Amitriptyline induced alterations in liver and kidney function and structure in male rats

ABSTRACT

Aims: Depression is a mental health issue that starts most often in early adulthood and it is a common and recurrent disorder causing significant morbidity and mortality worldwide. Amitriptyline is a tricyclic antidepressant that known to inhibit the presynaptic reuptake of serotonin, norepinephrine, inhibitor of mitochondrial functions and it induced apoptosis in several tissues. This study aims to identify the changes in liver and kidney structure and functions after the treatment of male rats with Amitriptyline drugs.
Materials and Methods: A total of 20 male albino rats were randomly and equally divided into 2 groups (G1, control group that included animals that did not receive any treatment during the experimental period. G2, Amitriptyline (Tryptizol; El Kahira Pharm And Chem Ind Co) group in which rats were injected intraperitoneally with Amitriptyline (70 mg/kg body weight/daily) for four weeks).
Results: The current results revealed that; Amitriptyline treatments were significantly (P <0.05) increased the levels of serum ALT, AST, ALP, urea, creatinine, sodium ions, chloride ions and liver and kidney damages as compared to control. In contrast; a significant (P <0.05) decrease in albumen, and total protein, potassium ions and calcium ions in Amitriptyline group when compared with control group.
Conclusion: Amitriptyline has many side effects on rat liver and kidney functions and structure. Physicians should be aware of Amitriptyline a differential diagnosis for hepatic and renal with an unknown etiology.

Key words: Amitriptyline**,** Antidepression**,** liver and kidney, Rats.

1. INTRODUCTION

Depression is a mental health issue that starts most often in early adulthood and it is a common and recurrent disorder causing significant morbidity and mortality worldwide [1]. Antidepressants drugs are all drug that used to treat depression and it works by balancing certain chemicals in your brain called neurotransmitters [2-4].
Tricyclic antidepressants (TCAs) are a class of antidepressant associated with sedation, dry mouth, blurred vision, constipation, urinary retention, and increased pressure in the eye. They are also associated with hypertension, abnormal heart rhythms, anxiety, insomnia, seizures, headache, rash, nausea, and vomiting, abdominal cramps, weight loss, and sexual dysfunction [5-7]. Amtriptyline is a tricyclic antidepressant (TCA) that known to inhibit the

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presynaptic reuptake of serotonin (5-HT) and norepinephrine (NE) and thus increase the concentrations of both neurotransmitters at the synaptic cleft used to treat a number of mental illnesses include major depressive disorder and anxiety disorder, and less commonly attention deficit hyperactivity disorder [8,9]. Amitriptyline was found to be an inhibitor of mitochondrial functions and it induced oxidative stress and apoptosis in several tissues, including brain, in a dose-dependent manner [10]. Therefore; the current study aimed to study the effect of treatment with amitriptyline in on liver and kidney structure and functions in male rats.

2. MATERIAL AND METHODS

The animal house of the College of Sciences at Tanta University in Tanta, Egypt, provided 6 albino rats for the experiments, with a weight of 110-130 g and age of 9-10 weeks. The rats were kept in cages in suitable environmental conditions (22-24°C, 12-hour light/dark cycle) and were put on a diet of commercial pellet, without water restrictions. Animal maintenance and treatments were conducted in accordance with the Faculty of Science, Tanta University guide for animal, as approved by Institutional Animal Care and Use Committee (IACUC-SCI-TU-0050).

The experiments were initiated 14 days after the animals were procured to allow them to become accustomed to the laboratory setting.

2.1. Experimental design and animal groups:

A total of 20 male rats were equally divided into 2 groups.

G1, Control group included animals that received no treatment.

G2, Amitriptyline group included animals that received Amitriptyline orally by Stomach tube with a dose of 100 mg/Kg body weight daily for four weeks according to Tousson et al. [9].

At the end of the experimental period, mice were euthanized with intraperitoneal injection of sodium pentobarbital and subjected to a complete necropsy. Blood samples were individually collected from the inferior vena cava of each rat in non-heparinized glass tubes for estimation of liver and kidney functions biomarkers [11]. Blood samples were incubated at room temperature for 10 minutes and left to clot then centrifuged at 3000 r.p.m for 15 min and the serum were collected, serum was separated and kept in clean stopper plastic vial at –80°C until the analysis of serum parameters.

2.2. Liver function Biomarker:

Serum aspartate transaminase (AST) and alanine transaminase (ALT) were estimated in the rat sera according to Moustafa et al. [12] and Al-Rasheed et al. [13] respectively while alkaline phosphatase (ALP) was estimated in the rat serum according to El-Moghazy et al. [14]. Serum albumin was estimated according to Basuony et al. [15] while serum total proteins level was estimated according to Tousson et al. [16].

2.3. Electrolytes and kidney functions Biomarker:

Serum urea and creatinine respectively were determined in the rat sera according to Oyouni et al. [17] and Eldaim et al. [18] respectively. The approach proposed by El-Masry et al. [19] was followed to measure the levels of serum electrolytes (Potassium, sodium, calcium and chloride ion) by using commercial kits (Sensa core electrolyte, India) according to Tousson et al. [20].

2.4. Histological preparation

After necropsy the liver and kidney were immediately removed and fixed by immersion in 10% neutral buffered formalin solution for 24-48 hours. The specimens were then dehydrated, cleared and embedded in paraffin. Serial sections of 5 µm thick were cut by mean of rotary microtome (Litz, Wetzlar; Germany) and stained with haematoxylin and eosin [21,22].

2.5. Statistical Analysis

Data were expressed as mean values±SD and statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) tests

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to assess significant differences among treatment groups. The criterion for statistical significance was set at $p<0.05$. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS® Inc., USA).

3. RESULTS

3.1. Effects of Amitriptyline in liver functions

Alanine amino transferase (ALT), Aspartate amino transferase) and alkaline phosphatase (ALP) levels were significantly increased in Amitriptyline group when compared with control group (Figures 1). On the other hand; a significant decrease in serum albumen and total proteins were detected in Amitriptyline group when compared with control group (Figure 1).

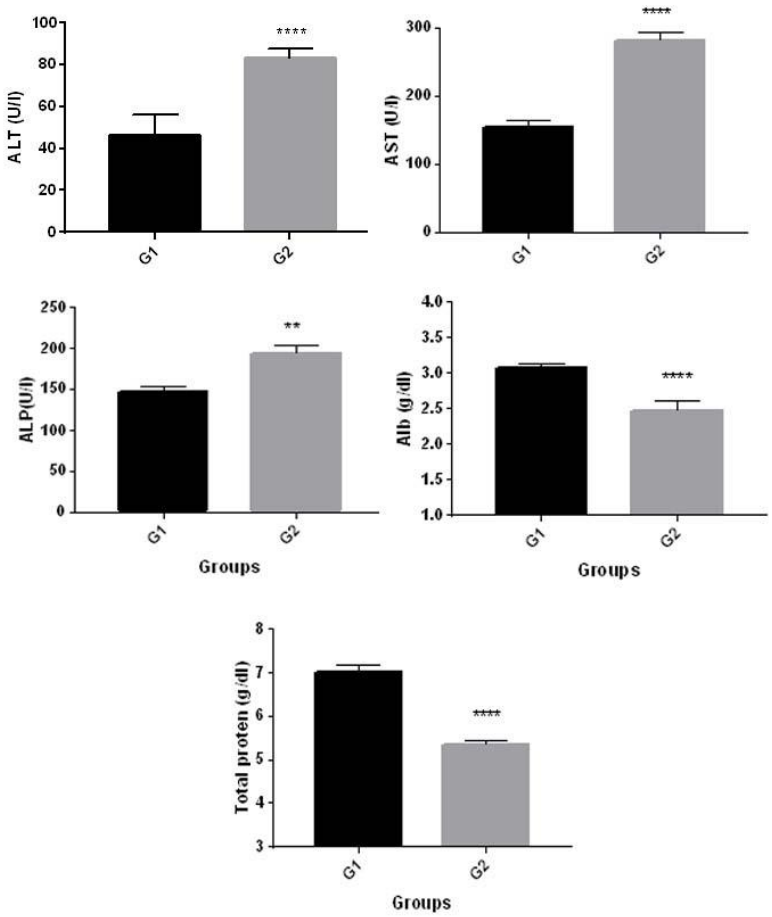


Figure 1: Changes in ALT (U/L), AST (U/L), alkaline phosphatase (ALP; U/L), albumen (g/dl) and total proteins (g/dl) levels in different groups under study. The significant difference was

analyzed by T-test unpaired. Values are expressed as mean± SEM. T-test was significant at p<0.05. T-test unpaired was significant from corresponding amitriptyline at NSP=0.1234; *P=0.0332; **P=0.0021; ***P=0.0002; ****P<0.0001. G1, control group; G2, Amitriptyline group.

3.2. Effects of Amitriptyline in kidney functions and electrolytes

Serum urea, creatinine, sodium and chloride ions levels were significantly increased in Amitriptyline group when compared with control group (Figures 2&3). On the other hand; a significant decrease in serum potassium and calcium ions were detected in Amitriptyline group when compared with control group (Figures 2&3).

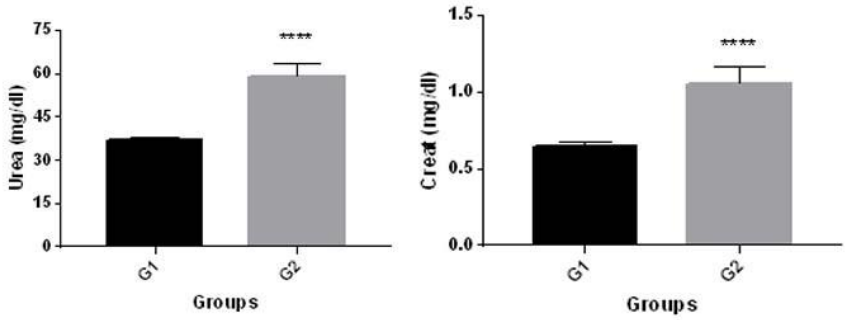


Figure 2: Changes in serum urea (mg/dl) and creatinine (mg/dl)levels in different groups under study. The significant difference was analyzed by T-test unpaired. Values are expressed as mean± SEM. T-test was significant at p<0.05. T-test unpaired was significant from corresponding amitriptyline at NSP=0.1234; *P=0.0332; **P=0.0021; ***P=0.0002; ****P<0.0001. G1, control group; G2, Amitriptyline group.

3.3. Effects of Amitriptyline on liver structure

Histological examination of haematoxylin and eosin stained on liver sections in control (G1) group showed that the structural unit of the liver is the hepatic lobule which is made up of radiating plates, cords, or strands of hepatocytes forming a network around central vein (Figure 4A&4B). The hepatocytes are polygonal in shape with prominent round nuclei, eosinophilic cytoplasm, and few spaced hepatic sinusoids arranged in-between the hepatic cords with fine arrangement of Kupffer cells (Figure 4A&4B).

Section of liver of Amitriptyline group (G2) showed lose of liver architecture as disturbance of the hepatocytes radially arranged cords, marked degenerated and vacuolated hepatocytes, congestion in central veins and portal vein, surrounded by leucocytoc infiltrations, cytoplasmic vaculation and the nuclei are pyknotic indicating apoptosis, moderate fibrosis, and marked diffuse necrosis of hepatic tissue (Figures 4C&4D).

3.4. Effects of Amitriptyline on kidney structure

Rat kidney is differentiated into two regions; an outer cortex and an inner medulla (Figures 5A&5B). The cortex consists of Malpighian corpuscles that consist of tuft of blood capillaries, the glomerulus and Bowman's capsule and both proximal and distal convoluted tubules while the medulla consists mainly of the descending and ascending limbs of Henle's loop. However, the collection tubules are located in both the cortical and medullary regions

(Figures 5A&5B). Kidney sections of treated rat treated with Amitriptyline group showed some histopathological lesions such as variable pathological changes in glomeruli and some parts of the urinary tubules (Figures 5C&5D). The most severe changes were in the Malpighian corpuscles lost their characteristic configuration and the renal tubules appeared with wide lumen, marked cortical and medullar tubular epithelial degeneration, focal tubular epithelial necrosis, moderate hemorrhage, mild to moderate atrophic glomerulus and degenerated epithelium and marked congestion in the renal blood vessels (Figures 5C&5D).

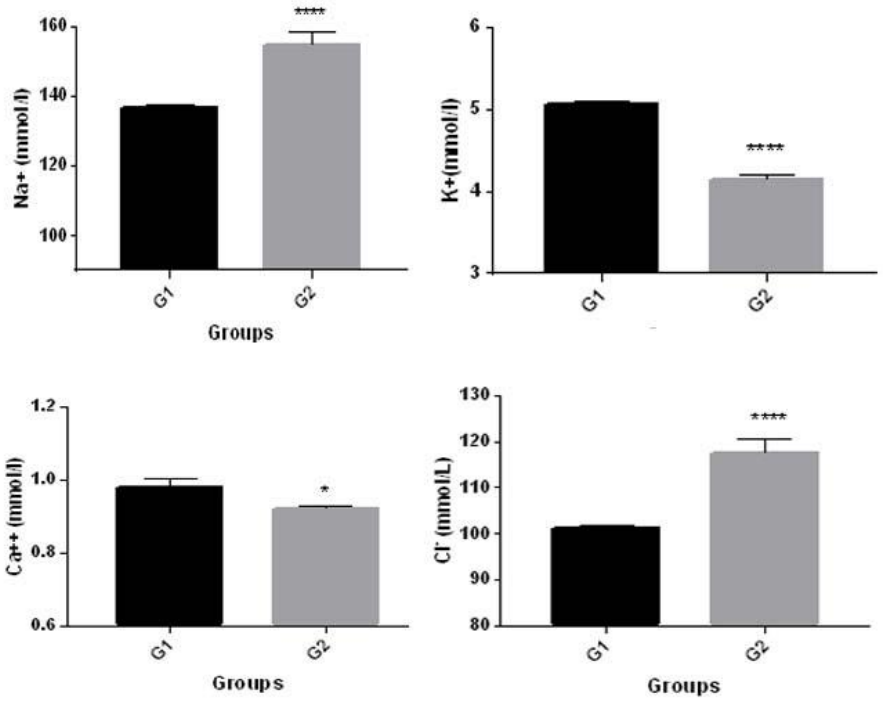
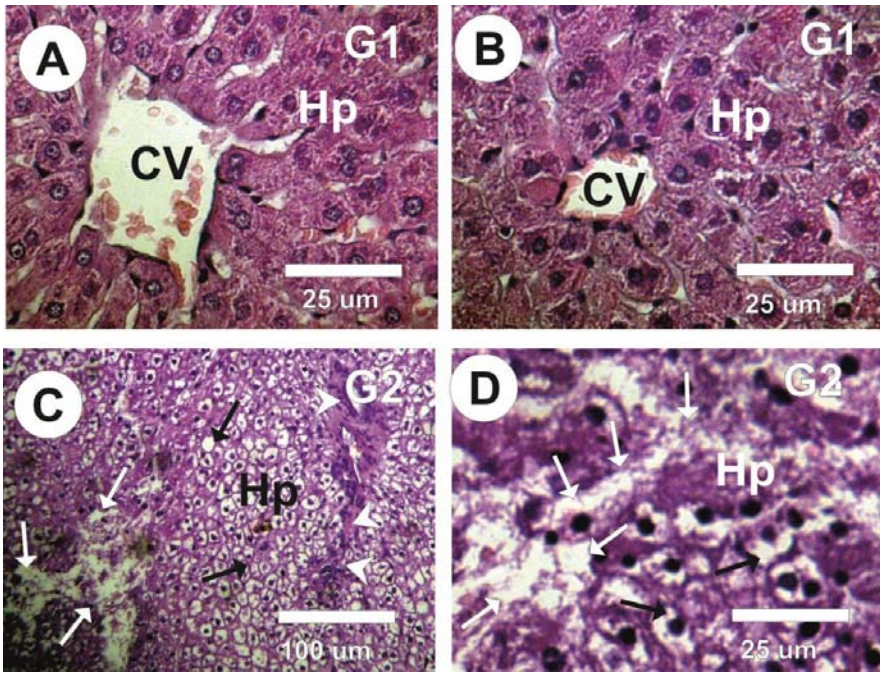
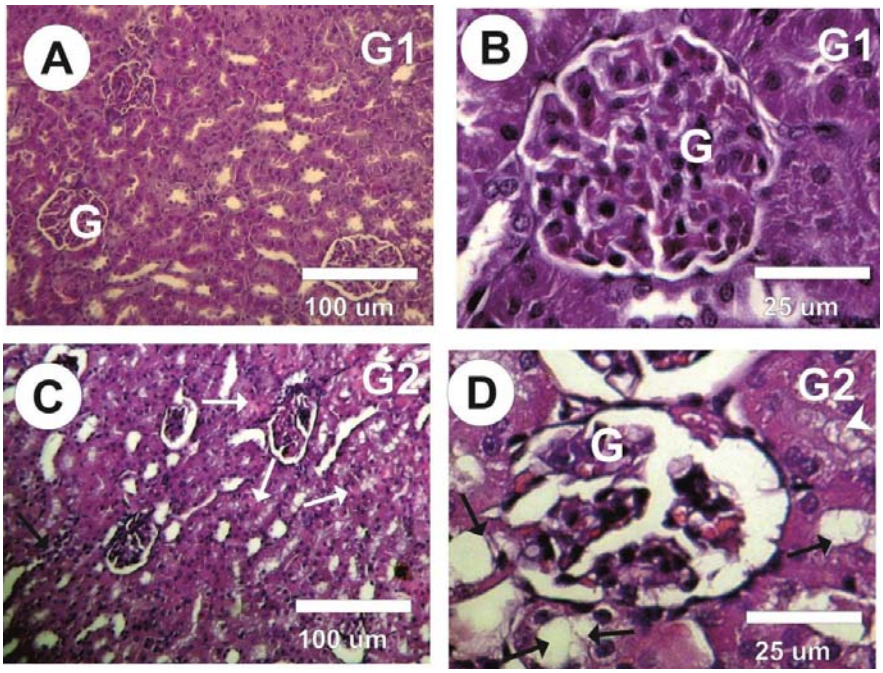


Figure 3: Changes in serum sodium ion (mmol/l), potassium ion (mmol/l), calcium ion (mmol/l) and chloride ion (mmol/l) levels in different groups under study. The significant difference was analyzed by T-test unpaired. Values are expressed as mean± SEM. T-test was significant at $p<0.05$. T-test unpaired was significant from corresponding amitriptyline at NSP=0.1234; *P=0.0332; **P=0.0021; ***P=0.0002; ****P<0.0001. G1, control group; G2, Amitriptyline group.



Figures 4: Photomicrographs of rat liver sections stained with Haematoxylin & Eosin. **A&B:** Liver sections in control group (G1) revealed normal structure of hepatocytes (Hp) with normal central veins (CV). **C&D:** Liver sections in Amitriptyline group (G2) revealed a disturbance of the hepatocytes radially arranged cords, marked vacuolated hepatocytes, cytoplasmic vaculation and the nuclei are pyknotic (Black arrows), moderate fibrosis (arrow heads), and marked diffuse necrosis of hepatic tissue (White arrows)**.**



Figures 5: Photomicrographs of rat kidney sections stained with Haematoxylin & Eosin. **A&B:** Kidney sections in control group (G1) revealed normal structure of glomerulus (G) and renal tubules**. C&D:** Kidney sections of treated rat treated with Amitriptyline (G2) showed severe changes were in the Malpighian corpuscles (G) lost their characteristic configuration and the renal tubules appeared with wide lumen, mild atrophy (arrows), tubular epithelial degeneration with focal tubular epithelial necrosis (arrow heads).

4. DISCUSSION

Antidepressants are psychiatric drugs which are available on receipt and are authorized to treat depression by altering chemical imbalances of neurotransmitters in the brain. Antidepressants have been in use for a long period of time. Although it has been used effectively to treat depression, its side effects are also known. The current study is aimed to determine the effects of antidepressants on vital organs such as liver and kidney.

The liver is the largest and very important organ in the body. It assists the body in breaking down drugs, including antidepressants. The liver has enzymes to help with its functions. AST and ALT are enzymes that are normally found within liver cells. Some drugs cause liver enzymes to leak from liver cells into the blood, causing the counts of liver enzymes in the blood to rise [14,16,23].

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It is also an organ of excretion, essential in the removal of the wastes and the toxic products from the blood [24]. It has great capacity to detoxicate toxic substances and synthesize useful principles [25]. Hepatocytes, which make up the majority of the liver structure, are very active in the metabolism of exogenous chemicals, and this is one of the major reasons why the liver is a target for toxic substances. The liver is

necessary for survival; there is currently no way to compensate for the absence of liver function over long term, although liver dialysis can be used short term.

Some drugs can cause these enzymes to leak from the cells and into the blood, thus elevating the blood levels of the enzymes [11,26,27]. Antidepressants are medications used to treat major depression, dysthymia or chronic low-grade depression, and anxiety disorders such as obsessive compulsive disorder and social anxiety disorder [4].

Chronic exposure to stress contributes to the etiology of mood disorders, and the liver as a target organ of antidepressant and antipsychotic drug metabolism is vulnerable to drug-induced toxicity.

In the current study; significant increase in ALT, AST and ALP levels in treated rat with Amitriptyline when compared with control group. On the other hand; a significant decrease in serum albumen and total proteins were detected in Amitriptyline group when compared with control group. The histopathological changes in the liver structure occur either during the hepato-cellular failure or the parenchymal damage caused due to various physiological and pathological conditions. Antidepressant-induced liver injury is generally considered to be dose independent. DeSanty and Amabile [28] reported that; antidepressant-induced liver injury.

Cunningham [29] who reported that treatment with amitriptyline and diazepam induced acute hepatic necrosis. The results were consistent with Ebuehi and Asonye [30] who find that; a significant increase in alkaline phosphatase, aspartate transaminase (AST) and alanine transaminase (ALT) activities in rabbits administered sertraline, clozapine, Amitriptyline. Anttila et al. [31] reported that; selegiline induced marked effect of liver and kidney function. Antidepressant-induced liver injury includes various biological and clinical presentations, ranging from isolated increases in liver enzyme levels to nonspecific symptoms such as fatigue, asthenia, anorexia, nausea, vomiting, and upper right abdominal pain, and also to more specific symptoms such as jaundice, dark urine or pale stool, progressive or even fulminant liver failure with hepatic encephalopathy, loss of hepatocellular functions, acute liver failure, and death.

The kidney is a compound tubular gland concerned with the important function of excretion [32]. It excretes urea and other nitrogenous waste products, eliminates substances foreign to the body and it maintains homeostasis by controlling the composition, volume and pressure of blood [33]. Approximately one and a half quarters of blood per minute are circulated through the kidneys, where waste chemicals are filtered out and eliminated from the body (along with excess water) in the form of urine. Medications are a common cause of kidney damage, also known as nephrotoxicity or, when severe, renal failure. This suggests a renal dysfunction and plasma creatinine were found to be high in correlation with the histological observation. The study concludes that any treatment with antidepressants may have negative effect on the vital organs. Thus these effects have to be considered while administering dose of the antidepressants the depression patients.

In the current study; a significant increase in the serum urea, creatinine, sodium and chloride ions levels was detected in the treated rats with Amitriptyline when compared with control. In contrast; a significant decrease in serum potassium and calcium ions were detected in Amitriptyline group when compared with control group. Our results were consistent with Tousson et al. [9] who reported that; amitriptyline induced an increase in sodium ions levels and decrease in potassium ions level.

Creatinine is primarily synthesized in the liver from the methylation of glycocyamine (guanidino acetate, synthesized in the kidney from the amino acids arginine, glycine, and methionine) by S-Adenosyl-L-Methionine. It is then transported through blood to the other organs, muscle, and brain where, through phosphorylation, it becomes the high energy compound phosphocreatine. Enzyme evaluation of changes in the activity of lysosomal enzymes in rat kidneys could be useful indicator of kidney damage as well as kidney failure [34-36]. Hence a biochemical assay of creatinine was carried out to ascertain the effects of Amitriptyline on kidney.

5. CONCLUSION

Our recommendation is Amitriptyline treatments induced changes in liver and kidney functions and structure. Physicians should be aware of Amitriptyline a differential diagnosis for hepatic and renal with an unknown etiology.

Conflict of interests

The authors declare no conflict of interest.

REFERENCES

1 Rashid T, Heider I. Life Events and Depression . Annals of Punjab Medical College 2008; 2 (1).

2 Fonseca AP, Leala V. Use of Antidepressants to Treat Postpartum Depression, During Breast Feeding. J Depress Anxiety 2014; 3:148.

3 Ghoneim M, Saber AL, El-Desoky H. Utility Spectrophotometric and Chromatographic Methods for Determination of Antidepressant Drug Sulpiride in Pharmaceutical Formulations and Plasma. J Anal Bioanal Tech 2014; 5:183.

4 El Atrash A, Tousson E, Gad A, Allam S. Hematological and Biochemical Changes Caused by Antidepressants Amitriptyline Induced Cardiac Toxicity in Male Rats. Asian Journal of Cardiology Research. 2019; 23:1-6.

5 Jespersen S. Antidepressant induced sexual dysfunction Part 2: assessment and management. S Afr Psychiatry Rev 2006; 9: 79-83.

6 Nazari M. Effect of Fluoxetine on the sexual behavior of Drosophila melanogaster, J. Postgr. Medi. Insti. (Peshawar Pakistan). 2011; 25(4).

7 Kavitha BB, Nischal R, Shashank B. Dose Dependent Amitriptyline Induced Sexual Dysfunction in a Migraine Patient. International Journal of Pharmacology and Clinical Sciences, 2016;5(2): 62-64.

8 George S, Acharya LD, Prabhu, AR, Mallayasamy S. Management and treatment outcome of complications of chronic kidney disease patients in a South Indian tertiary care hospital. Int. J. Pharmacol. and Clin Sci., 2013; 2:113-120.

9 Tousson E, Zaki S, Hafez, E Gad A. Biochemical and immunocytochemical studies of the testicular alteration caused by Amitriptyline in adult male rat. Journal of Bioscience and Applied Research 2018; 4 (4):418-424

10 Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol.2003; 552(2):335-344.

11 Saggu S, Sakeran M, Zidan N, Tousson E, Mohan A. Rehman H. Ameliorating effect of chicory (Chichorium intybus L.) fruit extract against 4-tert-octylphenol induced liver injury and oxidative stress in male rats. Food and Chemical Toxicology 2014; 72: 138–146

12 Moustafa AH, Ali EM, Moselhey SS, Tousson E, El-Said KS. Effect of coriander on thioacetamide-induced hepatotoxicity in rats. Toxicology and industrial health. 2014; 30(7):621-629.

13 Al-Rasheed NM, El-Masry TA, Tousson E, Hassan HM, Al-Ghadeer A. Protective Potential of Grape Seed Proanthocyandins Extract against Glivec (Imatinib Mesylate) Induced Liver Toxicity and Oxidative Stress in Male Rats. Annual Research & Review in Biology 2017;20(6): 1-9

14 El-Moghazy M, Zedan NS, El-Atrsh AM, El-Gogary M, Tousson E. The possible effect of diets containing fish oil (omega-3) on hematological, biochemical and histopathogical alterations of rabbit liver and kidney. Biomedicine & Preventive Nutrition 2014; 4: 371–377.

15 Basuony M, Hafez E, Tousson E, Massoud A, Elsomkhraty S, Eldakamawy S. Beneficial role of Panax ginseng root aqueous extract against Cisplatin induced blood toxicity in rats. American Journal of Biological Chemistry 2015;3: 1-7.

16 Tousson E, Tawfeek Z, Abu-Shaeir WA, Hassan H. Methotrexate-induced Hepatic and Renal Toxicity: Role of L-carnitine in Treatment. Biomedicine and Biotechnology, 2014; 2(4): 85-92.

17 Eldaim MA, Tousson E, El Sayed IE, El AE, Elsharkawy HN. Grape seeds proanthocyanidin extract ameliorates Ehrlich solid tumor induced renal tissue and DNA damage in mice. Biomedicine & Pharmacotherapy. 2019;115: 108908.

18 Oyouni AA, Saggu S, Ehab Toussonb, Rehman H. Immunosuppressant drug tacrolimus induced mitochondrial nephrotoxicity, modified PCNA and Bcl-2 expression attenuated by Ocimum basilicum L. in CD1 mice. Toxicology Reports 2018; 5: 687–694.

19 El-Masry TA, Al-Shaalan NH, Tousson E, El-Morshedy K, Al-Ghadeer A. P53 Expression in Response to Equigan Induced Testicular Injury and Oxidative Stress in Male Rat and the Possible Prophylactic Effect of Star Anise Extracts. Annual Research & Review in Biology 2017; 14(1): 1-8.

20 Tousson E, Bayomy MF, Ahmed AA. Rosemary extract modulates fertility potential, DNA fragmentation, injury, KI67 and P53 alterations induced by etoposide in rat testes. Biomedicine & Pharmacotherapy 2018; 98: 769–774.

21 Tousson E. Histopathological alterations after a growth promoter boldenone injection in rabbits. Toxicology and Industrial Health 2016; 32(2) 299–305

22 Tousson E, El-Moghazy M, Massoud A , El-Atrash A, Sweef O, Akel A. Physiological and biochemical changes after boldenone injection in adult rabbits. Toxicology and Industrial Health 2016; 32(1): 177–182

23 Tousson E, Ibrahim W, Barakat L, Abd El-Hakeem A. Role of Proplis administration in boldenone-induced oxidative stress, Ki-67 protein alterations and toxicity in rat liver and kidney. International Journal of Scientific & Engineering Research 2015;6(8): 660-664.

24 Tortora GJ, Grabowski SR. The digestive system (liver and gallbladder) In: Principles of Anatomy and Physiology, seventh edition, Harper Collins college publishers, New york, 2002; 24: 792-795.

25 Shanmugasundaram P, Venkataraman S. Hepatoprotective and antioxidant effects of Hygrophila auriculata (K. Schum) Heine Acanthaceae root extract. Journal of Ethnopharmacology 2006; 104: 124-128.

26 Salama A, Kasem S, Tousson E, Elsisy MK. Protective role of L-carnitine and vitamin E on the testis of atherosclerotic rats. Toxicology and Industrial Health 2015; 31(5) 467–474.

27 Bolkiny Y, Tousson E, El-Atrsh A, Akela M, Farg E. Costus Root Extract Alleviates Blood Biochemical Derangements of Experimentally-Induced Hypo-and Hyperthyroidism in Mice. Annual Research & Review in Biology 2019; 31(5):1-0.

28 DeSanty KP, Amabile CM: Antidepressant-induced liver injury. Ann Pharmacother 2007; 41:1201–1211

29 Cunningham ML: Acute hepatic necrosis following treatment with amitriptyline and diazepam. Br J Psychiatry 1965; 111: 1107–1109

30 Ebuehi OA, Asonye CL. Gender and alcohol consumption affect human serum enzymes, protein and bilirubin. Asian J Biochem. 2007;2:330-6.

31 Anttila M, Sotaniemi EA, Pelkonen O et al. Marked effect of liver and kidney function on the pharmacokinetics of selegiline. Clin Pharmacol Ther 2005; 77: 54–62.

357 32 Alm-Eldeen A, Tousson E. Deterioration of glomerular endothelial surface layer and the
358 alteration in the renal function in Rabbits after treatment with a growth promoter
359 Boldenone. Human & Experimental Toxicology, 2012;31(5): 465-472.
360 33 El-Moghazy M, Tousson E, Sakeran M. Changes in the hepatic and renal structure and
361 function after a growth promoter Boldenone injection in Rabbits. Animal Biology,
362 2012;62(2): 171-180.
363 34 Salama AF, Tousson E, Ibrahim W and Hussein MW. Biochemical and histopathological
364 studies in the PTU-induced hypothyroid rat kidney with reference to the ameliorating
365 role of folic acid. *Toxicology and Industrial Health* 2013; 29(7):600-608.
366 35 Salama AF, Kasem SM, Tousson E, Elsisy MK. Protective role of L-carnitine and vitamin
367 E on the kidney of atherosclerotic rats. Biomedicine & Aging Pathology 2012; 2: 212–
368 215.
369 36 Łąkowska H, Maciejewski R, Szkodziak P, Staśkiewicz G. Changes in the activity of
370 lysosomal enzymes in rat kidneys in the course of acute pancreatitis. Medical Science
371 Monitor. 2001;7(6):BR1193-7.

372