008. Desvenlafaxine was given an official agreement to use for the treatment of depression [3, 4]. The doses of desvenlafaxine approved to be used are 50 or 100 mg per day. There is some evidence that a high dose is more beneficial than a low dose [5]. The effectiveness and safety of desvenlafaxine in the treatment of depression has been determined in 3 randomized, controlled clinical studies [6-8]. Desvenlafaxine is metabolized extensively by the liver [9]. All SNRIs including desvenlafaxine have been linked to abnormalities in the liver and frequent hepatotoxicity [10]. When used in high doses, desvenlafaxine has been linked to hepatic enzyme elevation in some cases [11].

The liver is the primary site of drug metabolism, including that of antidepressants. As a result, it is critical to comprehend how a specific drug and its metabolites affect the structure and function of this organ [12]. Even at therapeutic doses, antidepressants used to treat various psychiatric disorders such as depression, schizophrenia, and anxiety can cause hepatotoxicity. Over 160 psychotropic drugs have been linked to hepatic side effects [13]. In a more recent study, psychotropic drugs were found to be responsible for 7.6% of liver disease cases in a cohort of 185 patients [14]. The fetal liver's health is critical because it is a key hematopoietic organ. It undergoes substantial functional and morphological alterations at birth with the volume and number of hepatocytes rising while the number of hematopoietic cells decreases. Extracellular matrix (ECM) components also provide structural strength and attachment sites for cell surface receptors, as well as roles in cellular proliferation, propagation, orientation, immune sensitivity, and tissue and organ phenotypic plasticity [15]. Several teratogens (xenobiotics) have been shown to impact the developmental processes of the fetus by increasing oxidative stress due to a lack of antioxidant defense during early stages of organogenesis, resulting in severe and long-term embryonic and fetal damage [16, 17]. Increased amounts of free radicals (free oxygen or nitrogen radicals) produced by the environment and/or maternal therapy may impact the developing organ by increasing lipid peroxidation, protein oxidation, DNA and RNA damage [17]. Oxidative stress is defined as a condition caused by an imbalance between the production of reactive oxygen species (e.g., superoxide anion, hydrogen peroxide, and hydroxyl radicals) and the activity of antioxidant defense systems such as superoxide dismutases (SOD), catalase, and glutathione [18]. Cells were found to be capable of dealing with low doses of oxidant stressors/reactive metabolites, but higher doses appear to overwhelm their barrier protection capacity and result in cell damage or death. Thereby, it has been demonstrated that oxidative stress can activate the mitochondrial apoptosis pathway by upregulating BAX and downregulating Bcl-2 [19]. In a previous study, desvenlafaxine was administered to adult female Swiss albino mice during gestation in the dose of 80 mg/kg body weight. Several changes were investigated to suggest the probable role of desvenlafaxine in causing neurodegenerative and oxidative damage to the cerebellum of Swiss albino mice [20].

Currently, there is no study published, investigated the histopathological, biochemical, and immunohistochemical effects of desvenlafaxine on the liver of albino rat fetuses. This study was designed firstly to investigate the possible hepatotoxic effects on rat fetuses through maternal exposure to desvenlafaxine in female Wistar rats during the gestation by investigating the histological possible toxic effects in the fetal livers, investigating the possible oxidative stress effects of desvenlafaxine on lipid peroxidation malondialdehyde (MDA) and glutathione (GSH) levels, catalase (CAT) and SOD activities in fetal

liver tissues, and studying the effect of desvenlafaxine on immunohistochemical analysis of BAX and caspase-3 protein expression in fetal rat livers. The results of this study will provide significant information about desvenlafaxine's safety during pregnancy.

Materials and methods

Animals and caring

Thirty (30) mature female Wistar rats (180–200 g) and fifteen (15) male Wistar rats (180–200 g) of 11-13 weeks were acquired from the Animal House at Mammalian Toxicology Department, Cairo, Egypt. All animals were acclimatized for one week in conventional animal house circumstances with a temperature-controlled environment (23°C, 12-hour light-dark cycle). Pelletized food and tap water were freely available. The treatment of the animals followed the standards for the use of laboratory animals. The experiment was conducted in accordance with the Organization for Economic Cooperation and Development's (OECD) standards 414 for testing chemicals and prenatal development. Overnight, the female rats were mated with a healthy untreated male rat. After successful mating, the male rats were removed, but the female rats were continuously exposed to the treatments.

Desvenlafaxine treatment

Desvenlafaxine was purchased from Pfizer (Pfizer Service company BVBA, Cairo, Egypt). The animals were separated into three groups with ten animals in each group. The control group received distilled water orally, while the low dose group received 5.14 mg/kg of body weight of desvenlafaxine supplied orally, and the high dose group received 10.28 mg/kg of body weight of desvenlafaxine provided orally. The doses used in this study (5.14 mg/kg and 10.28 mg/kg) were equivalent to human levels that had been adjusted to fit the weight of rats [21]. The duration of drug administration was 15 days from the 5th day to 19th day of gestation, once a day during gestation period. On the 20th day of gestation, the dams were completely anesthetized by ethyl ether inhalation, and the fetuses were removed individually from each horn of the uterus. The living fetuses were anaesthetized by ethyl ether inhalation thereafter, and fetal livers were removed and fixed for the following experiments. All experimental animals were then sacrificed by decapitation.

Histopathological examination

For light microscope examination, fetal livers of the control and experimental groups were fixed by immersion in 10% neutral formalin for 24 hours at room temperature. Specimens were then dehydrated in alcohol, cleared in xylol, and embedded in paraffin wax. Paraffin-embedded liver tissue for each fetus was cut into 3–5-µM sections and subjected to hematoxylin and eosin (H&E) stain for histological study [22] by using Leica RM2145 Microtome (Leica, Heidelberger, Germany).

Biochemical analysis

The fetal liver tissues were homogenized (1:10 w/v) in 50 mM potassium phosphate buffer, pH 7.5 containing 0.16 mg/mL heparin to eliminate any red blood cells and clots. The homogenate was then centrifuged at 10,000×g for 15 minutes at 4°C. The supernatant was collected for assay and then stored at −80°C. Colorimetric analysis was used to determine the protein concentration by using Hitachi U-1100 spectrophotometer (Hitachi, Tokyo, Japan) [23].

(1) Determination of lipid peroxidation malondialdehyde (MDA) level:

Malondialdehyde (MDA) is a lipid peroxidation end product that can react with thiobarbituric acid in acidic medium to form thiobarbituric acid reactive complex. As an index of oxidative stress, the formation of thiobarbituric acid reactive species (TBARS) was employed during an acid-heating reaction. Briefly, the samples were mixed with 1 mL of 10% trichloroacetic acid and 1 mL of 0.67% thiobarbituric acid followed by heating in a boiling water bath for 15 min. TBARS was determined by the absorbance at 535 nm using

1,1,3,3-tetramethoxypropane as an external standard [24].

(2) Determination of reduced glutathione (GSH) content:

GSH was determined by producing a yellow complex with a specific absorption at 412 nm in a reaction between 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and sulphydryl compounds. 0.5 mL of tissue homogenate was mixed carefully with 0.5 mL of Trichloroacetic acid (TCA) in a test tube. After 5 min of standing at room temperature, the reaction was centrifuged at 3,000 rpm for 15 min. 0.5 mL of supernatant was taken and pipetted into 1 mL of buffer and mixed with 0.1 mL of DTNB for 5-10 min. A reagent blank was also prepared by using 2 mL of water instead of tissue homogenate [25].

(3) Determination of catalase activity (CAT):

CAT was measured spectrophotometrically and was expressed in milligrams of protein per milligram of catalase activity. The homogenate was incubated in H_2O_2 substrate, and the enzymatic reaction stopped by adding 1 mL of ammonium molybdate. The intensity of the yellow complex formed by molybdate and H_2O_2 in the presence of cellular homogenates was measured as the exponential disappearance of H_2O_2 (10 mmol/L) at 240 nm [26].

(4) Determination of superoxide dismutase (SOD):

SOD activity was determined by using a modified competitive-inhibition assay that used the xanthine/xanthine oxidase–generated superoxide radical to reduce nitroblue tetrazolium (NBT) at a constant rate (absorbance, 0.02/min), that was monitored at 560 nm, spectrophotometrically. The homogenate samples were diluted to give an inhibition percent between 30 and 60. The working reagent was prepared right before the experiment by mixing 10 mL of phosphate buffer (pH 8.5), 1 mL of NBT, and 1 mL of NADH. The standard incubation system contained 1 mL of working reagent and 0.1 mL of H_2O. The reaction was initiated by adding 0.1 mL of phenazine methosulphate [27].

Immunohistochemistry

The immunohistochemical investigations for BAX and caspase-3 proteins were performed by the technique of Sati, *et al*. [28]. Rabbit BAX and caspase-3 antibodies were used as primary antibodies on paraffin embedded liver tissues cut into 4 μM thick sections. A biotinylated secondary antibody was used, followed by streptavidin-conjugated horse-radish peroxidase. Because streptavidin has a high affinity for biotin, it binds to the spot where the primary antibody covered the background, resulting in a brown color when a chromatogen is added. All slides are lightly counterstained with hematoxylin for 30s prior to dehydration and mounting. For observation and image capturing, an XSZ-N107 Biological microscope with a DCM130 USB2.0 digital camera (Everich Medicare, Nanjing, Jiangsu, China) were utilized. Positive cells had a cytoplasmic response to the caspase-3 and BAX antibodies. The modified Allred scoring system was used to undertake semi-quantitative analysis of positive-stained tissue slices [29]. The average number of positive cells was calculated after counting them in three different high-power fields (hpf) (400x). To get the final grades, the number grade of positive cells (0–5) and the staining intensity of the cytoplasm (0–3) were added together. The number grades of positive cells were set as follows: 1-less than 50 positive cells; 2-between 50 and 100 positive cells; 3-between 100 and 150 positive cells; 4-between 150 and 200 positive cells; and 5-over 200 positive cells. The staining intensity of positivity in the cytoplasm was graded as follows: 1-weak, 2-moderate, and 3-strong. A light microscope with a digital camera and ImagePro Plus 5.1 imaging software (Media Cybernetics, Rockville, Maryland, USA) was used to assess the combined intensity of immunostaining protein expressions in the liver.

Statistical analysis

IBM SPSS (version 25) (Armonk, New York, USA)

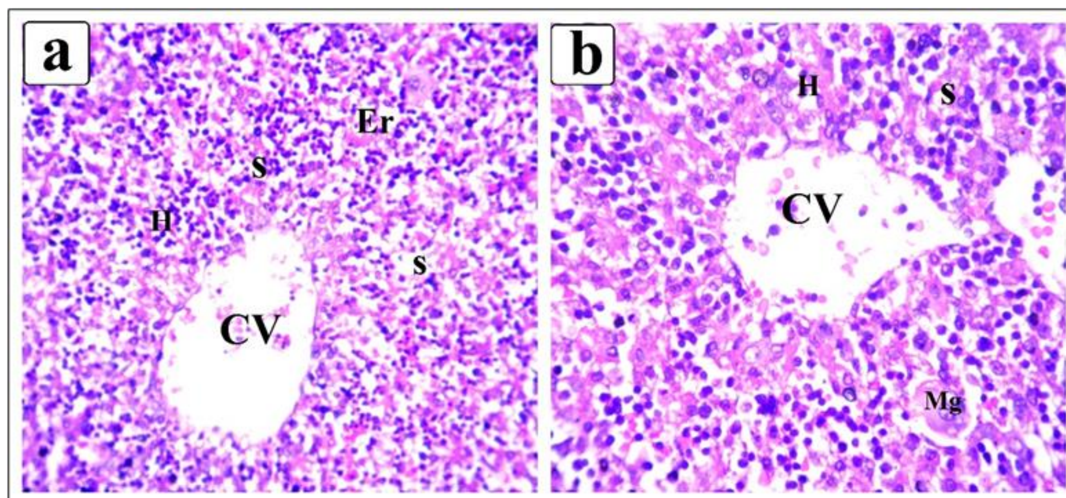

Figure 1. Photomicrographs of transverse H&E-stained sections of fetal liver of control group (400X). CV: central vein. H: cords of polygonal hepatocytes. S: blood sinusoids. Mg: megakaryocytes.

was used for statistical analysis. All data was presented in the form of mean (μ) ± standard errors of the means (S.E.M.). To compare more than two groups, a one-way analysis of variance (ANOVA) was employed, followed by Tukey's multiple comparison post hoc analysis [30]. The significance was determined at probability $p < 0.05$.

Results

Control group

The livers of control fetuses were histologically normal. It was made up of polygonal normal hepatocytes with coarsely granulated cytoplasm. Hepatic cells alternated with blood sinusoids, which converged on the central vein. Blood was found in the hepatic cords and sinusoids. Lymphocytes and erythroblasts were examples of different types of blood-forming cells. Rare megakaryocytes permeated the liver, and the sinusoid capillary architecture was preserved (Figure 1).

Low dose (5.14 mg/kg) group

Fetuses maternally administrated low dose of desvenlafaxine showed some histopathological alterations including a congested central vein surrounded by degenerative hepatocytes and inflammatory cells (Figure 2a-c). The portal vein became elongated and congested (Figure 2d). The normal architecture pattern of the classical lobule was disrupted. Hepatocytes showed signs of degeneration. Blood sinusoids were dilatated. In addition, megakaryocytes were found (Figure 2e). Hepatocytes were also seen to be enlarged, and the cytoplasm of the cells was light, foamy, and filled with vacuoles. Also, hemorrhagic areas were evident among the hepatocytes (Figure 2f).

High dose (10.28 mg/kg) group

Fetuses maternally administrated high dose of desvenlafaxine revealed a degree of histopathological changes including congested and dilated central veins which were surrounded by degenerative hepatocytes and inflammatory cells. Many erythroblasts were evident among the degenerated hepatocytes. There was a disruption of the normal architecture pattern of classical lobule along with severe blood sinusoids dilatation in many areas. Degeneration and vacuolation were evident in the hepatocytes (Figure 3a-d). The portal vein became elongated and surrounded by many inflammatory cells. The liver exhibited marked congestion of the portal veins and blood sinusoids, through which blood had escaped, producing hemorrhagic foci (Figure 3e, 3f).

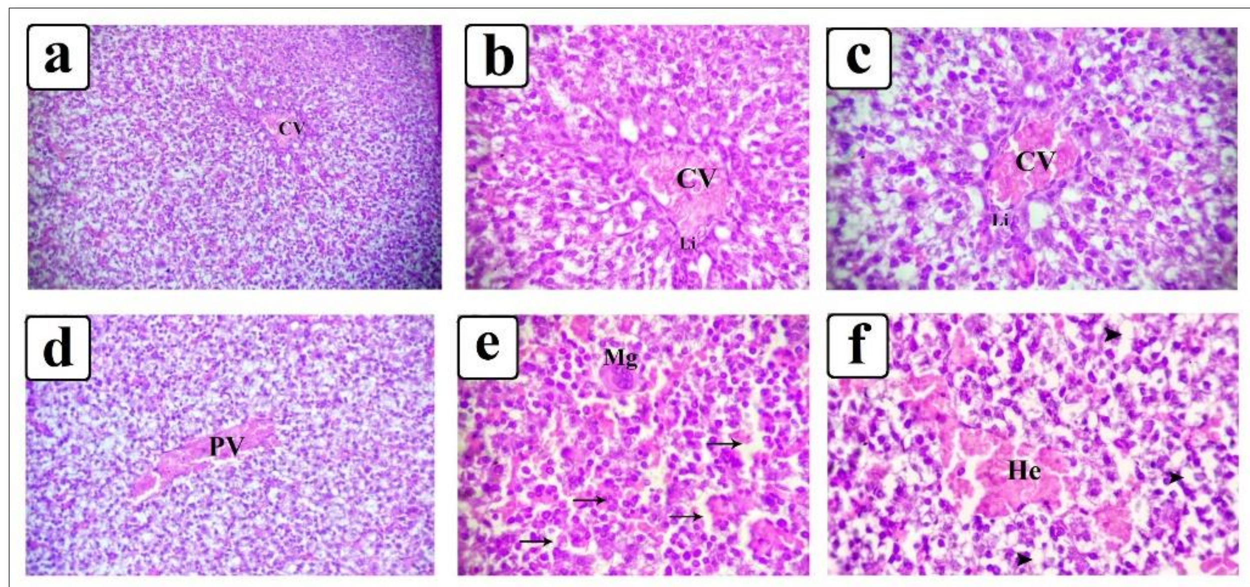

Figure 2. Photomicrographs of transverse H&E-stained sections of fetal liver of pregnant rats treated with a low dose of desvenlafaxine (5.14 mg/kg). **a-c**: congested central vein (CV) and leucocytic infiltration (Li). **d**: congested and dilated portal vein (PV). **e**: sinusoids dilatations (arrow) and megakaryocyte (Mg). **f**: hemorrhage (He) and hepatocytes with a vacuolated cytoplasm (➤). (a, d = 100X; b, c, e, f = 400X)

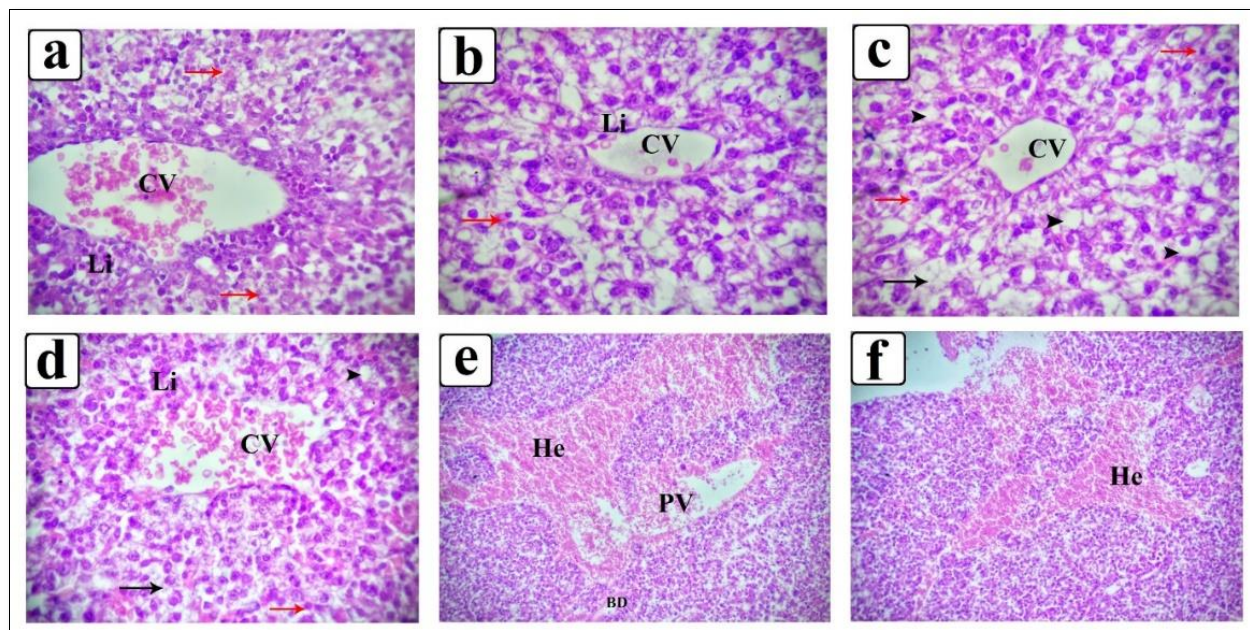

Figure 3. Photomicrographs of transverse H&E-stained sections of fetal liver of pregnant rats treated with a high dose of desvenlafaxine (10.28 mg/kg). **a-d**: congested and dilated central vein (CV), leucocytic infiltration (Li), erythroblasts (red arrow), sinusoids dilatation (black arrow), and vacuolations (➤). **e-f**: congested and dilated portal vein (PV), bile duct (BD), and hemorrhage (He). (a-d = 400X. e, f = 100X).

Oxidative stress

The levels of MDA that were noticeably increased in both low dose and high dose desvenlafaxine treated groups were detected as 7.08±0.11 and 11.06±0.11, respectively, comparing to control group detected 0.77± 0.16 ($p < 0.05$). On the other hand, GSH was remarkably dropped in the two treated groups with low dose and high dose

Table 1. Effects of desvenlafaxine on MDA and GSH levels, CAT and SOD activities in fetal liver tissues.

Group	MDA (nmol/mL)	GSH (mmol/L)	CAT (U/L)	SOD (U/mL)
Control	0.77± 0.16	107.16±1.5	5.82±0.19	120.13±1.08
Low dose (5.14 mg/Kg)	7.08±0.11[a b]	63.3±7.5[a b]	2.2±0.23[a b]	71.96±9.58[a b]
High dose (10.28mg/Kg)	11.06±0.11[a b]	20.8±4.7[a b]	0.64±0.19[a b]	28.71±4.52[a b]
F-value	1438.6	68.38	55.25	159.7
P-value	C-L=0.00 C-H=0.00 L-H=0.00	C-L=0.002 C-H=0.00 L-H=0.003	C-L=0.004 C-H=0.00 L-H=0.006	C-L=0.00 C-H=0.00 L-H=0.004

Note: Each value represented as means ± standard error of the mean. [a]Significant difference ($p < 0.05$) as comparing to control. [b]Significant difference between low and high treated groups. C: control group, L: low dose group, H: high dose group.

Table 2. The effect of desvenlafaxine on the immunohistochemical expression of BAX and caspase-3.

Groups	BAX	Score	Caspase-3	Score
Control	41.67±7.35	1+1=2	47±4.9	1+1=2
Low dose (5.14 mg/kg)	163±25.9[a]	4+2=6	191±5.56[a]	5+2=7
High dose (10.28 mg/kg)	213±13.3[a]	5+2=7	216±9.45[a]	5+3=8
F-Value	25.78		172.5	
P-Value	C-L = 0.006 C-H = 0.001 L -H = 0.18		C-L = 0.00 C-H = 0.00 L -H = 0.096	

Note: Each value represented as means ± standard error of the mean. [a]Significant difference ($p < 0.05$) as comparing to control. C: control group, L: low dose group, H: high dose group.

of desvenlafaxine to 63.3±7.5 and 20.8±4.7, respectively, comparing to that in the normal fetal liver tissues of control group detected 107.16±1.5 ($p < 0.05$). The fetal liver CAT level was significantly decreased in both low dose and high dose groups as 2.2±0.23 and 0.64±0.19, respectively, comparing to that in control group detected 5.82±0.19 ($p < 0.05$). In addition, the fetal hepatocyte level of SOD was significantly decreased in both low and high dose desvenlafaxine treated groups to 71.96±9.58 and 28.71±4.52, respectively, comparing to that in control group detected 120.13±1.08 ($p < 0.05$) (Table 1).

BAX protein expression

The immunohistochemical study showed that BAX was expressed in normal fetal liver tissues of the control group. The control group only had a few cells expressing BAX (Figure 4a). The average number of positive cells in fetal liver tissue was 41.67±7.35. The staining intensity of fetal liver tissues was 2. On the other hand, in the low dose group (5.14 mg/kg), some cells of the liver exhibited BAX moderate expression as comparing to that in control group (Figure 4b). The average number of positive cells in fetal liver tissue was 163±25.9 ($p < 0.05$). The staining intensity of fetal liver tissues was 6. In the high dose group (10.28 mg/kg), a strong BAX expression was detected in most cells of the fetal liver tissues. Protein expression was significantly increased comparing to that in control group ($p < 0.05$). The staining of BAX positive cells was observed in the cytoplasm and nucleus (Figure 4c). The average number of positive cells in the fetal liver tissue was 213±13.3. The staining intensity of fetal liver tissues was 7 (Table 2).

Caspase-3 protein expression

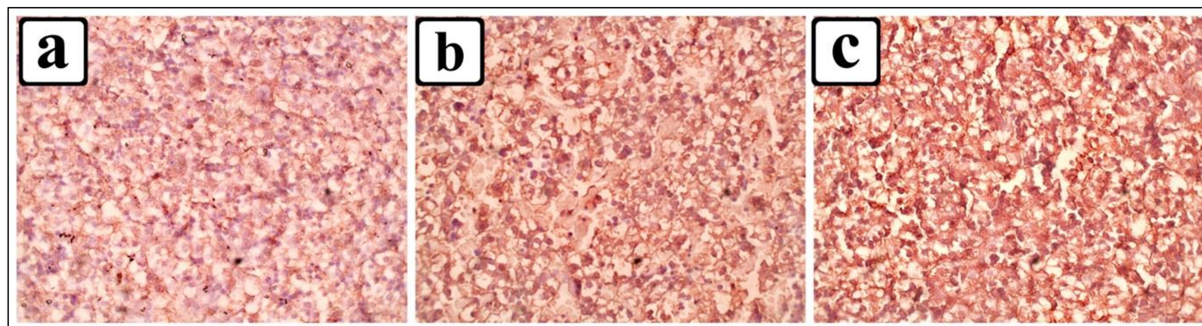

Figure 4. Photomicrographs of immunohistochemical reactions of BAX protein that was observed in the cytoplasm and nucleus of fetal liver tissue (400X). **a**: control group. **b**: low dose (5.14 mg/kg) group. **c**: high dose (10.28 mg/kg) group.

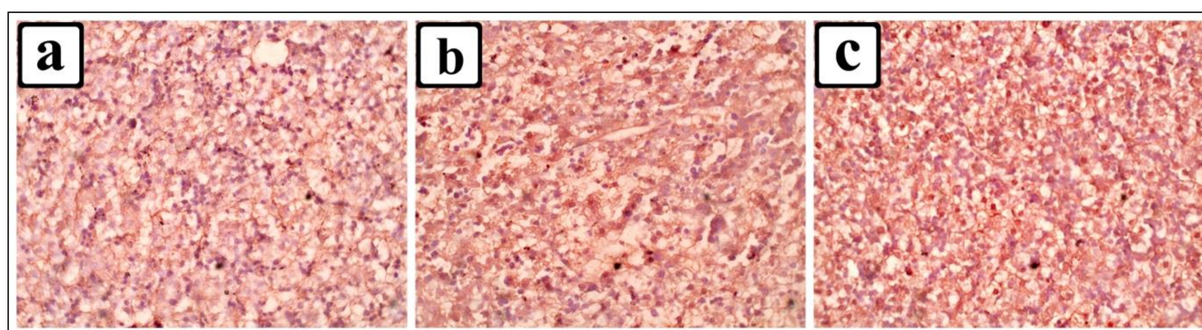

Figure 5. Photomicrographs of immunohistochemical reactions of caspase-3 protein that was observed in the cytoplasm and nucleus fetal of liver tissue (400X). **a**: control group. **b**: low dose (5.14 mg/kg) group. **c**: high dose (10.28 mg/kg) group.

The immunohistochemical results of the fetal liver tissues of the intoxicated group revealed a weak reaction for active caspase-3 (Figure 5a). The average number of positive cells in fetal liver tissue was 47±4.9. The staining intensity of fetal liver tissues was 2. However, in the low dose (5.14 mg/kg) group, some cells of the liver exhibited BAX mild reaction as comparing to control group (Figure 5b). The average number of positive cells in maternal liver tissue was 191±5.56 ($p < 0.05$). The staining intensity of fetal liver tissues was 7. In the high dose (10.28 mg/kg) group, a strong BAX reaction was detected in most hepatocytes (Figure 5c). The average number of positive cells in fetal liver tissue was 216±9.45, as it significantly increased comparing to that in the control group ($p < 0.05$). The staining intensity of fetal liver tissues was 8 (Table 2).

Discussion

Psychotropic medication prescriptions for the treatment of mental problems are becoming more widespread. Despite concerns regarding long-term safety and effects on the fetus, SSRIs and SNRIs are becoming increasingly widely used [31]. Desvenlafaxine was selected for being the antidepressant of choice for pregnant women who suffer from anxiety or depression [32]. The establishment of this study was supported by the scarcity of evidence available in the literature on the embryotoxic and hepatotoxic consequences of desvenlafaxine antidepressant during pregnancy.

The liver is one of the most important organs in the body, as it regulates various physiological processes in the body. It is involved in a variety of vital functions including metabolism, secretion, and storage. It is also an excretory organ,

necessary for the removal of wastes and toxic products from the blood [33]. It has a high capacity for detoxication of toxic substances, exogenous xenobiotics, and medications [34]. Hepatocytes which constitute the majority of the liver structure are highly active in the metabolism of exogenous chemicals, which is one of the primary reasons why the liver is a target for toxic substances [35]. The liver is required for survival. Currently, there is no way to compensate for the apparent lack of liver function over time, though liver dialysis can be used in the short term. The liver is stressed when completing multiple detoxifications, which leads to liver illnesses and may result in liver damage, major health problems, and death [36].

Histopathology is used extensively in pharmacology and toxicology. The examination of abnormal tissue may aid in determining the cause and agents of pathological abnormalities. Treatment with a specific toxicant, such as chemicals or drugs, can affect not only the microscopic structure but also the external morphology [37]. According to the present study, desvenlafaxine is found to cause a histopathological effect on the liver of albino rat fetuses. In this work, histological investigations of tissues demonstrated several changes in the maternal and fetal liver in all treated groups with low and high doses. The detected changes include dilatation in blood sinusoids, congestion in central and portal veins, activated Kupffer cells, a proliferation of bile ductules, hemorrhage, and alteration of hepatic architecture that consider evidence of cell irritability, inflammation, and hypersensitivity to the drug used. The accumulation of inflammatory cells around some central and portal veins may be due to the elimination of degenerated and necrotic hepatocytes and the protection of other hepatocytes. According to some authors, inflammation is a protective response designed to eliminate the initial cause of cell injury as well as the necrotic cells and tissues that result from the initial insult. It performs its protective function by diluting, destroying, or otherwise neutralizing harmful agents, allowing tissue repair to occur [38, 39].

A literature search revealed nothing significant about desvenlafaxine administration during the gestation period and histopathological changes in the liver in both animal and clinical trials that might be used to make a reasonable comparison. Though the previous work stated that SNRIs drugs, specifically venlafaxine and duloxetine revealed that they are strongly linked to hepatotoxic side effects. All these drugs cause a unique, unpredictable, and reversible hepatic injury. The injury can occur as soon as a few days or as late as 6 months after the drug is administered, and it usually ends when the drug is stopped [40]. This was in line with several other studies that have been made on the effect of fluoxetine, which caused toxic effects in rats, including hydropic degenerative changes, karyomegaly, steatosis, lobular inflammation, focal necrosis, apoptosis, disruption, twinning cell plates, cholestasis, portal area inflammation, few dead hepatocytes, congested blood vessels, and inflammatory cells. Hepatocellular hydropic vacuolar dysfunction was the most common histopathological change in all exposed rats [41]. In the contrast, another research investigated that the liver sections of fluoxetine-treated rats revealed that the majority of hepatocytes appeared normal, with no evident architecture change except for a few deteriorated hepatocytes, a little inflammatory infiltration of leukocytes, and congested blood vessels [42]. A previous investigation studied the effect of antidepressant drug, trazodone HCl on the rat neonates during the lactation period, showed some hepatic alterations including, blood sinusoids dilatation, pyknotic nuclei, extensive cytoplasmic vacuolization, necrosis and alteration of hepatic architecture, and mononuclear cells invasion [43]. Another former study on patients receiving combinations of venlafaxine and trazodone at normal therapeutic doses experienced fulminant hepatic failure [44]. The limited data on liver inflammatory changes caused by antidepressants are mostly related to SSRI drugs. However, liver inflammation,

including inflammatory cell infiltration and dilated sinusoids, was observed in rats treated with the SNRI venlafaxine as well [45].

Reactive oxygen species (ROS) play an essential role in the onset of apoptotic processes and the transition of mitochondrial permeability [46]. The young organ appears to have a considerably more critical role for ROS-regulated release of proapoptotic substances from mitochondria [47]. It's not surprising, then, that any environmental agents, chemicals, or drugs that produce more free radicals could harm the developing fetus by increasing protein oxidation, DNA fragmentation, chromatin condensation, and apoptotic cell death, which could lead to growth retardation, congenital anomalies, and, in the worst-case scenario, embryonic death [17, 18, 48, 49]. When an organ is exposed to high levels of oxidative stress for an extended period, low molecular weight antioxidants are depleted and enzyme activity is reduced, resulting in oxidative stress injuries that may cause increased apoptosis in the fetus and long-term consequences such as postnatal functional deficits [18, 49, 50]. In the present study, oxidative injury is scored in the fetal liver after maternal administration with desvenlafaxine as the level of MDA was remarkably elevated, while a severe reduction in GSH, enzymatic activities of SOD and CAT content was noticed. These findings suggested that oxidant-antioxidant system abnormalities may be an important mechanism by which desvenlafaxine promotes cellular damage, and histopathology findings corroborated these findings. A review of the literature found no evidence that desvenlafaxine treatment during pregnancy causes oxidative stress in the liver in both animal and human studies that could be utilized to draw a valid comparison. Nevertheless, there are a lot of evidence that certain teratogens, such as antidepressant medications, have been shown to impact embryo-fetal development by raising free radical load and oxidative stress by creating excessive reactive oxygen species (ROS) due to bioactivation [17]. Antidepressants and their metabolites can cause direct liver damage by accumulating ROS and causing mitochondrial dysfunction. They can also cause inflammation and cholesterol accumulation, impairing the structure and function of the liver [51]. The principal sources of ROS are oxygen, hydroxyl, and hydroperoxyl radicals, which interact with biological molecules and contribute to the breakdown of cellular membranes. Lipid peroxyl and hydroperoxide radicals are the major products of lipid peroxidation, while MDA is a subsequent result [52]. GSH is the primary component of non-enzymatic antioxidants and serves as a substrate and co-substrate for numerous antioxidant enzymes [53]. According to a prior study, the SNRI antidepressant medicine venlafaxine caused hepatotoxicity in isolated rat hepatocytes by inducing oxidative stress and consequent toxic consequences such as GSH depletion, lipid peroxidation, mitochondrial potential collapse, and lysosomal membrane leakiness [54]. Another SNRI, duloxetine, induced hepatotoxicity by inducing oxidative stress through hydroxylated and epoxide metabolites, which can release free radicals or ROS [55]. In agreement with the results of the present study, a previous study stated that both fluoxetine and sertraline increased MDA in the liver, although the lower dose of imipramine decreased it. By contrast, both fluoxetine and sertraline, as well as imipramine, increased glutathione levels in the liver [56]. Another study found that clozapine increased GST activity, decreased GSH level, and caused oxidative damage of lipids and proteins in the rat liver [57]. In the livers of fluoxetine-treated rats, increased total oxidant status was seen alongside a reduction in total antioxidant capacity [58]. Elgebaly, *et al*. agreed with the present study that it found a decrease in GSH levels as well as SOD and CAT activity, while the level of MDA increased in rat liver due to fluoxetine administration [59]. Another study found that fluoxetine downregulated the activity of superoxide dismutases and upregulated the activity of glutathione peroxidase in the liver of Wistar rats [60]. In rat and mouse livers, TCAs imipramine [61] and amitriptyline [62] were found to boost ROS generation and lipid

oxidation. Citalopram has also been linked to an increase in ROS generation and a reduction in antioxidant defenses in the rat liver inducing hepatotoxicity. In addition, risperidone-induced hepatotoxicity in rats was found, which was linked to oxidative stress, mitochondrial dysfunction, and lysosome damage [63]. Another study found that olanzapine raises hepatic GST activity, which is most likely due to the elimination of reactive olanzapine metabolites [64]. In isolated hepatic mitochondria, sertraline inhibited mitochondrial respiratory complexes by uncoupling oxidative phosphorylation [65]. Sertraline therapy for 10 days increased lipid peroxidation and nitric oxide (NO) generation in the liver of mice [66]. DNA damage has recently been discovered to have a role in sertraline-induced liver cytotoxicity. The parental form of sertraline was found to be the predominant cause of liver damage in the same investigation [67]. Mitochondria, the cell's powerhouse, contain proapoptotic molecules that play a role in programmed cell death (apoptosis). In general, two major mechanisms are implicated in apoptosis: the mitochondria-mediated intrinsic mechanism and the death receptor–mediated extrinsic mechanism [68–70]. The intrinsic mechanism is distinguished by the loss of mitochondrial membrane potential, mitochondrial dysfunction, and subsequent cytochrome c discharge [71].

BAX is a proapoptotic member protein that is implicated in the permeabilization of the mitochondrial membrane and cytochrome c release [72]. Antiapoptotic proteins are suppressed by proapoptotic proteins, and the apoptogenic factor cytochrome c is discharged from the mitochondria into the cytosol [73]. After that, cytochrome c triggers caspase-9, which stimulates caspase-3, the apoptosis "executioner," leading to cell death [74, 75]. ROS may cause cell death via interacting directly with the mitochondrion and/or members of the Bcl-2 family of proteins, such as BAX [76]. The expression of BAX and caspase-3 has been shown to be a predictor of increased apoptosis [77]. In the present work, an immunohistochemical study demonstrated that both BAX and caspase-3 proteins immunoreaction levels were significantly increased in the fetal liver cells gradually from normal tissues to treated ones with two different doses of desvenlafaxine, the highest expression was noticed in the fetal hepatocytes of rats given the highest dose of the drug. A search of the literature yielded nothing significant about in-utero Desvenlafaxine exposure and apoptotic gene alterations in the liver of both animal and clinical studies for valid comparison. Even though, antidepressant effects on hepatic mitochondrial homeostasis have largely been studied in the instance of SSRI fluoxetine. A previous study found that fluoxetine administration can induce oxidative stress, inflammation, apoptosis, and hepatotoxicity in rats. Pro-apoptotic BAXt and caspase-3 were upregulated whereas anti-apoptotic Bcl-2 was downregulated in hepatocytes [59]. Furthermore, Djordjevic, *et al*. demonstrated increased DNA fragmentation, a common event in apoptotic cells, in the liver of fluoxetine-induced rats [60]. Another previous study has been made on rat primary hepatocytes, HepG2, and Hepa1c1c7, treatment with 50 M sertraline elevated caspase-3 activity [71]. Several *in vitro* investigations have found that several antidepressants cause apoptosis [78–80]. It is not surprising that antidepressants' apoptotic activity eventually induced cell death in various types of cells due to their nonspecific mode of action [71]. The authors hypothesized that oxidative stress caused an imbalance between BAX and Bcl-2. ROS has been shown to suppress Bcl-2 and initiate pro-apoptotic mitochondrial signaling [81]. A similar finding was reported in which ROS downregulated Bcl-2 expression but elevated box expression, demonstrating ROS control over Bcl-2 family protein expression [82]. Recently, it was discovered that oxidative stress increased the expression of box and decreased the expression of Bcl-2 proteins in the liver of rats [83].

Conclusion

The results of this study confirmed that desvenlafaxine was embryotoxic to the fetuses of pregnant rats when treated with both low and high doses during the gestation period. The fetuses presented several histopathological alterations, oxidative injury, and immunohistochemical effects in the fetal livers. So, great caution should be taken when prescribing desvenlafaxine during pregnancy. Before recommending SNRIs in general, and desvenlafaxine, more extensive research is needed to extrapolate animal data to clinical settings and weigh the risks and benefits to both the fetus and the pregnant mother.

References

1. Burt V, Stein K. 2002. Epidemiology of depression throughout the female life cycle. J Clin Psychiatry. 7(7):9-15.
2. Yonkers KA, Wisner KL, Stewart DE, Oberlander TF, Dell DL, Stotland N, *et al*. 2009. The management of depression during pregnancy: a report from the American Psychiatric Association and the American College of Obstetricians and Gynecologists. Gen Hosp Psychiatry. 31(5):403–413.
3. DeMartinis NA, Yeung PP, Entsuah R, Manley AL. 2007. A Double-Blind, Placebo-Controlled Study of the Efficacy and Safety of Desvenlafaxine Succinate in the Treatment of Major Depressive Disorder. J Clin Psychiatry. 68(05):677–688.
4. Lieberman DZ, Montgomery SA, Tourian KA, Brisard C, Rosas G, Padmanabhan K, *et al*. 2008. A pooled analysis of two placebo-controlled trials of desvenlafaxine in major depressive disorder. Int Clin Psychopharmacol. 23(4):188–197.
5. Liebowitz MR, Manley AL, Padmanabhan SK, Ganguly R, Tummala R, Tourian KA. 2008. Efficacy, safety, and tolerability of desvenlafaxine 50 mg/day and 100 mg/day in outpatients with major depressive disorder. Curr Med Res Opin. 24(7):1877–1890.
6. Boyer P, Montgomery S, Lepola U, Germain J-M, Brisard C, Ganguly R, *et al*. 2008. Efficacy, safety, and tolerability of fixed-dose desvenlafaxine 50 and 100 mg/day for major depressive disorder in a placebo-controlled trial. Int Clin Psychopharmacol. 23(5):243–253.
7. DeMaio W, Kane C, Nichols A, Jordan R. 2011. Metabolism studies of desvenlafaxine. J Bioequivalence Bioavailab. 3(07):151-160
8. Tourian KA, Padmanabhan SK, Groark J, Brisard C, Farrington D. 2009. Desvenlafaxine 50 and 100 mg/d in the treatment of major depressive disorder: an 8-week, phase III, multicenter, randomized, double-blind, placebo-controlled, parallel-group trial and a post hoc pooled analysis of three studies. Clin Ther. 31(1):1405–1423.
9. Baird-Bellaire S, Behrle JA, Parker VD, Patat A, Paul J, Nichols AI. 2013. An open-label, single-dose, parallel-group study of the effects of chronic hepatic impairment on the safety and pharmacokinetics of desvenlafaxine. Clin Ther. 35(6):782–794.
10. Voican CS, Corruble E, Naveau S, Perlemuter G. 2014. Antidepressant-induced liver injury: a review for clinicians. Am J Psychiatry. 171(4):404–415.
11. Sedky K, Nazir R, Joshi A, Kaur G, Lippmann S. 2012. Which psychotropic medications induce hepatotoxicity? Gen Hosp Psychiatry. 34(1):53–61.
12. Aithal GP, Watkins PB, Andrade RJ, Larrey D, Molokhia M, Takikawa H. *et al*. 2011. Case definition and phenotype standardization in drug-induced liver injury. Clin Pharmacol Ther. 89(6):806–815.
13. Dumortier G, Cabaret W, Stamatiadis L, Saba G, Benadhira R, Rocamora J-F, *et al*. 2002. [Hepatic tolerance of atypical antipsychotic drugs]. L'Encephale. 28(61):542–551.
14. Licata A, Minissale MG, Calvaruso V, Craxì A. 2017. A focus on epidemiology of drug-induced liver injury: analysis of a prospective cohort. Eur Rev Med Pharmacol Sci. 21(1):112–121.
15. Badylak SF. 2002. The extracellular matrix as a scaffold for tissue reconstruction. Semin Cell Dev Biol. 13(5):377–383.
16. Ikonomidou C, Kaindl AM. 2011. Neuronal death and oxidative stress in the developing brain. Antioxid Redox Signal. 14(8):1535–1550.
17. Ornoy A. 2007. Embryonic oxidative stress as a mechanism of teratogenesis with special emphasis on diabetic embryopathy. Reprod Toxicol Elmsford N. 24(1):31–41.
18. Kohen R, Nyska A. 2002. Invited review: oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol. 30(6):620–650.
19. Herrera B, Fernández M, Alvarez A, Roncero C, Benito M, Gil J, *et al*. 2001. Activation of caspases occurs downstream from radical oxygen species production, Bcl-xL down-regulation, and early cytochrome C release in apoptosis induced by transforming growth factor β in rat fetal hepatocytes. Hepatol Baltim Md. 34(3):548–556.
20. Kumari A, Singh M, Mishra A. 2017. Histopathological changes induced by desvenlafaxine in cerebellum of Swiss albino mice. J Anat Sci. 25(1):5-7.
21. Reagan-Shaw S, Nihal M, Ahmad N. 2008. Dose translation from animal to human studies revisited. FASEB J. 22(3):659–661.
22. Buesa RJ, Peshkov MV. 2009. Histology without xylene. Ann Diagn Pathol. 13(4):246–256.
23. Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72(1):248–254.
24. Yoshioka T, Kawada K, Shimada T, Mori M. 1979. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. Am J Obstet Gynecol. 135(3):372–376.
25. Muller R. 1995. Use of 5,5'-Dithiobis (2-Nitrobenzoic Acid) as a Measure of Oxidation During Mashing. J Am Soc Brew Chem. 53(2):53–58.
26. Beers RF, Sizer IW. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem. 195(1):133–140.

27. Spitz DR, Oberley LW. 1989. An assay for superoxide dismutase activity in mammalian tissue homogenates. Anal Biochem. 179(1):8–18.
28. Sati L, Seval-Celik Y, Demir R. 2009. Lung surfactant proteins in the early human placenta. Histochem Cell Biol. 133(1):85–93.
29. Ilić IR, Stojanović NM, Radulović NS, Živković VV, Randjelović PJ, Petrović AS, *et al*. 2019. The Quantitative ER immunohistochemical analysis in breast cancer: detecting the 3 + 0, 4 + 0, and 5 + 0 allred score cases. Medicina (Mex). 55(8):461–472.
30. Ludbrook J. 1991. On making multiple comparisons in clinical and experimental pharmacology and physiology. Clin Exp Pharmacol Physiol. 18(6):379–392.
31. Andrade SE, Reichman ME, Mott K, Pitts M, Kieswetter C, Dinatale M, *et al*. 2016. Use of selective serotonin reuptake inhibitors (SSRIs) in women delivering liveborn infants and other women of child-bearing age within the U.S. Food and Drug Administration's Mini-Sentinel program. Arch Womens Ment Health. 19(6):969–977.
32. Deecher DC, Beyer CE, Johnston G, Bray J, Shah S, Abou-Gharbia M, *et al*. 2006. Desvenlafaxine succinate: a new serotonin and norepinephrine reuptake inhibitor. J Pharmacol Exp Ther. 318(2):657–665.
33. Bertrand N, Leroux J-C. 2012. The journey of a drug-carrier in the body: an anatomo-physiological perspective. J Controlled Release. 161(2):152–163.
34. Shanmugasundaram P, Venkataraman S. 2006. Hepatoprotective and antioxidant effects of *Hygrophila auriculata* (*K. Schum*) *Heine Acanthaceae* root extract. J Ethnopharmacol. 104(1):124–128.
35. Guillouzo A. 1998. Liver cell models in *in vitro* toxicology. Environ Health Perspect. 106(2):511–532.
36. Krishnappa P, Venkatarangaiah K, Venkatesh, Shivamogga Rajanna SK, Kashi Prakash Gupta R. 2014. Antioxidant and prophylactic effects of *Delonix Elata* l., stem bark extracts, and flavonoid isolated quercetin against carbon tetrachloride-induced hepatotoxicity in rats. BioMed Res Int. 2014:1–14.
37. Parrish AR, Gandolfi AJ, Brendel K. 1995. Precision-cut tissue slices: applications in pharmacology and toxicology. Life Sci. 57(21):1887–1901.
38. Salem M, Altayeb Z. 2017. Light and electron microscopic study on the possible protective effect of *Nigella Sativa* oil on cisplatin hepatotoxicity in Albino rats. Egypt J Histol. 40(1):68–79.
39. Elzoghby R, Ahlam F, Hamuoda A-F, Farouk M. 2014. Protective role of vitamin C and green tea extract on malathion-induced hepatotoxicity and nephrotoxicity in rats. Am J Pharmacol Toxicol. 9(3):174–185.
40. Todorović Vukotić N, Đorđević J, Pejić S, Đorđević N, Pajović SB. 2021. Antidepressants- and antipsychotics-induced hepatotoxicity. Arch Toxicol. 95(3):767–789.
41. Özden H, Bildirici K, Üstüner D, Üstüner C, Cengiz BP, Tülay A, *et al*. 2005. Histopathologic examination of rat liver after experimental application of fluoxetine. Türkiye Ekopatoloji Derg. 11(1):9–15.
42. Hamad S, Hassan DH, Rasul KH, Mahmood N. 2016. Histological interfaces of liver, kidney and cerebrum in male rats exposed to fluoxetine. ZANCO J Pure Appl Sci. 28(5):126–135.
43. Alduweesh N, Gamal A, Abd El Rahman H. 2020. Histopathological effect of antidepressant on the vital tissues of wistar rat neonates. Ciencia e Tecnica Vitivinicola. 35(3):105–129.
44. Detry O, Delwaide J, De Roover A, Hans MF, Delbouille MH, Monard J, *et al*. 2009. Fulminant hepatic failure induced by venlafaxine and trazodone therapy: a case report. Transplant Proc. 41(8):3435–3436.
45. Paulis MG, Hafez EM, El-Tahawy NF, Aly MKM. 2018. Toxicological assessment of venlafaxine: acute and subchronic toxicity study in rats. Int J Toxicol. 37(4):327–334.
46. Brodská B, Holoubek A. 2011. Generation of reactive oxygen species during apoptosis induced by DNA-damaging agents and/or histone deacetylase inhibitors. Oxid Med Cell Longev. 2011(1):1–7.
47. Blomgren K, Hagberg H. 2006. Free radicals, mitochondria, and hypoxia–ischemia in the developing brain. Free Radic Biol Med. 40(3):388–397.
48. Ornoy A, Zaken V, Kohen R. 1999. Role of reactive oxygen species (ROS) in the diabetes-induced anomalies in rat embryos in vitro: Reduction in antioxidant enzymes and low-molecular-weight antioxidants (LMWA) may be the causative factor for increased anomalies. Teratology. 60(6):376–386.
49. Zaken V, Kohen R, Ornoy A. 2000. The development of antioxidant defense mechanism in young rat embryos in vivo and in vitro. Early Pregnancy Online. 4(2):110–123.
50. Wells PG, McCallum GP, Chen CS, Henderson JT, Lee CJJ, Perstin J, *et al*. 2009. Oxidative stress in developmental origins of disease: teratogenesis, neurodevelopmental deficits, and cancer. Toxicol Sci. 108(1):4–18.
51. Ye H, Nelson LJ, Gómez Del Moral M, Martínez-Naves E, Cubero FJ. 2018. Dissecting the molecular pathophysiology of drug-induced liver injury. World J Gastroenterol. 24(13):1373–1385.
52. Yin H, Xu L, Porter NA. 2011. Free radical lipid peroxidation: mechanisms and analysis. Chem Rev. 111(10):5944–5972.
53. Schuliga M, Chouchane S, Snow ET. 2002. Upregulation of glutathione-related genes and enzyme activities in cultured human cells by sublethal concentrations of inorganic arsenic. Toxicol Sci Off J Soc Toxicol. 70(2):183–192.
54. Ahmadian E, Babaei H, Mohajjel Nayebi A, Eftekhari A, Eghbal MA. 2016. Venlafaxine-induced cytotoxicity towards isolated rat hepatocytes involves oxidative stress and mitochondrial/lysosomal dysfunction. Adv Pharm Bull. 6(4):521–530.
55. Mishra A, Keshari AK, Singh AK, Maity S, Nandy B, Saha S. 2016. Oxidative stress-based hepatotoxicity of duloxetine in wistar rats. Int J Pharm Pharm Sci. 8(11):28–32.
56. Czuczejko J, Zachara BA, Staubach-Topczewska E, Halota W, Kedziora J. 2003. Selenium, glutathione and glutathione peroxidases in blood of patients with chronic liver diseases. Acta Biochim Pol. 50(4):1147–1154.
57. Zlatković J, Todorović N, Tomanović N, Bošković M, Djordjević S, Lazarević-Pašti T, *et al*. 2014. Chronic administration of fluoxetine or clozapine induces oxidative stress in rat liver: a histopathological study. Eur J Pharm Sci Off J Eur Fed Pharm Sci. 59(1):20–30.

58. Yılmaz A, Elbey B, Yazgan ÜC, Dönder A, Arslan N, Arslan S, *et al*. 2016. Protective effects of caffeic acid phenethyl ester on fluoxetine-induced hepatotoxicity: an experimental study. BioMed Res Int. 2016(1):1–8.
59. Elgebaly HA, Mosa NM, Allach M, El-Massry KF, El-Ghorab AH, Al Hroob AM, *et al*. 2018. Olive oil and leaf extract prevent fluoxetine-induced hepatotoxicity by attenuating oxidative stress, inflammation and apoptosis. Biomed Pharmacother Biomedecine Pharmacother. 98(1):446–453.
60. Djordjevic J, Djordjevic A, Adzic M, Elaković I, Matić G, Radojcic MB. 2011. Fluoxetine affects antioxidant system and promotes apoptotic signaling in Wistar rat liver. Eur J Pharmacol. 659(1):61–66.
61. Duda W, Curzytek K, Kubera M, Iciek M, Kowalczyk-Pachel D, Bilska-Wilkosz A, *et al*. 2016. The effect of chronic mild stress and imipramine on the markers of oxidative stress and antioxidant system in rat liver. Neurotox Res. 30(2):173–184.
62. Bautista-Ferrufino MR, Cordero MD, Sánchez-Alcázar JA, Illanes M, Fernández-Rodríguez A, Navas P, *et al*. 2011. Amitriptyline induces coenzyme Q deficiency and oxidative damage in mouse lung and liver. Toxicol Lett. 204(1):32–37.
63. Eftekhari A, Ahmadian E, Azarmi Y, Parvizpur A, Hamishehkar H, Eghbal MA. 2016. *In vitro/vivo* studies towards mechanisms of risperidone-induced oxidative stress and the protective role of coenzyme Q10 and N-acetylcysteine. Toxicol Mech Methods. 26(7):520–528.
64. Todorović N, Tomanović N, Gass P, Filipović D. 2016. Olanzapine modulation of hepatic oxidative stress and inflammation in socially isolated rats. Eur J Pharm Sci Off J Eur Fed Pharm Sci. 81(1):94–102.
65. Li Y, Couch L, Higuchi M, Fang J-L, Guo L. 2012. Mitochondrial dysfunction induced by sertraline, an antidepressant agent. Toxicol Sci Off J Soc Toxicol. 127(2):582–591.
66. Abdel-Salam OME, Youness ER, Khadrawy YA, Sleem AA. 2013. Brain and liver oxidative stress after sertraline and haloperidol treatment in mice. J Basic Clin Physiol Pharmacol. 24(2):115–123.
67. Chen S, Wu Q, Li X, Li D, Fan M, Ren Z, *et al*. 2020. The role of hepatic cytochrome P450s in the cytotoxicity of sertraline. Arch Toxicol. 94(7):2401–2411.
68. Danial NN, Korsmeyer SJ. 2004. Cell death: critical control points. Cell. 116(2):205–219.
69. Kroemer G, Galluzzi L, Brenner C. 2007. Mitochondrial membrane permeabilization in cell death. Physiol Rev. 87(1):99–163.
70. Taylor RC, Cullen SP, Martin SJ. 2008. Apoptosis: controlled demolition at the cellular level. Nat Rev Mol Cell Biol. 9(3):231–241.
71. Chen S, Xuan J, Wan L, Lin H, Couch L, Mei N, *et al*. 2014. Sertraline, an antidepressant, induces apoptosis in hepatic cells through the mitogen-activated protein kinase pathway. Toxicol Sci. 137(2):404–415.
72. Chao DT, Korsmeyer SJ. 1998. BCL-2 family: regulators of cell death. Annu Rev Immunol. 16(1):395–419.
73. Marsden VS, O'Connor L, O'Reilly LA, Silke J, Metcalf D, Ekert P, *et al*. 2002. Apoptosis initiated by Bcl-2-regulated caspase activation independently of the cytochrome c/Apaf-1/caspase-9 apoptosome. Nature. 419(6907):634–637.
74. Degterev A, Boyce M, Yuan J. 2003. A decade of caspases. Oncogene. 22(53):8543–8567.
75. Zimmermann KC, Green DR. 2001. How cells die: apoptosis pathways. J Allergy Clin Immunol. 108(4 Suppl):99-103.
76. Singh M, Singh K p., Shukla S, Dikshit M. 2015. Assessment of in-utero venlafaxine induced, ROS-mediated, apoptotic neurodegeneration in fetal neocortex and neurobehavioral sequelae in rat offspring. Int J Dev Neurosci. 40(1):60–69.
77. Salakou S, Kardamakis D, Tsamandas AC, Zolota V, Apostolakis E, Tzelepi V, *et al*. 2007. Increased BAX/Bcl-2 ratio up-regulates Caspase-3 and increases apoptosis in the thymus of patients with myasthenia gravis. In Vivo. 21(1):123–132.
78. Chang H-C, Huang C-C, Huang C-J, Cheng J-S, Liu S-I, Tsai J-Y, *et al*. 2008. Desipramine-induced apoptosis in human PC3 prostate cancer cells: Activation of JNK kinase and caspase-3 pathways and a protective role of [Ca^{2+}]i elevation. Toxicology. 250(1):9–14.
79. Jan C-R, Su J-A, Teng C-C, Sheu M-L, Lin P-Y, Chi M-C, *et al*. 2013. Mechanism of maprotiline-induced apoptosis: role of [Ca^{2+}](i), ERK, JNK and caspase-3 signaling pathways. Toxicology. 304(1):1–12.
80. 80. Zhang Z, Du X, Zhao C, Cao B, Zhao Y, Mao X. 2013. The antidepressant amitriptyline shows potent therapeutic activity against multiple myeloma. Anticancer Drugs. 24(8):792–798.
81. Herrera B, Fernández M, Alvarez AM, Roncero C, Benito M, Gil J, *et al*. 2001. Activation of caspases occurs downstream from radical oxygen species production, Bcl-xL down-regulation, and early cytochrome C release in apoptosis induced by transforming growth factor beta in rat fetal hepatocytes. Hepatol Baltim Md. 34(3):548–556.
82. Li D, Ueta E, Kimura T, Yamamoto T, Osaki T. 2004. Reactive oxygen species (ROS) control the expression of Bcl-2 family proteins by regulating their phosphorylation and ubiquitination. Cancer Sci. 95(8):644–650.
83. Mahmoud AM, Hozayen WG, Ramadan SM. 2017. Berberine ameliorates methotrexate-induced liver injury by activating Nrf2/HO-1 pathway and PPARγ, and suppressing oxidative stress and apoptosis in rats. Biomed Pharmacother Biomedecine Pharmacother. 94(1):280–291.