Histological Changes of Liver, Kidney and Brain in Uninephrectomized Male Rats Exposed to Fluoxetine

Khder Hussein Rasul¹, Nazar Mohammed Shareef Mahmood², Sarkawt H. Hamad³, Dlshad Hussein Hassan³

1- Department of Biology, College of Science, Salahaddin University, Erbil, Kurdistan Region, Iraq.
3- Biology Department, Faculty of Science, Soran University, Soran, Erbil, Kurdistan Region, Iraq.

ARTICLE INFO

Article History:
Received: 10/08/2016
Accepted: 08/09/2016
Published: 10/01/2017

Keywords:
Nephrectomy;
Fluoxetine;
Liver;
Kidney;
Brain.

*Corresponding Author:
Khder Hussein Rasul
Email:
Khder.rasul@su.edu.krd

ABSTRACT

Kidney is the organ of humans and animals body which responsible for purification of blood from waste substances, the process of removing kidney or part of the kidney called nephrectomy. The depression disease mostly occurs in unhealthy person; it is commonly treated with fluoxetine. The purpose of this investigation to determine the histological effects of fluoxetine on liver, kidney, cerebrum and cerebellum of uninephrectomized male rats; rats were divided randomly into two groups, uninephrectomized control group and uninephrectomized fluoxetine treated group. After one month administration of fluoxetine, histological alteration observed in liver such as a little hydropic degeneration of the hepatocytes and inflammatory leucocyte infiltration. Hemorrhagic area and shrinking of glomerulus appeared in the cortex region of kidney, degeneration of kidney tubule epithelial cells and dilation in some kidney tubules were detected in medulla region as a result of exposing uninephrectomized rats to fluoxetine. Histological slides of cerebrum and cerebellum uninephrectomized rats which exposed to fluoxetine showed dead pyramidal and purkinje cells respectively. In conclusion, light microscopic examinations showed that fluoxetine had a little effect on the liver, kidney, cerebrum and cerebellum.

1. INTRODUCTION

The prevalence of kidney problems are increasing around the world; renal failure affects around 10 percent of the global population (Coresh et al., 2005), common and rose in those countries which they have developed industry (Coresh et al., 2007). Patients with chronic kidney disease showed a large number of neuropsychiatric alterations, including depression and behavioral changes (Kielstein et al., 2015); also more than a quarter of them have elevated depression symptoms (Balogun et al., 2012, Palmer et al., 2013). Patients with glomerular filtration rate problem revealed a 2-fold higher risk of depressive symptoms in comparison to the population with normal renal function (Campbell et al., 2013).

Organ donation is the operation of surgically getting an organ from donor and transplants it into recipient; nephrectomy is a process of removing kidney from healthy person which carries out to improve the recipient life or removing all or part of kidney...
to treat kidney disease and leaves healthy tissue (Salazar et al., 2005, Wilson et al., 2011). Increasing depression was significantly noted after the process of nephrectomy (Minz et al., 2005).

Depression is a medical condition which recognized by low mood, loss of interest, hopeless, feeling sad, disfavor to do activity and often is unable to live in a normal way; depressive symptoms are common in patients with end-stage renal disease (Palmer et al., 2013) and associated with mortality (Fan et al., 2014, Farrokhi et al., 2014). Developing of depression over eleven years period of nephrectomy were significantly reported (Liounis et al., 1988); after recipient death, donors had extreme depression (Indudhara et al., 1998). Review article reported that more than 5,000 living donors estimated that depression affects 5 to 23% of donors at an average of four years after nephrectomy (Clemens et al., 2006).

Like other diseases, depression needs medication, one of the main drugs which used to treat depression is fluoxetine which it is a selective serotonin reuptake inhibitor (Wernicke, 2004, Cipriani et al., 2005), one of the most common treatments for major depression, affects mood through changes in immune function and the effect of it depend on the quality of the living environment (Alboni et al., 2016).

Rising agreement of living organ transplantation and growing numbers of organ donors become serious to look for any opposite outcomes in the population (Minz et al., 2005). In the last few years, using of medications among them fluoxetine increased to treat depression which caused by kidney failure, organ donation especially kidney and industry grew in Kurdistan region – Iraq. So that, the purpose of current research to assess the histological effects of fluoxetine on liver, kidney, cerebrum and cerebellum of uninephrectomized male rats.

2. MATERIALS AND METHODS

2.1 Animals and housing

The current study was carrying out on sixteen male adult uninephrectomized rats, weighing about 200 – 270 gm and 8-10 weeks old at the time when the experiment started. Animals were obtained from animal house of Biology department, Faculty of Science, Soran University; under supervision and approval of local scientific committee and animal care rules. Rats housed at room temperature (22 ± 2°C); regular 12 hours light and 12 hours dark by using an automated light-switching device.

2.2 Experimental design

Rats were divided randomly into two groups and each group consisted of eight rats per plastic cage; group 1: uninephrectomized control group, which take normal diet (Krinke, 2000) and drink water ad libitum; group 2: uninephrectomized group rats were administrated with fluoxetine (10 mg/kg body weight/day) within drinking water for one month.

2.3 Nephrectomy

In the aseptic condition, rats were anesthetized by injection intraperitonal (80 mg/Kg) ketamine (GmbH, Germany)/ (12mg/Kg) Xilazine (Over SRL, Argentina). The right upper abdomen of rats was cleaned and shaved; then 2-3 cm incisions was made; pull out kidney gently by forceps, the renal artery and ureter tied by absorbable thread (Doğsan,Turkey) then cut the kidney and removed. Suture muscular layer by an absorbable (22mm ½ tapernTrocar Point)
thread and skin layer closed by non-absorbable (25mm ½round) thread (B. Braun, German).

2.4 Anesthesia, dissection and removal of the organs

At the end of the experiment, all rats were anesthetized with intraperitoneal injection of a mixture of Ketamine Hydrochloride (80 mg / Kg) and Xylazine (12 mg / Kg), sacrificed, and then liver, kidneys, cerebrum and cerebellum were removed, cut into smaller pieces (approximately 0.5cm in thickness) in petri dish which contained a fixative (Bouin’s fluid) and then put in Bouin’s fluid for fixation.

2.5 Histological preparation (Paraffin method)

Tissue samples (liver, kidneys, cerebrum and cerebellum) of all uninephrectomized rats removed from Bouin’s fluid and transferred into dehydrating solution (using a series of graded ethanol in ascending concentrations), then immersed in xylene for clearing, infiltrated with paraffin wax and the tissues were embedded in paraffin wax. Four to six micrometer thick paraffin sections were obtained by using rotary microtome (Bright, MIC, England) and stained by hematoxylin and eosin (H&E) (Bancroft et al., 1977). The specimens were examined and photographed under light microscope (digital binocular compound microscope 40x-2000x, built-in 3MP USB camera).

3. RESULTS AND DISCUSSIONS

3.1 Effect of fluoxetine on liver uninephrectomized rats

Liver is the organ of body animals which have many functions; one of the main functions of liver is detoxify the drugs; administration of fluoxetine for one month in uninephrectomized rats caused presence of some histological changes in liver such as presence of hydropic degeneration of hepatocytes which agreed with the findings of (Özden et al., 2014) and inflammatory leucocyte infiltration which also noted in the results of (Zlatković et al., 2014) when compared with the liver sections of non-administrated fluoxetine uninephrectomized rats which showed normal appearance of hepatocytes architecture which polyhedral in shape, normal shape nucleus and clearly defined plasma membrane as well as presence of normal blood sinusoids and central vein (Fig. 3.1 and Fig. 3.2). These histological changes in liver may be due to toxic effects of fluoxetine (Souza et al., 1994); previous studies showed that fluoxetine drop antioxidant levels and elevated oxidant stress by increasing superoxide anion levels, and producing oxidative stress (Park et al., 2011).

3.2 Effect of fluoxetine on kidney uninephrectomized rats

The outer region of the kidney is cortex, in uninephrectomized rats showed normal composition and structure such as glomeruli, kidney tubules (proximal convoluted tubule and distal convoluted tubule) and cells of the kidney tubules (Fig. 3.3). The kidney tubules (nephrons) extended into the medullary region of the kidney; uninephrectomized rats showed normal cells of the kidney tubules and normal diameter of the lumen of kidney tubules in the sections through the medullary region of kidney (Fig. 3.4). Histological alterations would be observed in the exposed fluoxetine cortex of uninephrectomized rats which include hemorrhage, widening of the lumen of kidney tubules, degeneration of some epithelial cells of kidney tubules and shrinking of glomeruli (Fig. 3.5). Other research showed that hemorrhage would happen due to antiplatelet
activity of serotonin reuptake drugs (Chan et al., 2011). As a result of administration of fluoxetine, degeneration of epithelial cells of kidney tubules and dilation in some kidney tubules were observed in the medullary region of uninephrectomized rats (Fig. 3.6). Fluoxetine can promote acute kidney disease (Carrero and Stenvinkel, 2010). (Herbet et al., 2016) findings showed that fluoxetine administration doesn’t change renal functions.

### 3.3 Effect of fluoxetine on brain uninephrectomized rats

One of the parts of brain is cerebrum, cerebrum paraffin sections of uninephrectomized rats showed normal architecture of grey matter with different layers, normal pyramidal cells and normal glial cells (Fig. 3.7); but the cerebrum of uninephrectomized rats which exposed to fluoxetine showed abnormality such as presence of dead neuronal and non-neuronal cells in the second and third layers of cerebral cortex (Fig. 3.8). Light Microscopic view examinations showed normal healthy structure of molecular layer, granular layer and purkinje cells in the cerebellum of uninephrectomized rats (Fig. 3.9), while in treated rats with fluoxetine, dead purkinje cells were clear in the cerebellum of uninephrectomized rats (Fig. 3.10). Experimental studies indicated that fluoxetine decreased antioxidant levels, increased oxidant levels by elevating superoxide anion levels, and induced oxidative stress (Chen et al., 2015, Sakr et al., 2015) and these consequences may be cause of neuronal damage of the brain. On the other hand, the neuroprotective of fluoxetine were reported by other findings (Jin et al., 2009, Vizi et al., 2013).

### 4. CONCLUSIONS

The administration of fluoxetine (10 mg / kg body weight / day) within drinking water for one month had a minor alteration of the histological structure on liver, kidney, cerebrum, and cerebellum.

**Conflict of Interest**

There is no conflict of interest.
Figure 3.1: Photomicrograph of liver sections of uninephrectomized rats showed normal appearance of hepatocytes architecture which polyhedral in shape, round or ovoid shape nucleus well known and clearly defined plasma membrane (Black arrow). Blood sinusoids (S) and central vein are normal (CV). A) H&E. 100X. B) H&E. 400X.
Figure 3.2: Histological sections of liver uninephrectomized rats which exposed to fluoxetine showing a little hydropic degeneration of the hepatocytes (D) and infiltration of inflammatory cells (I). A) H&E. 100X. B) H&E. 400X.
Figure 3.3: Cortex slides of kidney of uninephrectomized rats. Glomeruli (G), kidney tubules (T) (proximal convoluted tubule and distal convoluted tubule) and cells of the kidney tubules appeared normal. A) H&E. 100X. B) H&E. 400X.
Figure 3.4: Sections of medullary regions of uninephrectomized rat’s kidney in which the cells of kidney tubules and the diameter of the lumen of tubules (L) were appeared normal. A) H&E. 100X. B) H&E. 400X.
Figure 3.5: Hemorrhagic area (H), a few increases in the diameter of lumen of kidney tubules, degeneration of some epithelial cells of kidney tubules and shrinking of glomeruli (Black arrow) were appeared in the cortex region of kidney due to exposing uninephrectomized rats to fluoxetine. A) H&E. 100X. B) H&E. 400X.
Figure 3.6: Paraffin sections of the medullary regions of uninephrectomized rat’s kidney which exposed to fluoxetine showed degeneration of epithelial cells (Black arrow) of kidney tubules and dilation in some kidney tubules (T). A) H&E. 100X. B) H&E. 400X.
Figure 3.7: Slides of cerebrum of uninephrectomized rats showing the normal architecture of grey matter of cerebrum with different layers in low power magnification, while in higher power magnifications, normal pyramidal cells (black arrow) and glial cells (white arrow) were observed in cerebral cortex layer. A) H&E. 100X. B) H&E. 400X. C) H&E. 1000X.
Figure 3.8: Light microscopic views of cerebrum uninephrectomized rats exposed to fluoxetine, the cortex region of cerebrum with different layers and a few dead (black arrow) cells were observed in second and third layers of cerebral cortex. A) H&E. 100X. B) H&E. 400X. C) H&E. 1000X.
Figure 3.9: Cerebellum of uninephrectomized rats show the normal healthy structure of molecular layer, granular layer and purkinje cells (black arrow). A) H&E. 100X. B) H&E. 400X.
Figure 3.10: Dead Purkinje cells (black arrow) observed in cerebellum of uninephrectomized rats as a result of fluoxetine administration. A) H&E. 100X. B) H&E. 400X.
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