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Research Article

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The Preventive Effect of Nigella Sativa Oil on the Liver of Male Albino Rats Treated with Anti-Depressants (Olanzapine) is Histological Study

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ABSTRACT

Depression has been recently one of the most prevalent psychological disorders that affect various age groups. Olanzapine is an anti-depressant which exerts a systematic effect upon body organs in general. Many studies have illustrated the harmful impacts, organic changes, and side effects caused by taking tranquilizers. Many of those studies clearly indicated the liver, which plays a primary role in filtering toxins along with fatty tissues, as being the primary victim thereof. This particular study was conducted on Albino rats which were divided into several groups. The first group consisted of 10 male albino rats, considered as the negative group, which were fed on basal diet. The Second group included 10 male albino rats, considered as the positive group, in which the rats were orally given the drug Zyprexaata with the dose of 6mg/kg body weight/day for 8 weeks through the oral route via a gastric tube. The third group composed of 10 male albino rats, considered as the treated group, and they were orally given Nigella Sativaata with the dose of 2ml/kg body weight/day for a period of four weeks after being medicated for four weeks via a gastric tube. At the end of the experimental period, the autopsy of the liver specimens was taken and fixed in formaldehyde based on the standard procedures for the preparation of microscopic examination. The examination of various sections of hepatic tissues revealed pathological and histological changes, where the liver cells appeared deformed in addition to the occurrence of hemostasis, hemorrhages and inflammation of hepatic cells.

Keywords: *Olanzapine-Hepatic cells-Blood Sinusoids-Kupffer cells, Nigella Sativa oil, Bile Duct-Hepatic Vein-Hepatic artery (Ha)*

INTRODUCTION

Lately, depression has been considered as one of the most prevalent psychological disorders. In the United States of America, a scientific study discovered the striking prevalence of this disorder among the American population and expected in other countries, those motivating physicians of various disciplines to conduct studies centered on the effect of sedatives and tranquilizers in this regard Andrade L, et al.[2]. Olanzapine has been used in cases of Schizophrenia, psychological disorders and depression. It has been considered as an unconventional treatment for psychosis and a highly effective agent controlling the food assimilation process in patients, causing both internal and external changes, such as marked weight gain and hyperglycemia as a result of decreased glucose metabolism in the blood [11]. However, the effects of Olanzapine are not only limited to gaining weight, but also they are strongly associated with Diabetes Mellitus[16]. As a result of increased body weight, the patient is more susceptible to diabetes, with the exception of a few patients with less than the proportion of diabetes. According to many studies,

few patients had diabetes that occurred not because of an increase in the body weight, once the patients stopped taking the Olanzapine, there was a change in plasma blood due to the low blood glucose [14,15,26,28].
In many experiments conducted on animals, the results indicated that the drug Olanzapine led to the increase in the body weight as well as the blood sugar level [5]. In 2000, an experimental by [7] revealed an until then undetected side effect of the drug Olanzapine, namely acute pancreatitis.

The Liver
The liver is the largest body organ and the main organ, where the various fatty acids are metabolized. Hepatocytes (Hc), which represent the basic function and the structural unit of the liver tissues are polygonal in their normal state, containing one or more central nuclei. Hepatocytes (Hc) are highly sensitive to alkaline dyes. Other cells of note are Kuppfer cells, which project into the cavities of blood Sinusoids, as well as sinusoidal cells which are endothelial cells that line blood Sinusoids and constitute a barrier against pathogens and act as a selective screen for the materials that pass from the blood stream to Hepatocytes (Hc)[21].

Nigella Sativa oil
Nigella Sativa is an annually flowering plant of South West Asia. It is a black seed that is classified as a member of the family of Ranunculaceae. It has been considered as the key plant of both nutritive and medicinal values. The black seed and the oil which is extracted from it, are used in the treatment of various diseases, such as Arthritis, Digestive System Disorders [1].
In Islamic tradition, this black seed has been narrated to be a cure for many diseases [24, 25].
Treatment with the black seed has also been of the great importance in the world of herbal medicine. This therapeutic importance is greatly attributable to the substance known as Thymoquinone, which is a major constituent of Nigella Sativa oil [27].
Nigella sativa oil has many benefits as proven by several studies. Such beneficial effects include the marked improvement of cancer, Diabetes Mellitus, hypertension, and it has also been beneficial for the digestive system and central nervous system. These highlighted the great value of Nigella sativa oil, and made it a prime component of a new generation of drugs that have been used to treat various diseases [1].
Additionally, many studies indicated the strong impact of the Nigella Sativa oil on the immune system, which has been highly effective by improving the capability of the natural killer cells, which are protecting the body against the development of malignant tumors, infections, hepatic viruses, and other immune deficiency disorders.
Preventive effect of Nigella Sativa oil
Nigella Sativa oil is highly effective by preventing hepatotoxicity, improving hypercholesterolemia and reducing hyperglycemia [8]. The effectiveness of Nigella Sativa oil in protecting liver is attributable to the presence of Thymoquinone that acts as a natural antioxidant and protects the liver from lipid inhibition, elevated catalase, quinone reduction and inhibition of both cyclic oxidative enzymes through various mechanisms [10].

MATERIALS AND METHODS
First: Materials:
Drug: Zyprexa
The Chemical Name: Olanzapine; 132539-06-1Zyprexa(C17H20N4S)
Nigella Sativa seeds were purchased from local market from Saudi Arabia.
Methods:
Structural Formula of Olanzapine
The drug Olanzapine was prepared by dissolving a 6 gm tablet of Olanzapine in 10 ml of the distilled water, then stirred by a magnetic stirrer and given to test rats orally with the dose of 6mg/kg body weight/day for 8 weeks through the oral route via a gastric tube, ensuring the daily preparation of the test drug on a daily basis [23].
2-Nigella Sativa Oil
Nigella sativa Oil was orally administered via a gastric tube with the amount of 2ml/kg body weight/day for a period of four weeks.
Biological Experimental Animals
The study was conducted on Swiss Albino rats.

The first group was consisted of 10 male albino rats, it was considered as the negative group, and the rats were fed by the basal diet.

The second group included 10 male albino rats, it was considered as the medicated group, and the rats were given the drug Zyprex at a dose of 6mg/kg body weight/day for 8 weeks through the oral route via a gastric tube.

The third group was composed of 10 male albino rats, it was considered as the treated group, and the rats were only orally given Nigella sativa oil at a dose of 2ml/kg body weight/day for four weeks after being medicated for four weeks via a gastric tube.

Histological studies

The test rats were anesthetized and autopsied, and the liver specimens were collected and cut into small pieces, which were then fixed in 10% buffered formaldehyde solution, embedded in paraffin, sectioned into 3 micron thick sections of the control and medicated tissue specimens, as well as tissue cross sections, dyed with Hematoxylin and Eosin, to be used to study the histological changes in the liver. The autopsy of the liver specimens was taken and fixed in formaldehyde, following the standard procedures for the preparation of microscopic examination as per Bancroft and Stevens [3].The technique of getting fixed tissue into paraffin is called tissue processing. In this study, the main steps in this process were dehydration and clearing. The clearing consisted of the removal of the dehydrated substance that will be miscible with the embedding medium (paraffin). The commonest clearing agent has been xylene according to [4,19].

RESULTS

Control Group

The general appearance showed that the hepatic tissue with visualized central veins (Cv) and the blood sinusoids, the arrangement of hepatocytes in cords around central veins, the visualization of blood Sinusoids(S) and hepatocytes (Hc) in the cords. Mono-nucleated cells(N) and two nucleic cells(2N) were clearly visualized, as well as the blood sinusoids, which were clearly seen between the cords, inside which an increased number of Kupffer cells, widening sinusoids and two nucleated cells(2N) with clearly visible nuclei(N) and nucleoli(Nu) were observed.

Treatment Group G2

The hepatic tissues which were treated with Zyprexa (Olanzapine) in male rats:

The portal area showed the static RBC appeared inside the Central vein (Cv), acute inflammation of the Hepatic vein and Bile duct(Bd), increased number of Kupffer cells inside blood Sinusoids, breakdown of Hepatocytes (Hc), nuclear Necrosis(Ne), deformed Hepatocytes (Hc), widening of blood Sinusoids(S), Fibrosis of the portal area(Pa), where there were static RBC's and Endothelial Fibrosis (E), breakdown of Hepatocytes (Hc)(Py), and increased number of Kupffer cells(K).The Olanzapine medicated group exhibited severe deformity of the Portal area due to the increased fat deposition, Fibrosis of the Hepatic artery(Ha), acute infiltration inside the Portal vein , breakdown of Hepatocytes (Hc), Kariolysis(Ka), cellular desquamation inside the Bile duct(Bd) and static RBC inside the Portal vein(Pv), Dilated Hepatic artery(Ha), proliferation of Bile duct due to the failure of fat digestion, increased inflammatory cells(arrow), and appearance of cellular compact, illustrating the severity of inflammation involving the portal area (Pa), associated with the appearance of bloody infiltrate inside the Portal vein(Pv), static RBC's, acute Cellulitis(Arrow), Cellular Compact(Cc) and Bile Duct Fibrosis (Bd), due to the aggravated acute inflammation, breakdown of Hepatocytes (Hc), and the appearance of different areas in the cellular compact(Cc), increased number of Macrophages due to the severity of inflammation(Ma), widening of Sinusoids, and the presence of Lipocytes (Lp), and further illustrating the severely deformed Central vein (Cv), static RBC's inside the Central vein (Cv), bloody infiltration(O), cellulitis(Arrow), extensive nuclear necrosis(Ne) , break down of blood sinusoids(Py) (S), severely deformed hepatic tissue processed with Zyprex, as reflected by the acute changes occurring in the Portal area, deformed Central vein (Cv), static RBC's, widening of Portal area(Pa), breakdown of Bile duct(Bd) and Fibrosis of the Portal vein.

There was also the appearance of acute inflammatory cells around the Central vein (Cv) , dilation of the Hepatic artery (Ha), breakdown of Hepatocytes (Hc) along with separation/sloughing of the Endothelial lining of the Central vein(E), widening of blood Sinusoids(S), breakdown of cellular nuclei(N), dilation of the Portal vein(Pv), abnormal cellular breakdown(Py), the appearance of numerous cellular compact(Cc), and Macrophages(M) as a result of hepatocellular hepatitis.

Treated Group G3

The hepatic tissues of Nigella Sativa oil in treated male rats showed a marked improvement in the shape of hepatocytes(Hc), restoration of cords and re-appearance of nuclei(N), as well as blood Sinusoids and its lining Kupffer cells, and Endothelial lining of the Central vein (Cv). The portal area showed restored normalcy, regression of inflammation of the Portal vein (Pv), decreased proliferation of Bile duct, increased number of Kupffer cells and bi-nucleated cells(2N), improvement of Hepatocytes(Hc), decreased Pyknosis with appearance of prominent nuclei (N), bi-nucleated cells(2N), and prominent Kupffer cells(K). There was also marked improvement in the portal area with restored normal shape to Hepatocytes (Hc), decreased nuclear atrophy and decreased RBC Stasis, decreased inflammation of endothelial cells, and appearance of many bi-nucleated cells (2N).

Control Group G1

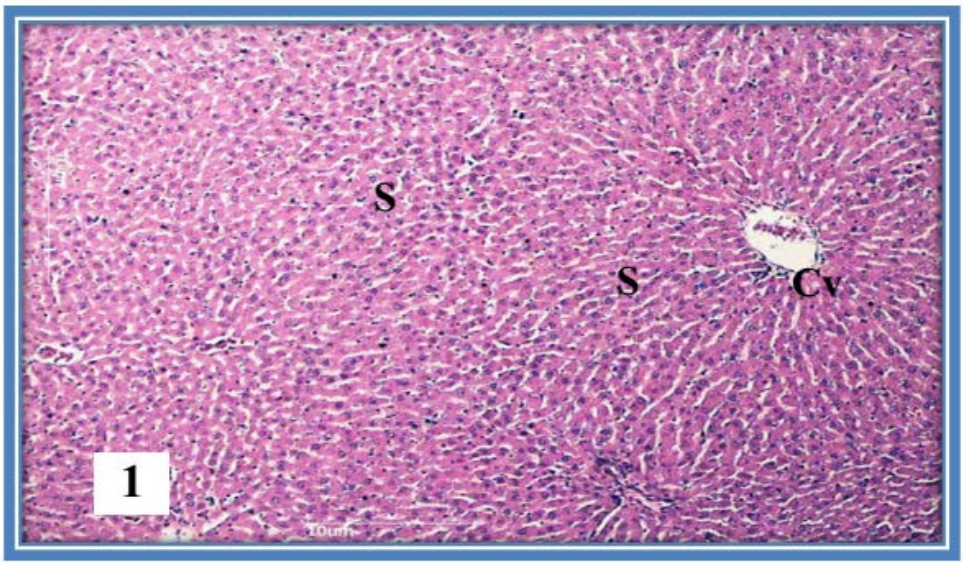


Figure1. cross section of hepatic tissue of male rats, illustrating the general appearance of hepatic tissue and demonstrating Central veins (Cv) and Blood Sinusoids(S), H&EX400

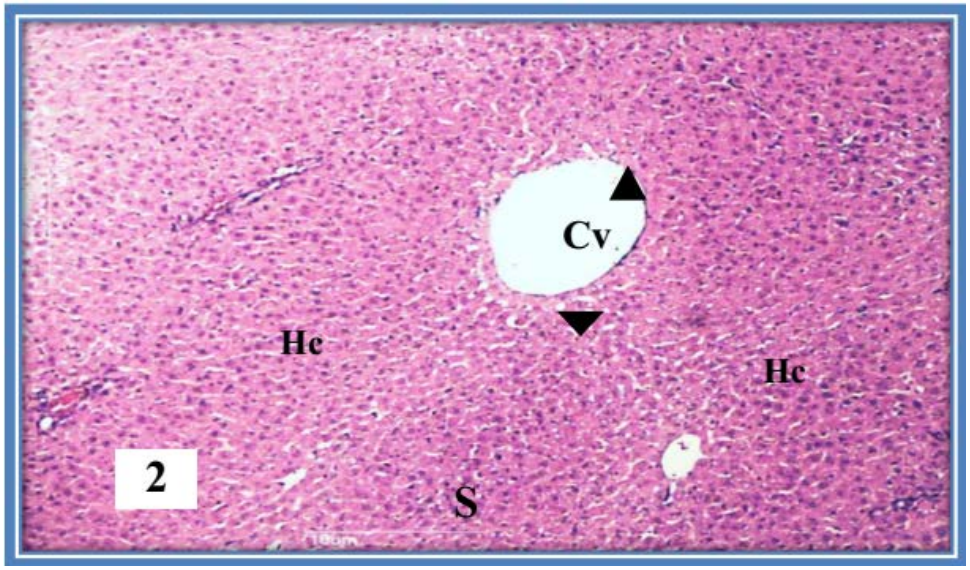


Figure 2. cross section of hepatic tissue of male rats, illustrating the general Arrangement of Hepatocytes (Hc) in cords around Central vein (Cv), in which lining squamous epithelium was clearly visualized(Arrow Head), as well as Blood Sinusoids(S) H&E X400,

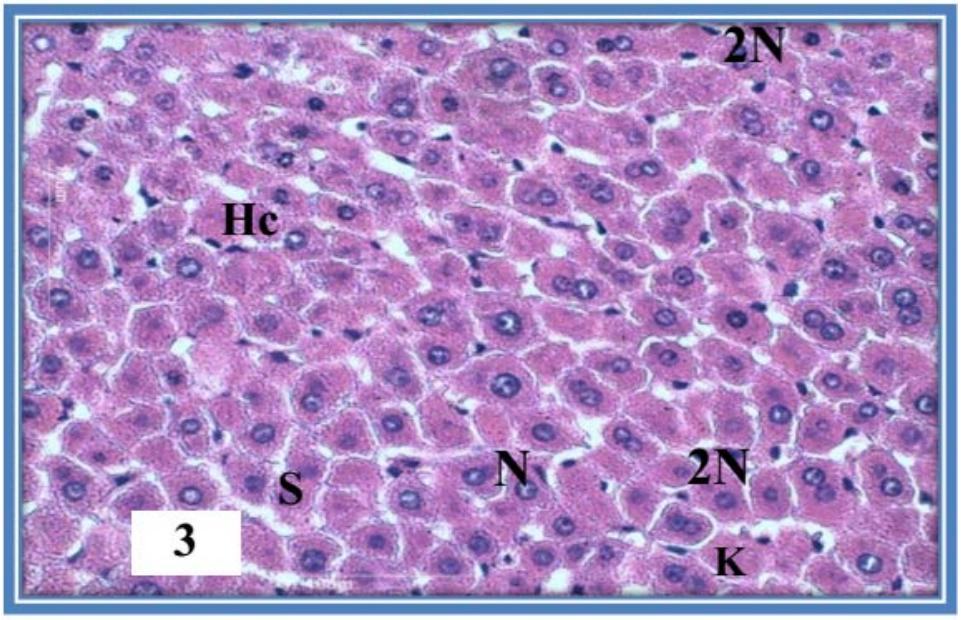


Figure 3. cross section of hepatic tissue of male rats, illustrating well-formed, mono-nucleated(N) arranged in cords. H&E X1000,

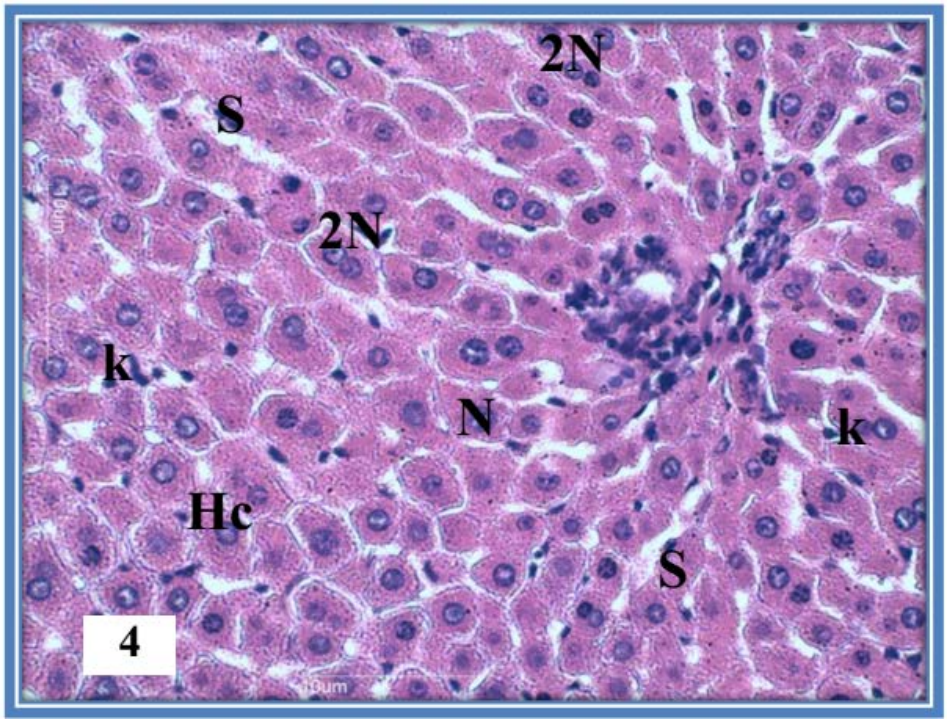


Figure 4. Cross section of hepatic tissue of male rats, , illustrating well-formed, nucleated(N) and bi-nucleated (2N)Hepatocytes (Hc) and clearly visualizing Blood Sinusoids(S) in between cords of Hepatocytes (Hc), as well as Kupffer cells inside Blood SinusoidsH&E X4000

Treatments Group G2

The microscopic picture of Cross sections of the hepatic tissue of the male rats, medicated with Zyprexa (Olanzapine)

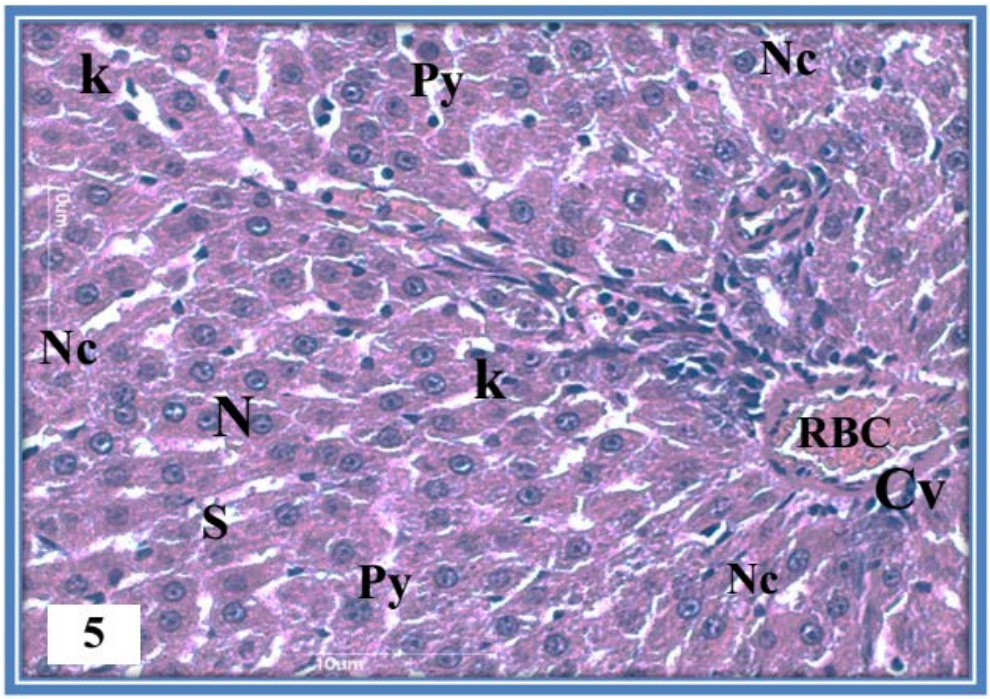


Figure 5. Cross section of hepatic tissue of male rats, medicated with Zyprexaillustrating the liver's portal area(Pa), where stasis was visualized inside the Central vein (Cv), acute inflammation of the Portal Vein(Pv) and bile Duct(Bd), as well as an increased number of Kupffer cells(K) inside Blood Sinusoids(S), breakdown of Hepatocytes (Py) and Nuclear necrosis(Ne)H&E X4000

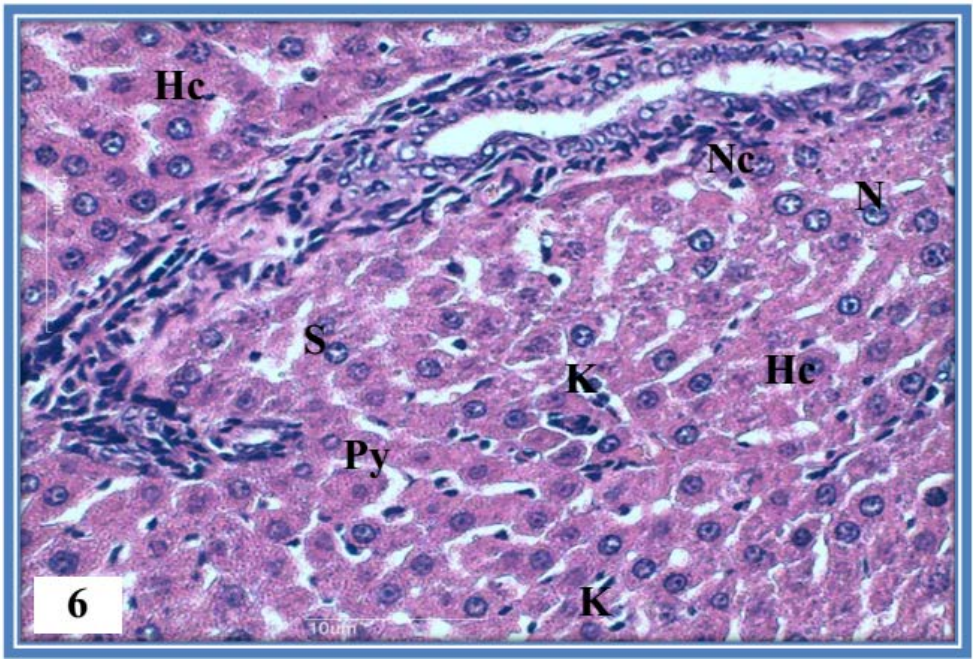


Figure 6. Cross section of hepatic tissue of male rats, medicated with Zyprexa illustrating deformed hepatocytes (Hc), as well as an increased number of Kupffer cells(K) inside Blood Sinusoids(S), atrophic Nuclei and Cellular Necrosis (Py), fibrosis of Hepatocytes (Hc), widening of blood Sinusoids(S), as well fibrosis of the portal area and cellular necrosis.H&EX1000,

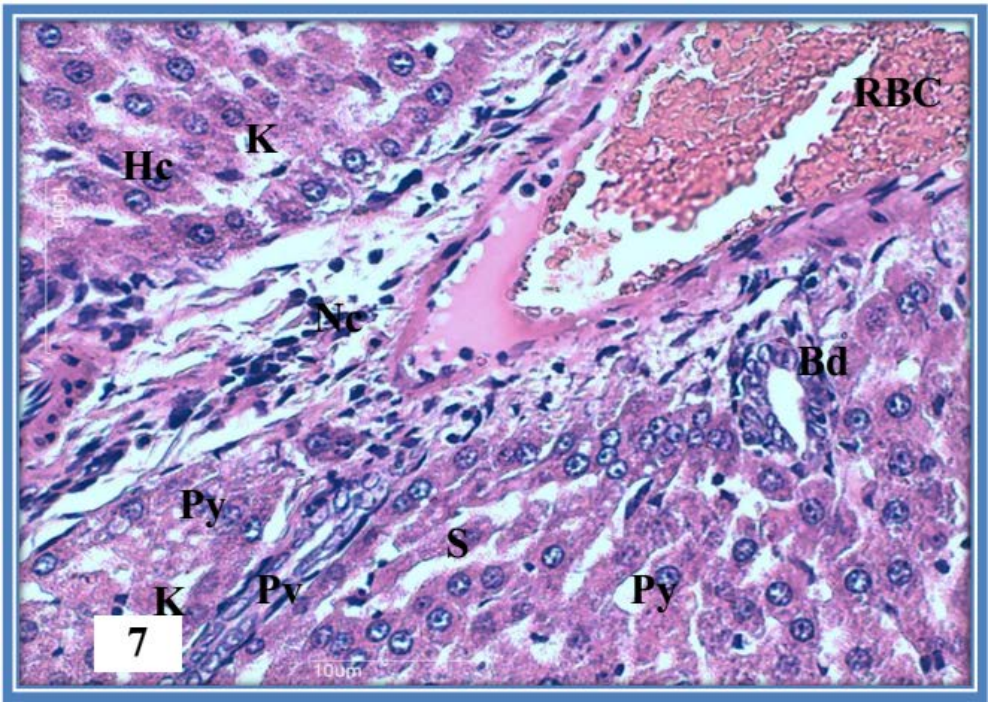


Figure 7. Cross section of hepatic tissue of male rats, medicated with Zyprexa, illustrating the severely deformed portal area, where stasis (RBC), Endothelial Fibrosis(E), Breakdown /pyknosis of Hepatocytes (Hc)(Py) and increased number of Kupffer cells(K) visualized H&E X4000

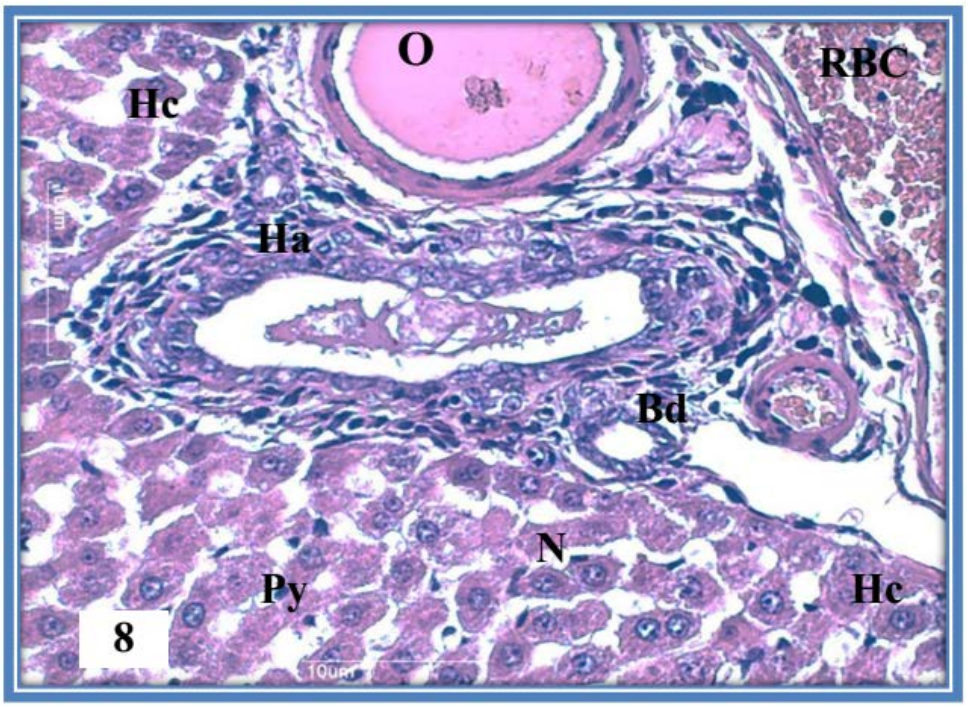


Figure 8. Cross section of hepatic tissue of male rats, medicated with Zyprexa illustrating the severely deformed portal area, where there was marked Fibrosis of Hepatic artery(Ha) (Ha), Acute infiltration inside the Portal Vein(Pv), karyolysis=breakdown of nuclei(Ka) and desquamation of cells inside the Bile duct(Bd)H&EX1000,

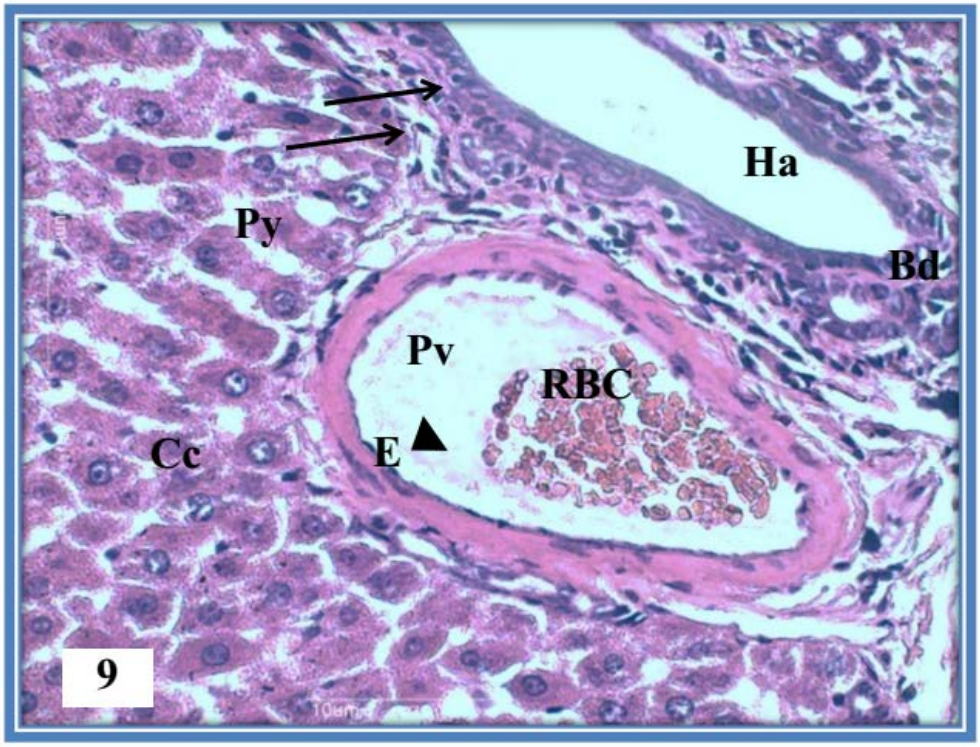


Figure 9. Cross section of hepatic tissue of male rats, medicated with Zyprexa illustrating RBC stasis inside the Portal vein(RBC), dilation of Hepatic artery(Ha), Proliferation of Bile duct(Bd), increased number of inflammatory cells(arrow) and the cellular compact(Cc).H&EX1000,

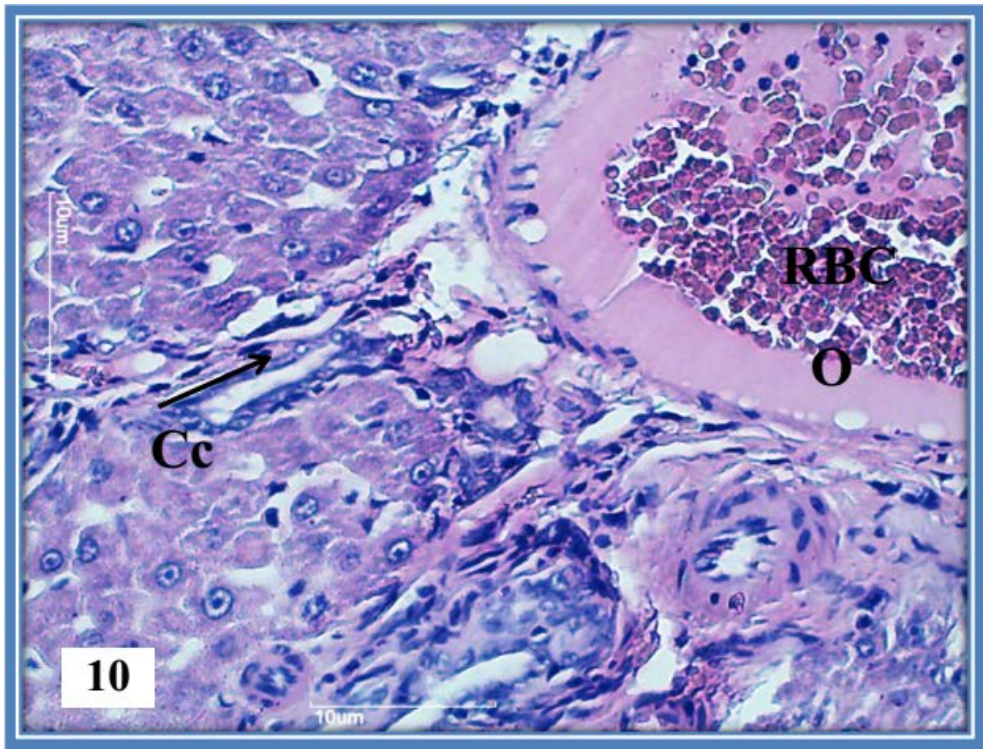


Figure 10. Cross section of hepatic tissue of male rats, medicated with Zyprexa, , magnified from picture No.(40), illustrating severe inflammation of the Portal area (Pa), where a bloody infiltrate(O) was visualized inside the Portal vein(Pv), as well as(RBC) stasis, acute cellular inflammation=Cellulitis (Arrow), Cellular Compact (Cc) and Fibrosis of Bile duct(Bd) H&EX1000

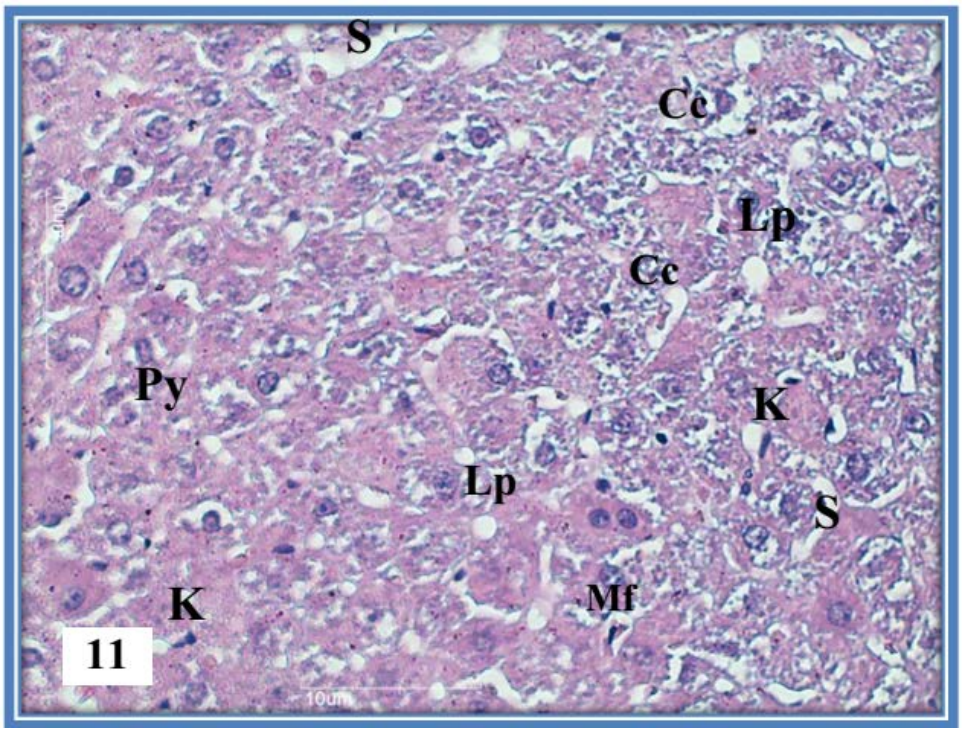


Figure 11. Cross section of hepatic tissue of male rats, medicated with Zyprexa illustrating severe breakdown of Hepatocytes (Py), appearance of different areas in the cellular compact(Cc), increased number of Macrophages(M), widening of Blood Sinusoids and presence of lipocytes(Lp, H&EX1000,

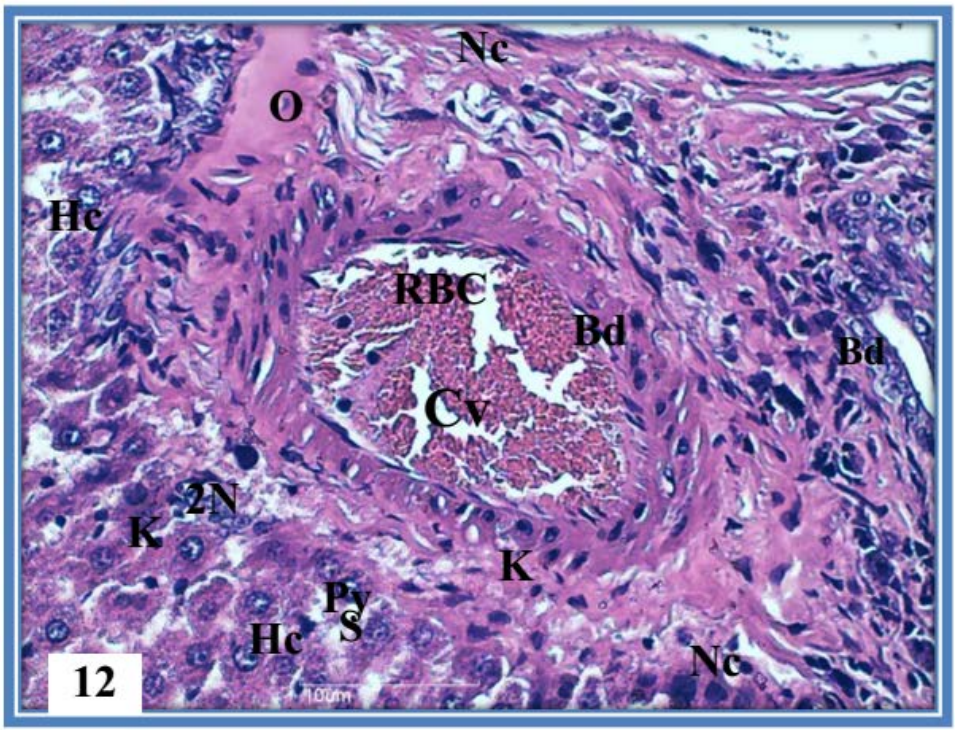


Figure 12. Cross section of hepatic tissue of male rats, medicated with Olanzapine, illustrating severely deformed Central vein (Cv), deformity of stasis(RBC) inside Central vein, bloody infiltrate(O), increased number of inflammatory cells (arrow), widening of blood Sinusoids (S) and Nuclear necrosis(Ne) and Breakdown of Hepatocytes (Py), H&EX1000

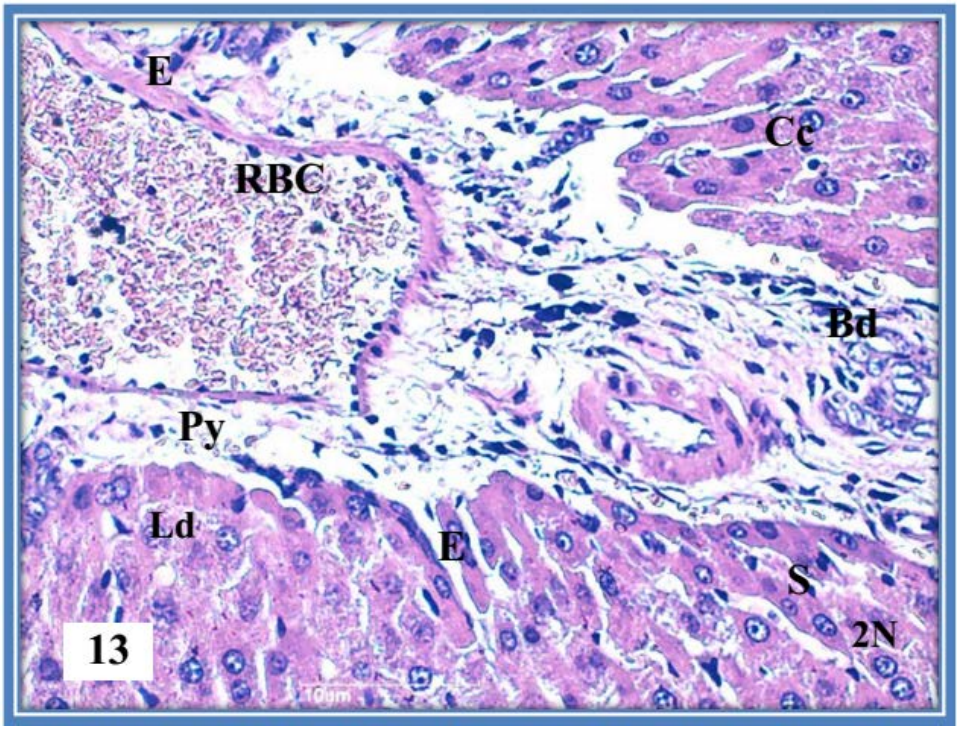


Figure 13. Cross section of hepatic tissue of male rats, medicated with Zyprexa, illustrating severely deformed Hepatic tissue, medicated with Zyprex, where there was an acute change in the Portal area, where there was a marked deformity of Central vein (Cv), stasis(RBC), widening of the Portal area(Pa), breakdown of the bile duct(Bd) as well as fibrosis of the Portal vein(Pv)H&EX1000

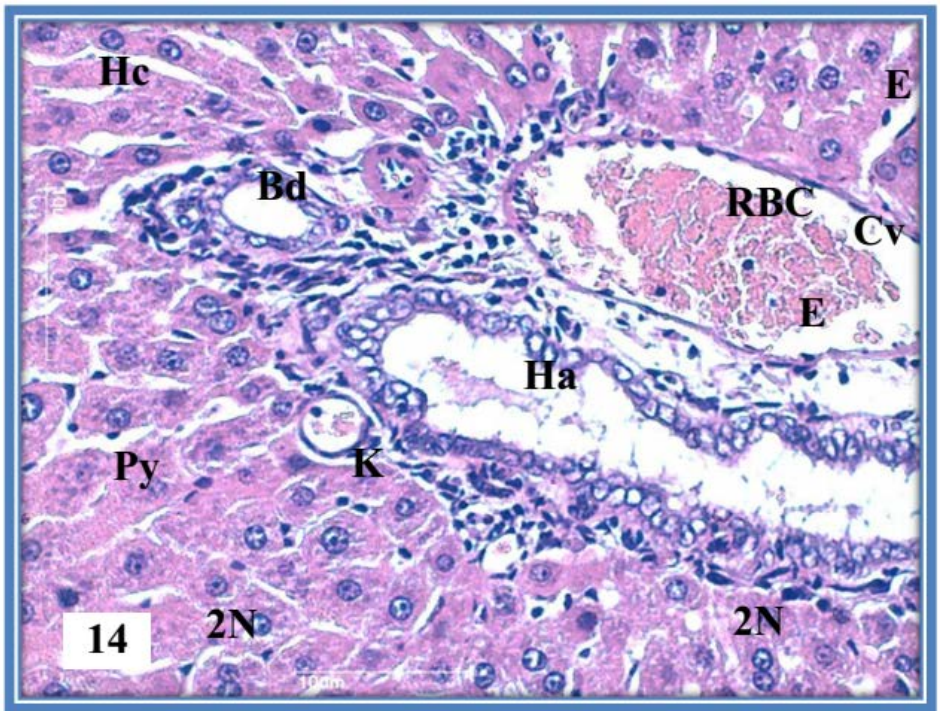
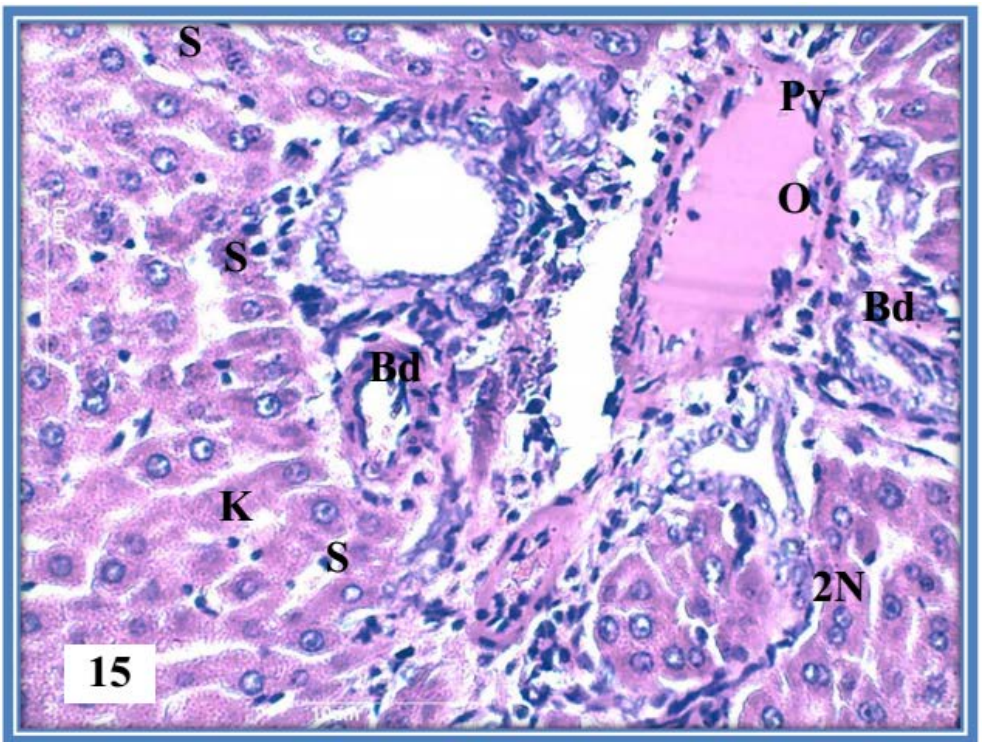


Figure 14. Cross section of hepatic tissue of male rats, medicated with Zyprexa, illustrating acute changes in the Portal area, where there was marked stasis of RBC inside Central vein (Cv), Appearance of acute inflammatory cells around the Central vein (Cv), dilation of the Hepatic artery(Ha), Breakdown of Hepatocytes (Hc)(Py) and separation/sloughing of the endothelial lining of the central vein (Cv)(E, H&E) X4000



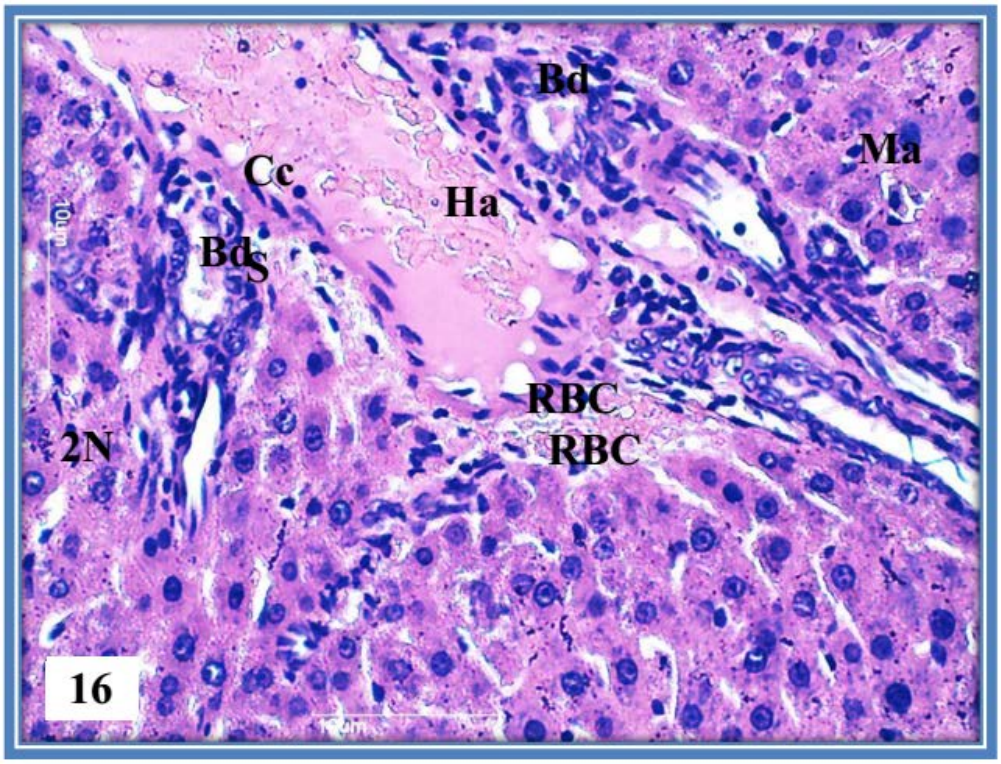


Figure 15-16. Cross section of hepatic tissue of male rats, medicated with Zyprexa, illustrating changes in the Portal area, associated with the appearance of a bloody infiltrate(O) inside the Central vein (Cv), proliferation of Bile ducts (Bd), acute widening of blood Sinusoids(S), appearance of a large number of inflammatory cells in the Portal area(Pa)., dyed with H&E X4000

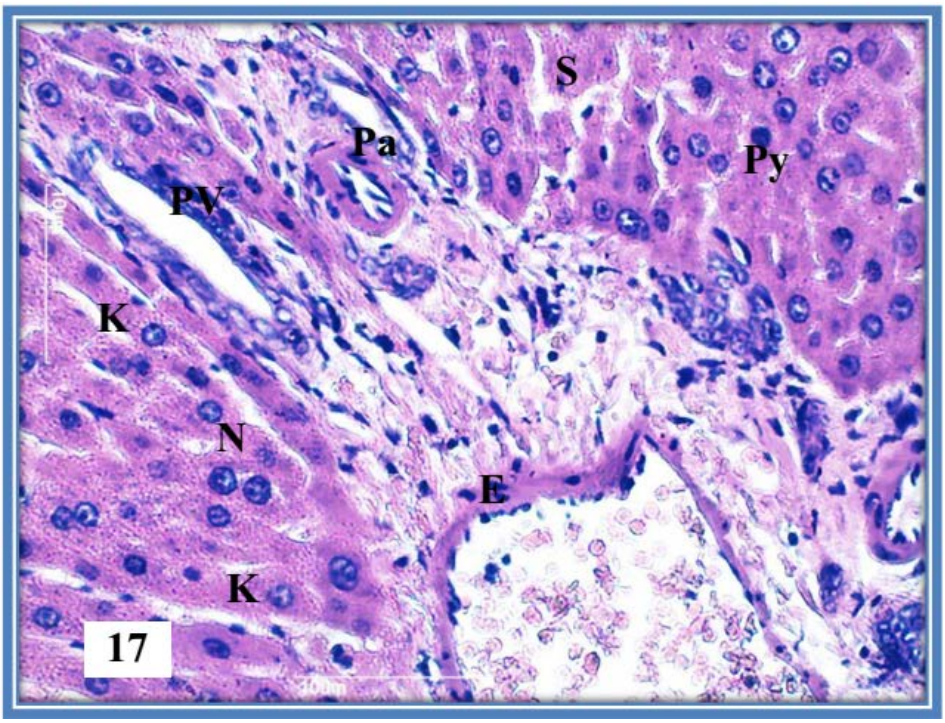


Figure 17. Cross section of hepatic tissue of male rats, medicated with Zyprexa, illustrating an increased number of Kupffer cells (K) inside blood Sinusoids, intracellular nuclear breakdown(N), dilation of the Portal vein (Pv), widening of blood Sinusoids (S) and Nuclear necrosis(Ne)H&E X1000,

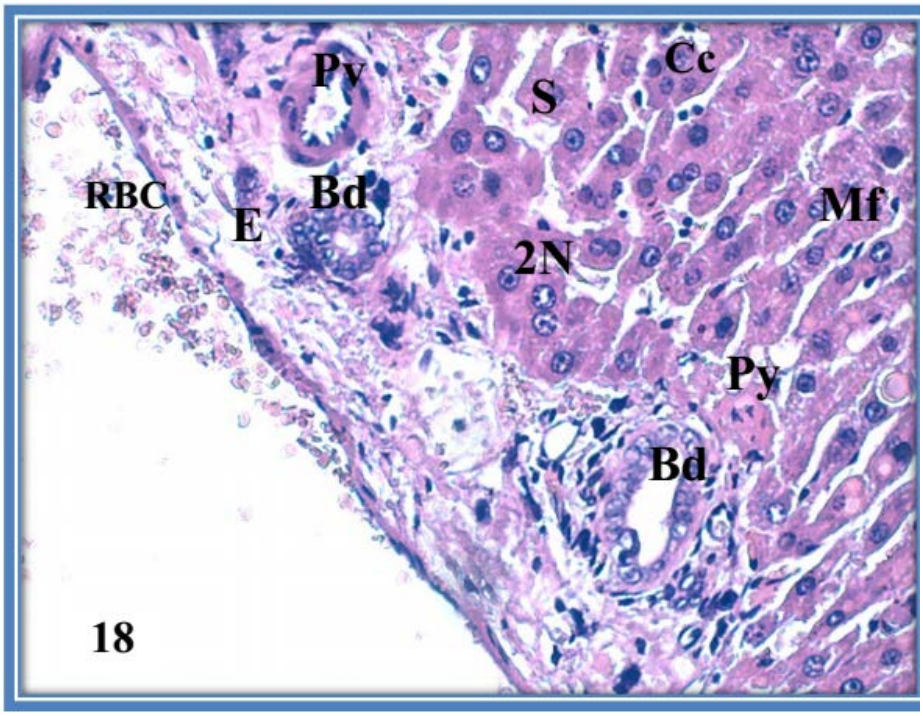


Figure 18. Cross section of hepatic tissue of male rats, medicated with Zyprexa, of a magnified part of the portal area, , illustrating acute cellulitis(arrow), Fibrosis of the Portal vein(Pv), abnormal cellular lysis (Ka) (Py), acute widening of blood Sinusoids(S), appearance of Cellular Compact (Cc) and Macrophages(M)H&E X1000

Treatment Group G3

The microscopic picture of a cross section of hepatic tissue of male rats, of the group treated with Nigella Sativa oil

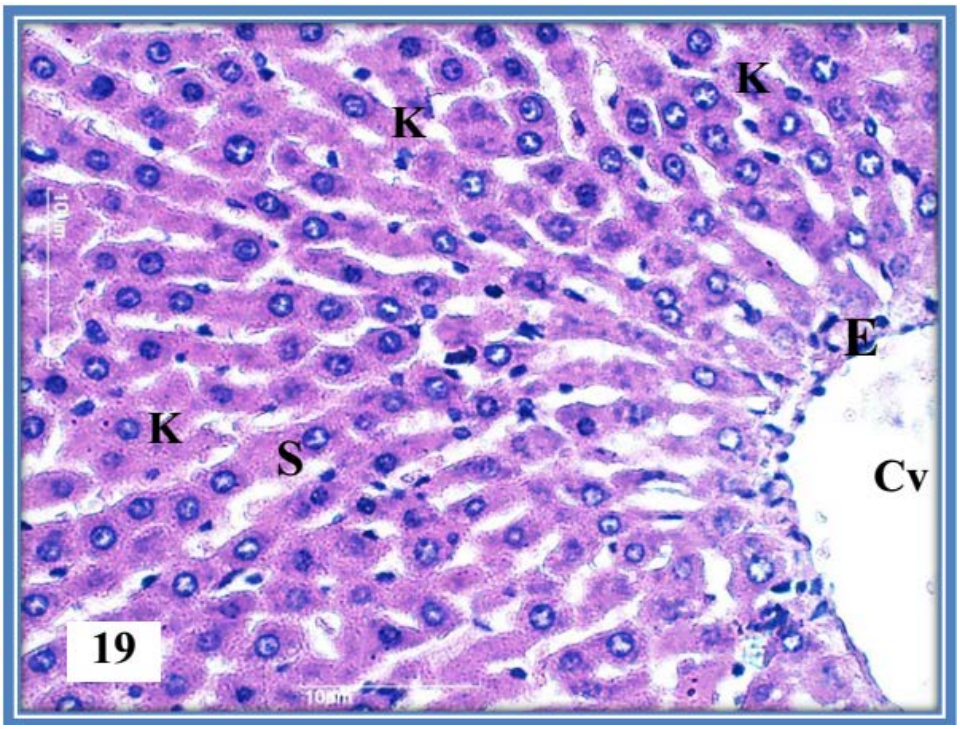


Figure 19. Cross section of hepatic tissue of male rats, of the group treated with Nigella Sativa oil and Olanzapine where the section showed an improved shape of Hepatocytes, appearance of clearly visualized nuclei, (N) and nucleoli (Nu), blood Sinusoids(S) with lining Kupffer cells(K) and Epithelial cells(E) inside the Central vein(Cv), H&E X100,

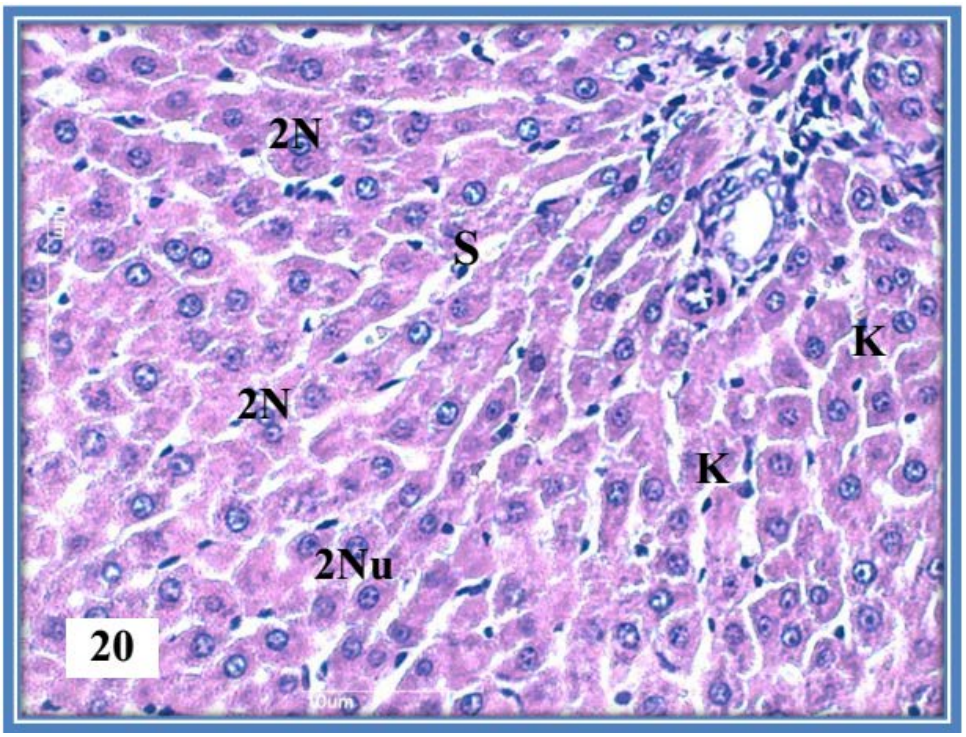


Figure 20. Cross section of hepatic tissue of male rats, of the group treated with Nigella Sativa oil and Olanzapine, shows the portal area, where there is reduced inflammation of the portal vein and the bile duct and increased number of Kupffer cells, as well as bi-nucleated cells., H&E X100

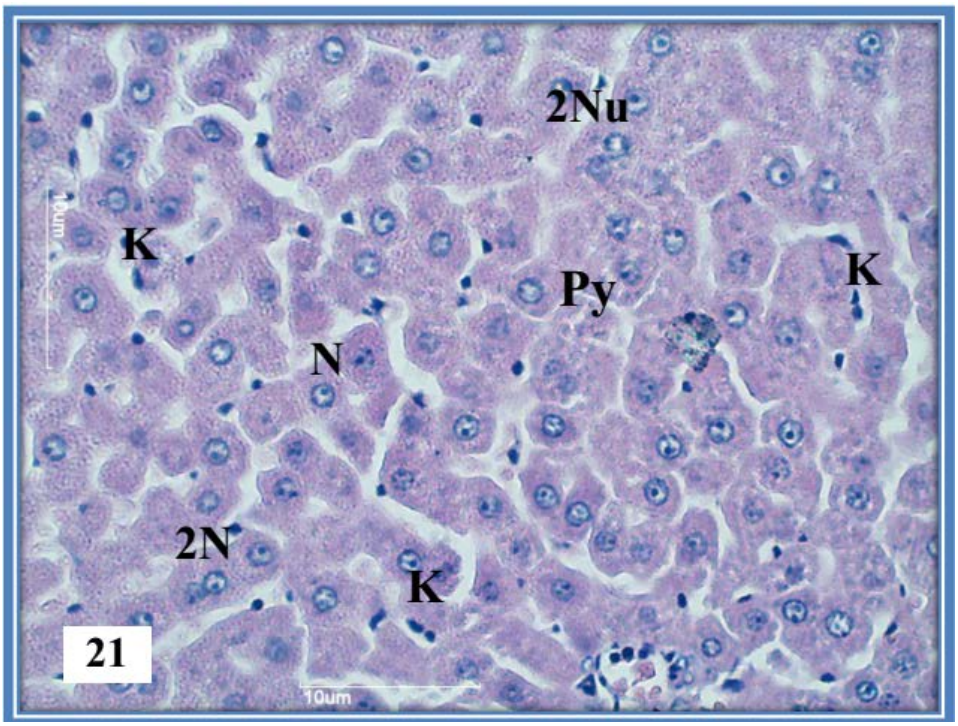


Figure 21. Cross section of hepatic tissue of male rats, of the group treated with Nigella Sativa oil, shows improved hepatocytes(Hc), reduced cellular breakdown/Pyknosis(Py), clearly visualized nuclei(N), appearance of bi-nucleated cells and Kupffer cells(K)H&E X4000,

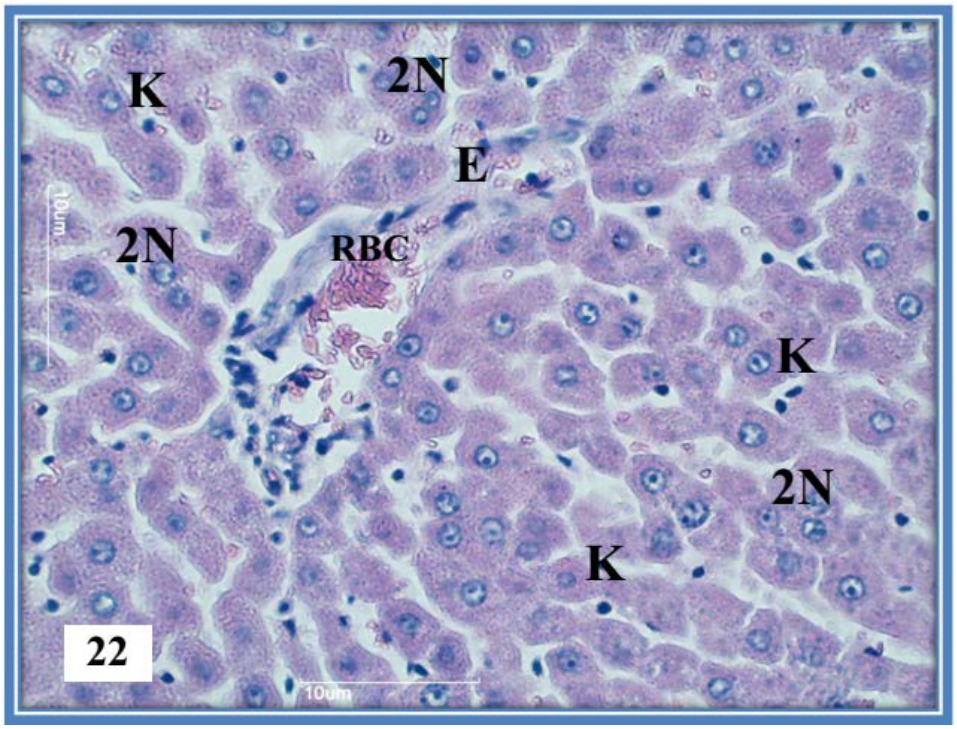


Figure 22. Cross section of hepatic tissue of male rats, of the group treated with Black seed oil, where the section showed an improved portal area (Pa), improved shape of Hepatocytes, less nuclear atrophy(N), improved portal area, where there is less RBC Stasis, less inflammation of endothelial cells and appearance of many -nucleated cells., H&E X4000

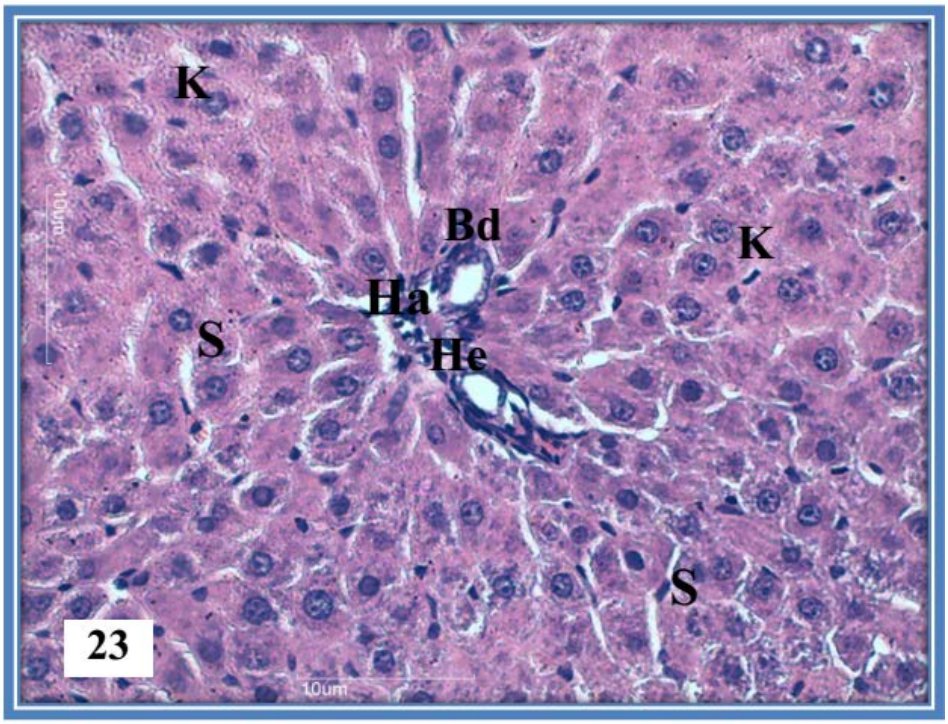


Figure 23. Cross section of hepatic tissue of male rats, of the group treated with Black seed oil, , where the section shows markedly improved portal area (Pa), less RBC Stasis, less inflammation of endothelial cells and appearance of many bi-nucleated cells(2N).H&E X4000

DISCUSSION

Olanzapine is an anti-depressant drug with strong hepatic impact. Many studies proved that ingestion of that drug is associated with the occurrence of inflammatory changes in the liver, hyperlipidemia and hyperglycemia. In this study, the researchers treated a group of rats with the antidepressant Olanzapine at a dose of 6ml/kg body weight for 8 weeks and then with Nigella Sativa oil at a dose of 2ml/kg bodyweight via a gastric tube, based on previous studies. Test rats were administered the above-mentioned doses without deprivation of food or water, simulating a normal person ingesting regular doses of medicine.

The histological structure of the livers of the test rats of the negative control group in the current study corresponded to the results of the microscopic examination conducted by [6,12],who found that the hepatic tissue was seen to be composed of un-trabeculated hepatic lobules. Hepatic cells were mostly arranged in one cell thick radial cords around the Central vein, located at the center of the hepatic lobule, lined by squamous epithelial cells, further corresponding to the results of the examination conducted by [13]. Hepatic lobes were separated by the portal area, containing the thin walled and relatively wide-lumen portal vein, as well as a relatively thicker walled, narrower-lumen hepatic artery, round or oval, wide lumen bile duct, lined with the simple cubical epithelium [9,18,20]

Autopsy of the livers of the medicated male rats showed that the hepatic cellular damage, increased the number of Kupffer cells, as confirmed by [22] who concluded that Olanzapine causes stasis and cellular hemorrhage, leading to hepatitis. Periodic Autopsy of liver sections of male rats treated with Nigella Sativa oil showed the improvement of hepatic cells as proven by [17], who confirmed the efficacy of Nigella Sativa oil against the infection and hyperlipidemia. Such efficacy was attributable to Thymoquinone that played a key role in the improvement of hepatocytes, decreasing the incidence of infection as proven by [27]. This illustrated the efficacy of Nigella Sativa oil as a prophylactic treatment, which reduced the adverse effects of Olanzapine.

CONCLUSION:

Olanzapine has a potent effect in causing the development of hepatitis, leading to hepatic dysfunction, deformity of hepatocytes, stasis of RBC's and acute sinusoidal bleeding. On the other hand, use of Nigella Sativa oil leads to the improved function of hepatocytes, stoppage of bleeding, RBC stasis, pyknosis and nuclear breakdown, thus improving hepatic function.

RECOMMENDATIONS:

1-Olanzapine is an anti-depressant, commonly used in the treatment of depression and psychosis. However, caution must be exercised, and the ingestion of large doses must be avoided.

2-Avoiding excessive ingestion of anti-depressants and regular exercise would make the occurrence of liver damage less likely.

3- The nutritional awareness of the members of the community on the importance of the daily ingestion of Nigella Sativa oil by the patients suffering from the drug induced-hepatitis should be raised.

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