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The Histopathological study of Oxidative Stress by H_2O_2 on hepatic and renal tissues of female mice

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***Abstract*— The aim of this study was to determine the histopathological study of oxidative stress by H2O2 on hepatic and renal tissues of female mice. Liver histological section for a control positive group animal showed moderate to severe central vein congestion loss of regular arrangements of hepatic cords with hepatocytes vacuolation .Whereas, renal histological section revealed atrophy of glomeruli with severe interstitial congestion and tubular epithelia necrosis. Furthermore, liver histological section for a Fadrozole treated group animal revealing the mild to moderate portal vascular congestion, with mild hepatocytes vacuolation and mild inflammatory cells infiltration in portal area; whereas, kidney histological section for this group animal revealed the characteristic close to normal glomeruli, with intact renal tubules epithelia, but characteristic close to normal glomeruli , with intact renal tubules epithelia with significant brush borders. Whereas, liver histological section for a PLGA loaded Fadrozole treated group animal revealed the slight vascular congestion ,with mild hepatocytes swelling; but kidney histological section for this group animal showed the normal glomeruli , with degenerative renal. Obtained data and review of literature suggested that H_2O_2 and estrogen preparations could be responsible for toxic damage of mice liver and kidney, described as fatty change, vascular disorders, hepatic, and focal nodular hyperplasia., tubules epithelia. the normal glomeruli with degenerative renal tubules epithelia. This study concluded that PLGA loaded Fadrozole had the ability in ameliorating harmful oxidative stress.**

***Keywords* — Histopathological, oxidative stress, H_2O_2 , hepatic, renal, mice.**

INTRODUCTION

The liver is a important organ responsible for regulating the body's homeostasis. the liver has a huge impact on metabolism as it metabolises each endogenous molecules (including cholesterol, fatty acids and proteins) and exogenous ones (together with medicines). It converts these substances into less risky chemical substances or eliminates them totally (1). Extended publicity to external materials, including H_2O_2, narcotics, and alcohol, might also bring about liver damage. Research has demonstrated that consuming excessive amounts of H_2O_2 over a prolonged period would possibly bring about intense liver complications due to elevated stages of reactive oxygen species (ROS) in liver cells, leading to oxidative pressure (2). ROS together with hydroxyl radical (HO%), superoxide anion radical (O2 −%), singlet oxygen, and hydrogen peroxide (H_2O_2), are produced throughout the regular metabolism of human cells. They have a great effect on cell signal transmission and proliferation (3).

An excess of ROS can disrupt the stability among oxidation and antioxidant outcomes, resulting in an oxidative stress response in residing organisms. This occurs while the ROS stages surpass the body's potential to effectively respond with antioxidants (4). ROS manufacturing in hepatic cells can lead to oxidative stress, that is characterized by way of the oxidation of important macromolecules together with DNA, proteins, and lipids, resulting in damage (5).Apoptosis may occur as a result of inflammation caused by oxidative stress. Under typical

circumstances, endogenous antioxidant enzymes function to maintain equilibrium in the quantity of ROS by converting these free radicals into a less detrimental chemical compound. Glutathione peroxidase (GPX) is a vital antioxidant enzyme that converts H_2O_2 into water (6). Unaddressed liver injury has the potential to result in numerous life-threatening illnesses. Liver illnesses result in 2 million fatalities annually on a global scale, with cirrhosis being responsible for half of these deaths (7). The components of the genetic machinery that either facilitate or inhibit apoptotic cell death are being gradually understood. However, the entire process is still not fully comprehended and may indeed differ depending on the specific stimuli and types of cells involved (8). Since necrosis is a passive process, it is likely that this type of cell death does not trigger the chemical reactions that are involved in apoptosis. Nevertheless, the molecular mechanisms of necrosis had not been well investigated. Remarkably, a solitary stimulus has the potential to induce either apoptotic or necrotic cell death in a controlled environment, contingent upon the intensity of the stimulus, the cellular growth stage, and the circumstances of the culture (9). The same results were observed in living organisms in the liver, where injury caused by ROS resulted in simultaneous death of hepatocytes through both necrosis and apoptosis (10). The Neutrophil extracellular traps (NETs) that are discharged in the liver blood vessels remain firmly attached to the walls of the blood vessels. The liver injury in this instance was ascribed to the activity of polymorphonuclear neutrophil elastase (PMN-ELA), with at least 80% of the resulting damage being caused by the creation of NETs (11). Fibrogenesis refers to the procedure wherein cells deposit abnormal extracellular matrix (ECM). According to Wynn and Vannella (12), it's far regularly regularly occurring that that is a wound-recuperation response to tissue damage that dose not leave. The excessive accumulation of ECM outcomes within the lack of organ shape and the impairment of organ feature. Fibrosis changed into located to be usually related with persistent organ disorder, particularly in the kidneys and liver (13).

Macrophages are the primary starting place of numerous types of matrix metalloproteinase (MMPs) and tissue inhibitors of MMPs (TIMPs). The MMPs are chargeable for each the breakdown of ECM and the enhancement of the inflammatory reaction, therefore affecting the advancement of tissue remodelling. Certain MMPs play a sizeable function in promoting fibrosis. One example is MMP-12, which is an elastase launched by means of macrophages. It is strongly stimulated by IL-13 inside the kidney and liver at some point of the development of IL-13-based fibrosis (12). Activated macrophages can recruit myofibroblasts and get worse the infiltration of inflammatory cells to regions of tissue damage. This results in a sizeable production of various chemokines, growth elements and cytokines (14). Research has established that hepatic macrophages cell the survival of myofibroblasts by way of activating NF-κB in fibroblasts. This activation is critical for the progression of renal and hepatic fibrosis (12). Fibroblasts derived from sick kidneys showcase particular methylation patterns that lead to the suppression of the RAS intracellular signalling system and the extended presence of myofibroblasts (15). The non-stop activation of fibroblasts with the aid of clearly happening seasoned-fibrotic cytokines has been shown in systemic sclerosis, as well as in liver and kidney fibrosis (16). The patience and intensity of NETs, whether in an acute or chronic phase, can either fight ailment or potentially make contributions to the development of sickness (17). PMN serine-proteases located outside of cells in tissues can also make a contribution to an immoderate inflammatory reaction, damage specific to positive tissues, and ultimately the formation of fibrosis inside the liver and kidney (18,19).

Regardless of the initial causes, if this process becomes dysregulated or if the tissue damaging stimuli continues, it can result in the formation of a persistent fibrotic "scar" at the location of tissue injury. This condition is marked by an excessive buildup of substances outside of cells called ECM components. These components include hyaluronic acid, proteoglycans, fibronectin and notably collagen I (COL1) (12). Fibrosis production due to persistent inflammation has been seen in various human tissues, including the kidney (20).

Poly lactic-co-glycolic acid (PLGA) is a usually employed biodegradable substance utilised for encapsulating diverse therapeutic agents, consisting of hydrophilic and hydrophobic small molecule drug treatments, proteins and DNA. In addition, it has confirmed high-quality biocompatibility (21). Managing inflammatory ailments is tough for the reason that sufferers range of their reaction and the irritation process is complex. this approach that remedy wishes to the tailor-made to each man or women affected person. Theranostics has the ability to permit the well timed assessment of the effectiveness of a remedy and the evaluation of its safety and toxicity, leading to personalized remedy methods. Macrophages, which are crucial in inflammatory disorders, have diverse functions in both resolving and worsening the diseases. Researchers have investigated the use of nanostructures made from PLGA to specifically target and control the disease characteristics and natural ability of macrophages to engulf particles, which ultimately helps resolve inflammation in many diseases (22). The toxicity effects of PLGA NPs have been assessed on various cell lines, demonstrating minimal or no toxicity in laboratory conditions (23). Additionally, the invivo toxicity of PLGA-based nanostructures has been investigated on visceral organs (such as the intestine, liver, spleen, brain and kidney) following oral exposure to nanoparticles for a duration of less than seven days. A prior investigation examined the toxicity of both PLGA and surface-modified PLGA.

The enzyme responsible for the conversion of testosterone to estradiol is P450 aromatase. This enzyme is not only found in the ovaries but also in other tissues such as brain, bone, , blood vessels and adipose tissue. The synthesis of oestrogen by aromatase in local tissues outside of the gonads becomes the primary source of oestrogen in patients who have gone through menopause. Aromatase has been diagnosed in diverse cellular sorts within the brain, which include astrocytes, neurons,

smooth muscle cells, and endothelial cells. Aromatase inhibitors (AIs), which have been administered in medical practice to treat breast cancer, had the capability to elevate the incidence of cardiovascular activities. this became partially because of the beneficial impact of oestrogen on plasma lipids (24). Aromatase inhibitors, along with Fadrozole and Afema, had been created to treat estrogen-established malignancies. These inhibitors are used particularly for post-menopausal women with malignant breast tumours and to enhance vascular endothelial function (25). Fadrozole exhibited inhibitory outcomes at the production of adrenal cortisol and aldosterone (26). The inhibitory consequences of fadrozole were generally reversible, and the blockading of oestrogen turned into reliant on the drug being constantly gift (27). The assessment of nanoparticles became conducted following their oral transport to rats. The biodistribution of each PLGA and modified PLGA tested similar outcomes, indicating negligible hepatotoxicity and shortage of nephrotoxicity (28).

Materials And Methods

Ethical Approval: The study was conducted in adherence to the ethical principles out-lined in the Helsinki Declaration. The study protocol, subject information, and per-mission form were examined and authorized by the local ethics committee under the reference number UOK.VET. PH.2024.086

Experiment One: Induction of oxidative stress (OS)

Fifty-five adult female mice were provided food and water ad libitum and would be induced oxidative stress by injection 17 B-estradiol (E2) mixed with olive oil given (I/M) at a dose (0.003) mg/kg/once per week according to (29). In addition, exposed (0.4) % of hydrogen peroxide (H2O2) with drinking water, according to (30) for (8) weeks. After the end period of induction five mice were taken randomly and the histopathological section of liver and kidney, OS was induced.

Experiment Two: comparative effects between Fadrozole alone and PLGA Fadrozole on oxidative stress.

Fifty adult female mice were used in this experiment and divided into five equal groups, oxidative stress was induced in all groups except the negative control group, the third group were given PLGA alone daily dose (1.1) mg/ kg according to (31), the fourth group were given dose of (0.601) mg/kg Fadrozole. the fifth group were given (0.484) mg/kg of PLGA Fadrozole (32), the period of experiment was 30 days.

Histopathological study

After the end of the treatment period, the animals were killed, and the kidney and liver tissues were examined and histopathological. The samples were placed in formalin solution (10%), then stained with hematoxylin and eosin, and the method was followed according to (33).

RESULT & DISCUSSION

Histopathological changes with liver and kidney

Photomicrograph of hepatic histological section for a control negative group animal showed the normal histological arrangements of hepatocytes in cords around the central vein shown in (Figure 1); the normal histological appearance of hepatocytes with significant granular cytoplasm and prominent nuclei surround the central vein were shown in (Figure 2); as well as, Photomicrograph of renal histological section for a control negative group animal showing the normal histological appearance of the cortex with significant glomeruli (white arrow) and normal renal tubules were shown in (Figure 3); the normal histological morphology of the glomeruli with normal renal tubules endothelia were shown in (Figure 4); Whereas, Photomicrograph of liver histological section for a control positive group animal showing the significant histological alteration manifested by moderate to severe central vein congestion, loss of regular arrangements of hepatic cords with hepatocytes vacuolation and presence of perivascular inflammatory cells infiltration were shown in (Figure 5); the significant histological alteration manifested by portal vein congestion, with hepatocytes vacuolation and inflammatory cells infiltration in portal area were shown in (Figure 6).Whereas photomicrograph of renal histological section for a control positive group animal revealing the remarkable histological alteration characterized by atrophy of glomeruli , with severe interstitial congestion and hemolysis, inflammatory cells infiltration and tubular epithelia necrosis were shown (Figure 7); the remarkable histological alteration characterized by atrophy of the glomeruli, with significant cortical tubules epithelia degeneration, inflammatory cells infiltration ,were shown in (Figure 8); Photomicrograph of liver histological section for a PLGA treated group animal showing the histological alteration represented by mild vascular congestion, with marked hepatocytes vacuolation (fatty degeneration) ,were shown in (Figure 9) ; but figure 10, shown photomicrograph of liver histological section for a PLGA treated group animal showing the histological alteration represented by mild to moderate perivascular inflammatory cells infiltration, with marked hepatocytes vacuolation. Whereas Figure (11) showed kidney histological section for a PLGA treated group animal revealing the remarkable histological alteration characterized by atrophied glomeruli, with severe interstitial and tubular tissues necrosis, inflammatory cells infiltration; Figure (12) revealed the histological changes characterized by atrophied glomeruli, with severe interstitial and tubular tissues necrosis ; Furthermore, Figure (13) Photomicrograph of liver histological section for a Fadrozole treated group animal revealing the mild to moderate portal vascular congestion, with mild hepatocytes vacuolation and mild inflammatory cells infiltration in portal area; Figure (14) showed slight hepatocytes vacuolation , whereas, Figure (15) Photomicrograph of kidney histological section for a Fadrozole treated group animal revealing the characteristic close to normal glomeruli, with intact renal tubules epithelia,but Figure (16) revealed characteristic close to normal glomeruli, with intact renal tubules epithelia with significant brush borders; whereas Figure (17) Photomicrograph of liver histological section for a PLGA and Fadrozole treated group animal revealed the slight vascular congestion, with mild hepatocytes swelling; Figure (18) revealed normal central vein , with mild hepatocytes swelling (degeneration), but Figure (19) Photomicrograph of kidney histological section for PLGA and Fadrozole treated group

animal showing the normal glomeruli , with degenerative renal tubules epithelia ; Figure (20) showed the normal glomeruli, with degenerative renal tubules epithelia.

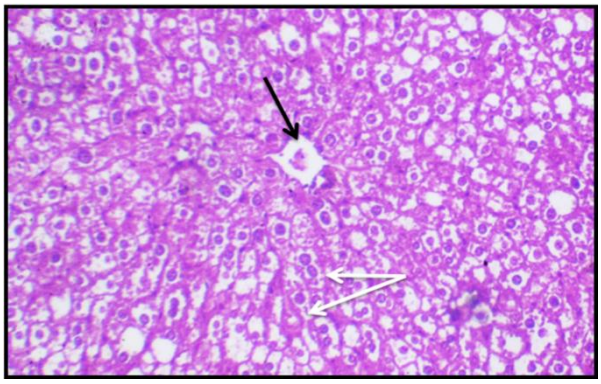

Figure 1. Photomicrograph of hepatic histological section for a control negative group animal showing the normal histological arrangements of hepatocytes in cords (white arrow) around the central vein (black arrow). (E and H, 10X)

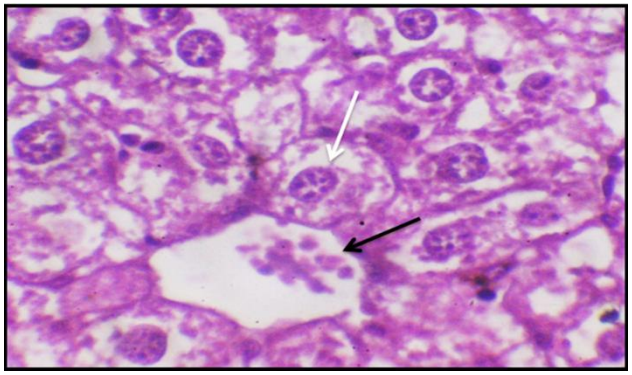

Figure 2. Photomicrograph of hepatic histological section for a control negative group animal showing the normal histological appearance of hepatocytes with significant granular cytoplasm and prominent nuclei (white arrow) surround the central vein (black arrow). (E and H, 40X)

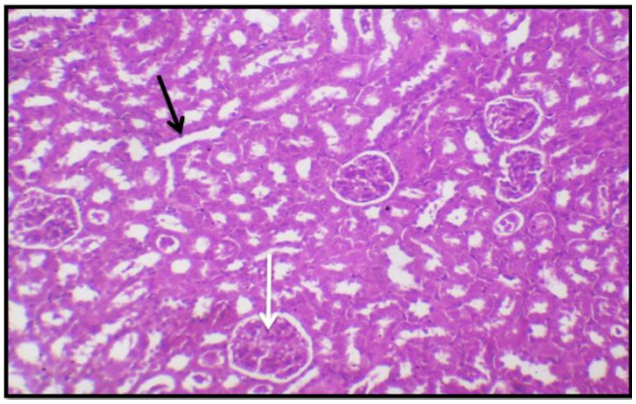

Figure 3. Photomicrograph of renal histological section for a control negative group animal showing the normal histological appearance of the cortex with significant glomeruli (white arrow) and normal renal tubules (black arrow). (E and H, 10X)

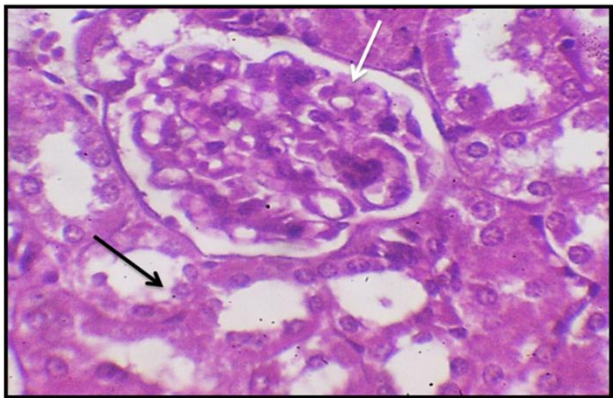

Figure 4. Photomicrograph of kidney histological section for a control negative group animal showing the normal histological morphology of the glomeruli (white arrow) with normal renal tubules endothelia (black arrow). (E and H, 40X)

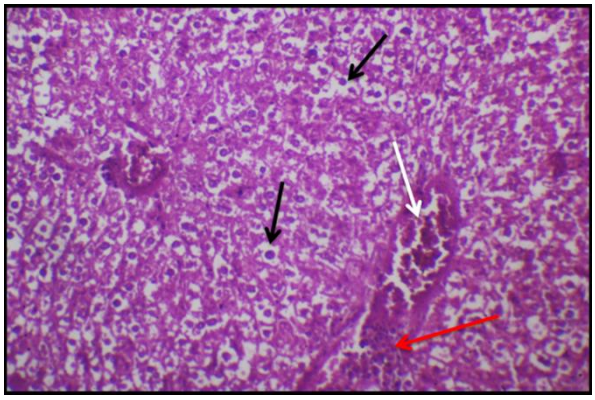

Figure 5. Photomicrograph of liver histological section for a control positive group animal showing the significant histological alteration manifested by moderate to severe central vein congestion (white arrow), presence of perivascular inflammatory cells infiltration (red arrow) and loss of regular arrangements of hepatic cords with hepatocytes vacuolation (black arrow). (E and H, 10X)

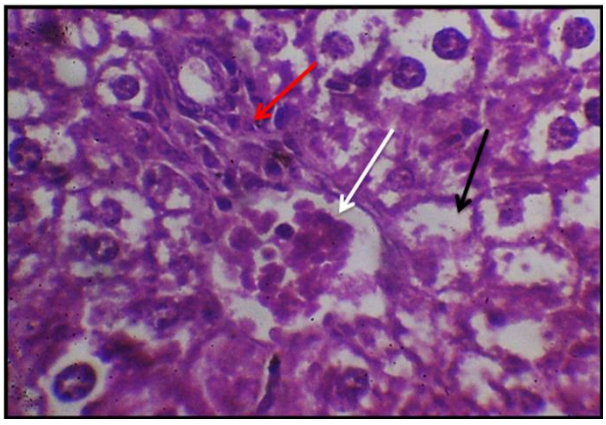

Figure 6. Photomicrograph of liver histological section for a control positive group animal showing the significant histological alteration manifested by portal vein congestion (white arrow), with hepatocytes vacuolation (black arrow) and

inflammatory cells infiltration in portal area (red arrow). (E and H, 40X)

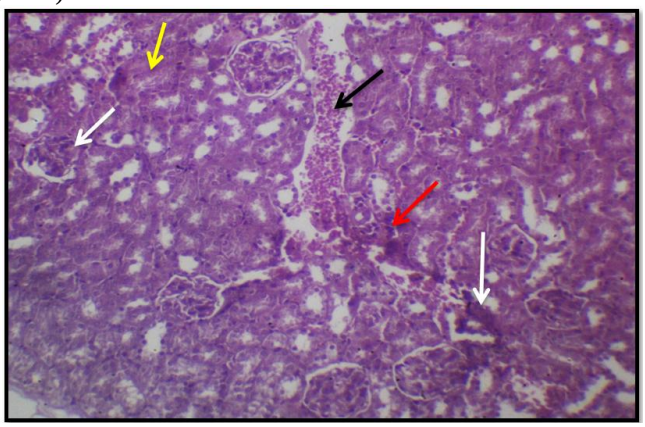

Figure 7. Photomicrograph of renal histological section for a control positive group animal revealing the remarkable histological alteration characterized by atrophy of glomeruli (white arrow) ,with severe interstitial congestion and hemolysis (black arrow) , inflammatory cells infiltration (red arrow) and tubular epithelia necrosis(yellow arrow) .(H and E, 10X)

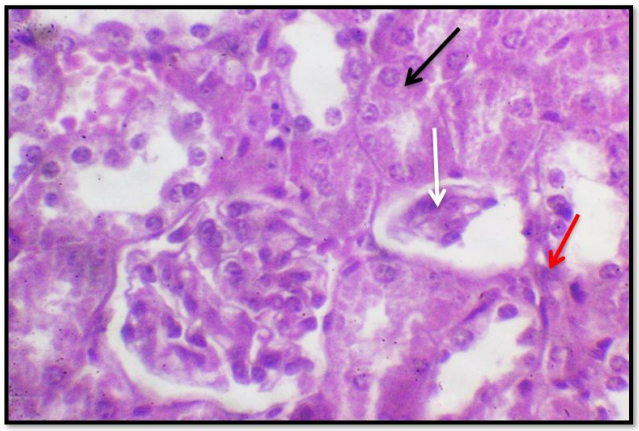

Figure 8. Photomicrograph of renal histological section for a control positive group animal revealing the remarkable histological alteration characterized by atrophy of the glomeruli (white arrow) , with significant cortical tubules epithelia degeneration (black arrow) , inflammatory cells infiltration (red arrow).(H and E, 40X)

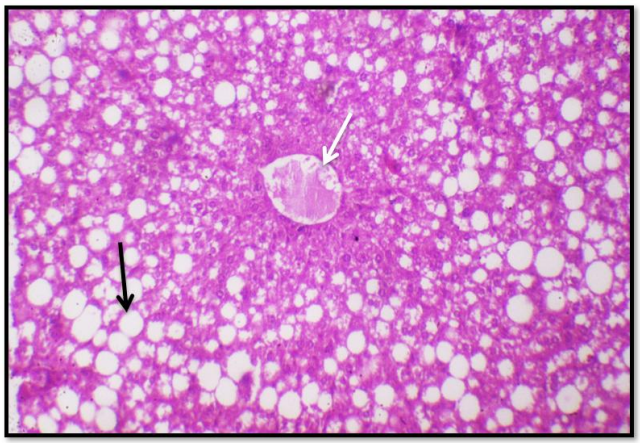

Figure 9. Photomicrograph of liver histological section for a PLGA treated group animal showing the histological alteration represented by mild vascular congestion (white arrow) , with marked hepatocytes vacuolation (fatty degeneration)(black arrow).(H and E, 10X)

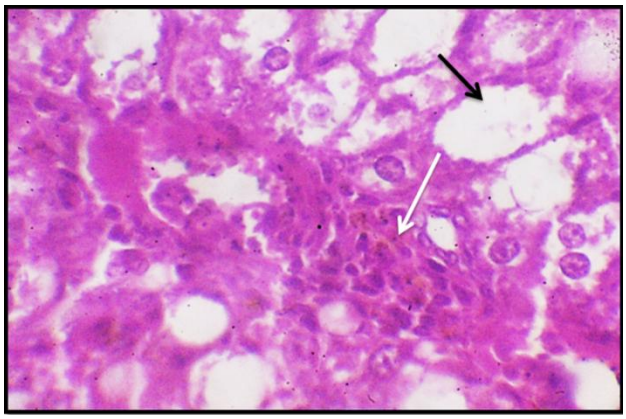

Figure 10. Photomicrograph of liver histological section for a PLGA treated group animal showing the histological alteration represented by mild to moderate perivascular inflammatory cells infiltration (white arrow), with marked hepatocytes vacuolation (black arrow). (E and H, 40X)

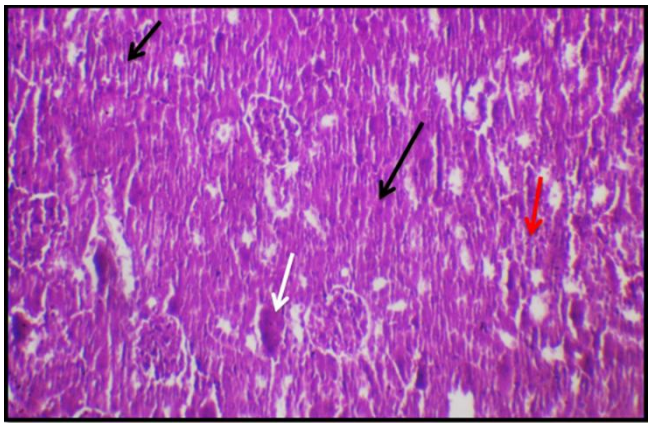

Figure 11. Photomicrograph of kidney histological section for a PLGA treated group animal revealing the remarkable histological alteration characterized by atrophied glomeruli (white arrow) , inflammatory cells infiltration (red arrow), with severe interstitial and tubular tissues necrosis (black arrow). (E and H, 10X)

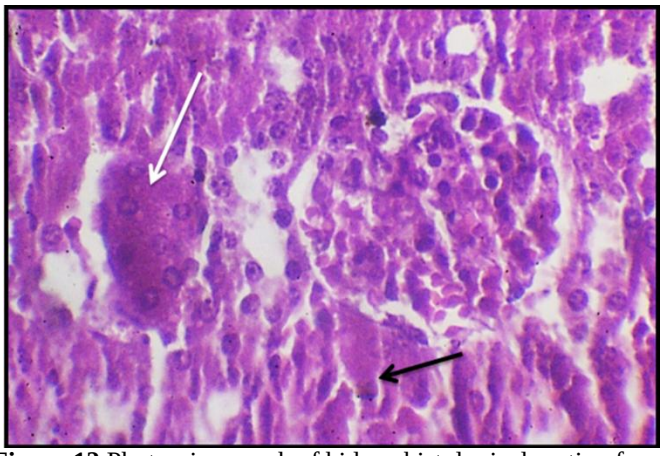

Figure 12. Photomicrograph of kidney histological section for a PLGA treated group animal revealing the histological changes characterized by atrophied glomeruli (white arrow), with severe interstitial and tubular tissues necrosis (black arrow). (E andH, 40X)

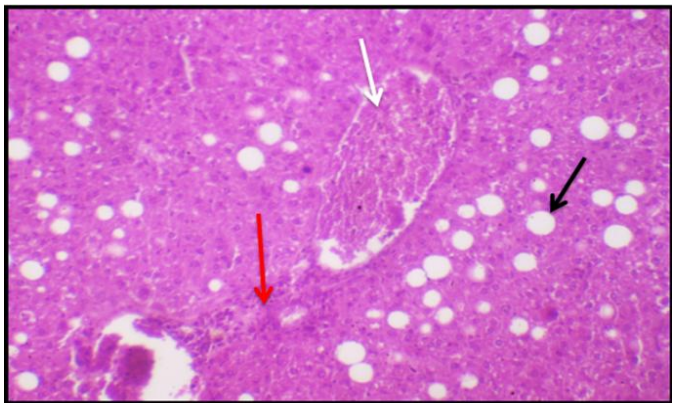

Figure 13. Photomicrograph of liver histological section for a Fadrozole treated group animal revealing the mild to moderate portal vascular congestion (white arrow), with mild hepatocytes vacuolation (black arrow) and mild inflammatory cells infiltration in portal area (red arrow). (E and H, 10X)

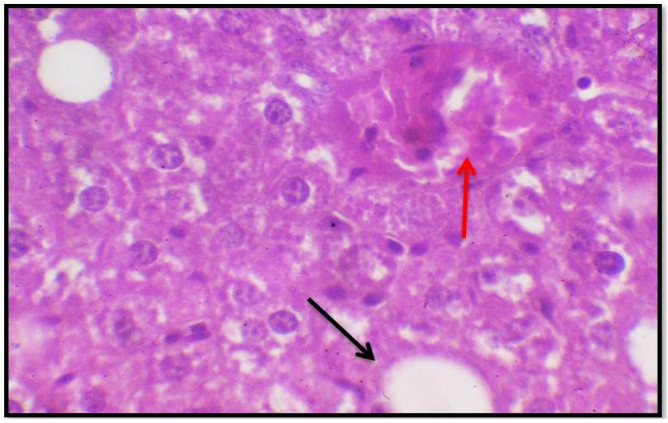

Figure 14. Photomicrograph of liver histological section for Fadrozole treated group animal showing slight hepatocytes vacuolation (black arrow) with area of focal necrotic hepatocyte (red arrow). (H and E, 40X)

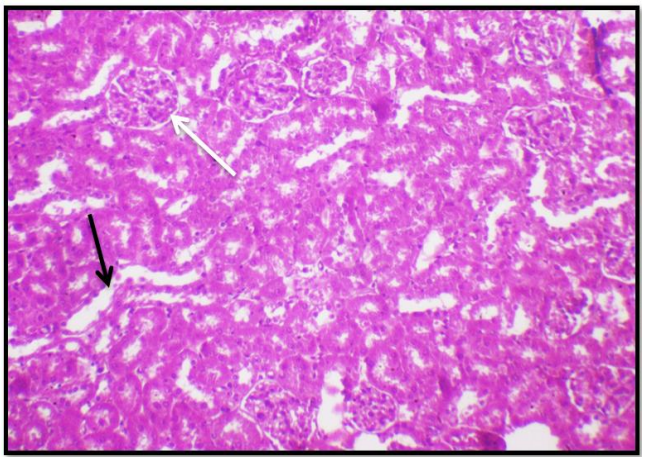

Figure 15. Photomicrograph of kidney histological section for a Fadrozole treated group animal revealing the characteristic close to normal glomeruli (white arrow) , with intact renal tubules epithelia (black arrow) .(H and E, 10X)

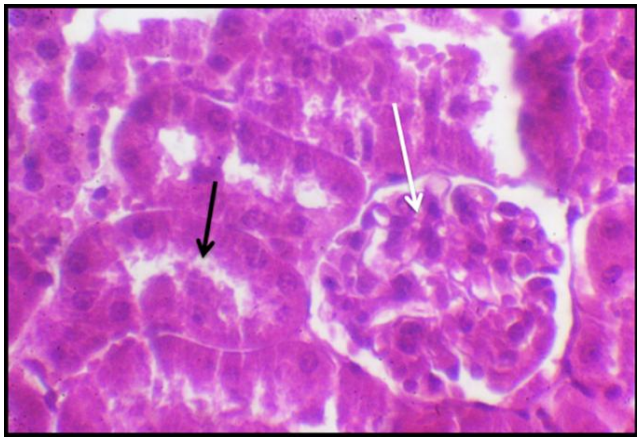

Figure 16. Photomicrograph of kidney histological section for a Fadrozole treated group animal revealing characteristic close to normal glomeruli (white arrow), with intact renal tubules epithelia with significant brush borders (black arrow).(E and H, 40X)

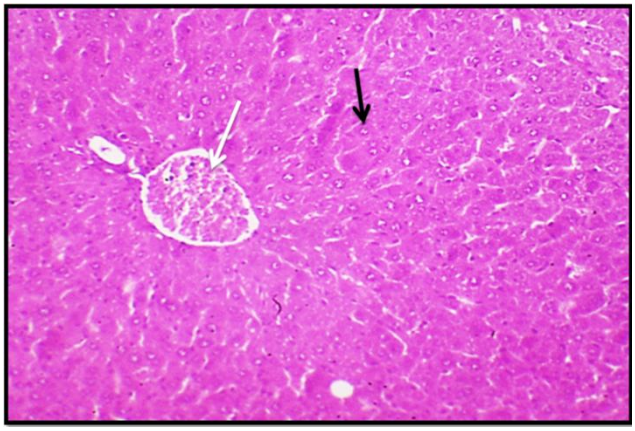

Figure 17. Photomicrograph of liver histological section for a PLGA and Fadrozole treated group animal revealing the slight vascular congestion (white arrow), with mild hepatocytes swelling (black arrow). (E and H, 10X)

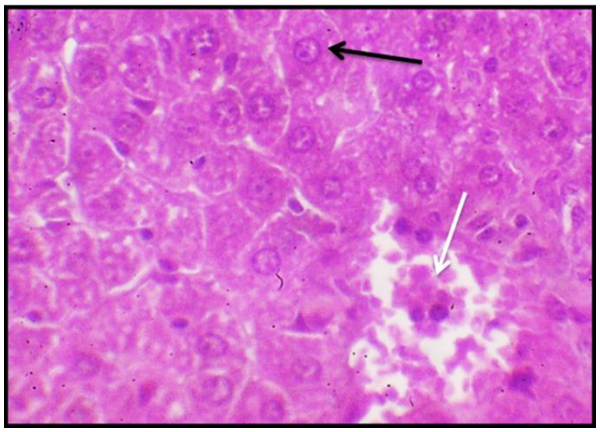

Figure 18. Photomicrograph of liver histological section for a PLGA and Fadrozole treated group animal revealing normal central vein (white arrow), with mild hepatocytes swelling (degeneration) (black arrow). (E and H, 40X)

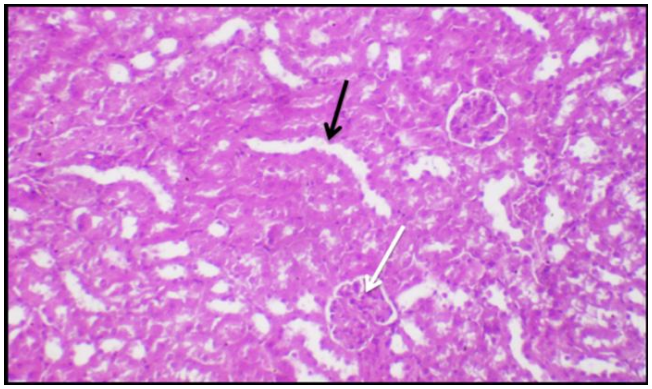

Figure 19. Photomicrograph of kidney histological section for PLGA and Fadrozole treated group animal showing the normal glomeruli (white arrow), with degenerative renal tubules epithelia (black arrow). (E and H, 10X)

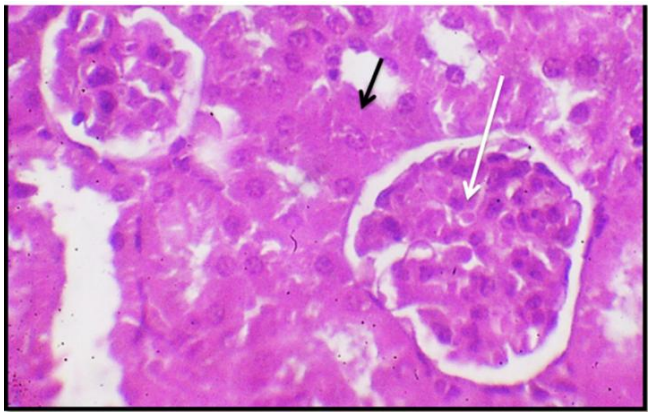

Figure 20. Photomicrograph of kidney histological section for PLGA and Fadrozole treated group animal showing the normal glomeruli (white arrow), with degenerative renal tubules epithelia (black arrow). (H and E, 10X)

The histopathological outcomes of mice subjected to H_2O_2 and etradiol to induce oxidative pressure observed the modifications that characterized through the huge histological alteration manifested by way of slight to intense vital vein congestion, lack of everyday preparations of hepatic cords with hepatocytes vacuolation and presence of perivascular inflammatory cells infiltration. The effects of induction group the in modern examine agreed with the preceding look at regarding the hepatotoxicity impact of estrogen management, executed on the same group of rats, vascular lesions had been mentioned (34). Vascular lesions were discovered inside the animal organizations that obtained large doses of oestrogens. Neither venules nor arterioles have been affected, and no aneurysms were visible. Significant inflammatory infiltrations and vasculitis affecting small-sized vascular channels had been found. The inflammatory infiltration turned into composed of eosinophils and mononuclear cells. indicates that these vascular abnormalities can function defining functions of necrotizing hypersensitivity. Individual necrotic liver cells have been found. These findings align with the conclusions of other researchers who proposed that necrotizing vascular lesions can rise up in unique kinds of allergy angiitis (35). It is crucial to acknowledge that the timing of management and the onset of vasculitis can vary. Equivalent changes of a vasculitis nature were cited. In the observe being discussed, drug hypersensitivity isn't encouraged with the aid of the dosage or duration of drug publicity. These findings align with the results reported in current literature. The facts presented assist the belief that vasculitis is caused by interactions among antigens and antibodies (36). Our investigation discovered the presence of spherical, blood-filled cavities allotted at some stage in numerous areas of the liver. These regions had been every now and then next to regions of euphoric sinusoids. These niches are packed with erythrocytes. The modifications had been compared to the consequences obtained with the aid of (37).
The authors occasionally located areas of organised bleeding with tissue dying, which we additionally observed, in person cases the usage of each light and electron microscopy. The sluggish increase inside the quantity of red blood cells within liver cells, together with the loss of life of liver cells, ends in the improvement of gaps full of blood.

An improvement in the histopathological changes in uterus of mice treated with Fadrozole perhaps related to the role of Fadrozole as mechanism of action through inhibition of aromatase enzyme, reduced estradiol synthesis due to aromatase was primarily works by creating a hypoestrogenic state. Aromatase p-450 inhibition might be suppressed endometrial cell growth while increasing apoptosis. The successful treatment with an Fadrozole could be attributed to at least two causes. First, peripheral (i.e. adipose tissue and skin) aromatase activity was suppressed and second, high levels of decreased after treatment with the Fadorzole. Apart from Fadorzole's expected direct reduction of aromatase activity in EPF, the absence of aromatase mRNA expression in the lesion could be attributable to a drop in estrogen, which was known to boost local PGE2 production, which stimulated aromatase expression, this study agreed with Meresman *et al.* (38).

When treated with PLGA-Fadrozole, PLGA renowned for its low risk of toxicity and prolonged release qualities, the hepatic and renal histological alterations improved. Fadrozole-PLGA nanoparticle therapy had a controlled anti-estrogenic, anti-

inflammatory, anti-angiogenic, and antioxidant effect in OS animals, resulting in disease regression, these finding agreed Jana *et al.* (39) who studied non-human primate pre-clinical research to assess the safety and efficacy of these nanoparticles at low doses in OS. So, the loaded and formation by nanoparticle techniques gave therapeutic effect with apperantely minimum side effects in lesser dose in sustained release mannar than Fadrozole treated group that needed seemingly high dose and long duration of action.

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