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Histological Evaluation of the Effect of Clopidogrel (Plavix) on the Brain, Liver, and Kidney: Histopathology Study

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ABSTRACT

Clopidogrel is a prodrug that is absorbed in the intestines and 85% of it is metabolized in the liver, while 15% is the active part of the drug as it works to inhibit platelet aggregation and thus not forming a blood clot. The current study aims to evaluate the histological effect of Clopidogrel on the tissues of the, brain, liver and kidney. Experiment design: The experiment included fifteen albino rats divided randomly into three groups and each group included five animals . The first group was considered the control group. It was received tap water and natural food. As for the second group, the animals were received 15 mg/kg of Clopidogrel once a day with water and food, while the animals of the third group were received 20 mg/kg of Clopidogrel once a day with water and food. The experiment lasted for a month. After the end of the experiment, the animals were anesthetized, and the organs were surgically obtained, and the organs were placed in a 10% formalin solution, and then the tissue preparation techniques were applied to them. Results: The results of the study showed that a drug had several effects in tissues, including atrophy, caseous necrosis, apoptosis of some cells, foamaus appearance, inflammation, and degradation of other cells.

KEYWORDS: Clopidogrel, atrophy, apoptosis, degradation, Brain , liver, kidney.

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INTRODUCTION

Clopidogrel, a prodrug with anti-thrombotic activity, acts as an irreversible antagonist of the P2Y12 receptor on platelet surface, Recent studies have indicated that after being absorbed in the intestines, around 85% of the Clopidogrel prodrug was metabolized by esterase to conversion to an sedentary form. Only 15% of Clopidogrel undergoes transformation into the active metabolite through the hepatic cytochrome P450 (CYP450) system, with CYP2C19 playing a crucial role as an enzyme. Though, extensive research has revealed significant inter-individual erraticism in the effectiveness of Clopidogrel as an antiplatelet agent. It is important to note that impaired platelet responsiveness to Clopidogrel has been related with an increased hazard of cardiovascular occasions [1,2,3,4]. Clopidogrel is a prodrug that calls for hepatic bioactivation to generate the active metabolite answerable for inhibiting platelets [5]. Clopidogrel has received FDA approval for the scientific control of thrombotic sicknesses, along with unstable angina, myocardial infarction, stroke, and percutaneous coronary interventions. The metabolism of Clopidogrel in the liver produces multiple distinct metabolites. However, none of these metabolites are accountable for constraining the platelet P2Y12 receptor [2].

Clopidogrel works simplest when it is absorbed through the gut after being taken orally. Evidence has shown that the absorption of clopidogrel is restrained via the intestinal P-glycoprotein efflux transporter [1]. Taupert et al. First established that altered intestinal uptake of copidogrel is affected by the ABCB1 C3435T polymorphism in 60 patients with coronary artery disorder [3].

When Clopidogrel was initially approved, the individuality of the lively metabolite and the enzymes responsible for its creation, namely the CYP450s, remained unknown. To determine the clinical pharmacokinetics of Clopidogrel, the plasma attentiveness of the primary circulating metabolite (SR26334) was used as a surrogate marker. However, SR26334, a carboxylic acid derivative formed through esterase-dependent metabolism, does not contribute to the inhibition of platelet aggregation [6,7]. Despite this progress,

the pharmacology of SR26334 and other Clopidogrel metabolites has not been lengthily studied. Nevertheless, there is substantial evidence suggesting that these abundant metabolites may have significant biological effects[8].

The use of Clopidogrel, like any medication, carries certain risks, Some of the potential risks associated with Clopidogrel which included ischemic events can be beneficial in preventing unwanted blood clots, it also increases the risk of bleeding, In some cases, bleeding can be severe and may require medical intervention [9]. Diabetic patients exhibit a diminished antiplatelet effect when treated with Clopidogrel, in contrast to non-diabetic patients [10,11]. Additionally, individuals with chronic kidney disease have a higher likelihood of experiencing recurrent cardiovascular events and bleeding incidents despite receiving treatment [12,13]. Patients who have an elevated production of certain Clopidogrel metabolites and/or reduced defense mechanisms may consequently be at risk of developing Clopidogrel-related toxicity [14].

The administration of Clopidogrel and pravastatin significantly reduced intimal hyperplasia [15]. Interestingly, Clopidogrel alone did not have a significant effect on intimal hyperplasia [16]. Clopidogrel increased the overall antioxidant volume of the kidney. Moreover, pretreatment with Clopidogrel reduced the number of CD41-positive cells. Therefore, Clopidogrel exhibits defensive belongings on renal ischemia-reperfusion injury in mice by preventing renal cell apoptosis through enhanced renal antioxidant capacity, mitigating severe tubular dilation, necrosis, histological damage [17,18].When Clopidogrel is metabolized by CYP3A4, it produces hepatotoxic metabolites that can be sequestered by glutathione. Elevated CYP3A4 activity and depleted cellular glutathione stores may constitute risk factors for hepatocellular toxicity associated with Clopidogrel [19].Due to the lack of previous studies investigating the impact of clopidogrel on heart, brain, liver, and kidney tissues, the present study was conducted to assess and observe the histological effects of Clopidogrel on these specific tissues for the first time.

MATERIALS AND METHODS

The experiments were conducted using healthy female rats weighting between 170 and 200 grams. The rats were housed in cages under standard laboratory conditions, with a temperature of 22 ± 2°C. They had free access to standard rat chow and tap water. All procedures involving the animals were conducted in accordance with the approved animal experimental protocols of the Animal Care and Use Committee at Tikrit University. Fifteen albino rats were randomly divided into three groups, with each group containing of five animals. The first group served as the control group and received tap water and regular food. The second group received a daily dose of 15 mg/kg of Clopidogrel along with water and food. The third group conventional a daily dose of 20 mg/kg of Clopidogrel along with water and food. The duration of the experiment was one month. At the end of the experiment, the animals were anesthetized, and their organs were surgically extracted. The organs were then fixed in 10% formaldehyde and embedded in paraffin. Five-micrometer sections were ready and stained with haematoxylin and eosin (H&E).

RESULTS

The results showed that is the control group appeared brain cortex was invested by normal pail membrane which associated with normal meningeal blood vessels which traverse the brain cortex toward the medulla, the molecular layer had few small pyramidal cells and glial cells, the external granular layer was formed by multiple pyramidal small cells and glial cells and the deeper layer of brain cortex was the polymorphic cells which had different size of pyramidal cells, surrounded by the vacuolar zone, glial cells also occupied the space in between pyramidal cells, whereas the whit matter of the brain had myelinated nerve fibers which was associated with many glial cells of different size and the whole cells were surrounded by vacuolar zone (fig 1, 1:1-1:3). In the second group showed that the pail meningeal membrane was detached from the brain cortex which had macrophages and the molecular layer appeared a spongy appearance with absence of most the pyramidal cells and few glial supporting cells were demonstrated scattered in different region of this layer. Also the external granular layer had few pyramidal cells, the external pyramidal cells were present in small groups with atrophic condition of surrounded by wide vacuoles, the myelinated nerve fibers were degraded with presence of great space in between those fibers. The polymorphic layer of deeper layer of brain cortex had atrophic pyramidal nerve cells and the adjacent white matter was containing degraded nerve fibers with presence great cavities scattered glial cells which appeared with dark bluish color stain of pyknotic nuclei and The white matter had basal ganglia which reflected by presence of great nerve pyramidal cells, the nuclei of those cells were ill-defined, vacuolar zone was surrounding those cells and many glial cells were distributed in the network of myelinated nerve fibers. Whereas, the molecular layer and external granular layer of brain cortex was traversed by micro blood vessels which were surrounded by protoplasmic astrocytes, those micro blood vessels seen contracted and great cavities around it, small pyramidal cells and many pyknotic nuclei of glial cells were demonstrated, surrounded by foamy- appearance nerve fibers plexus. (fig1, 2:1-2:3). In the third group the results showed that the pail meningeal membrane was thick and detached in certain regions from the brain cortex with presence of many macrophages with other white blood cells on the outer surface of brain and beneath the pail membrane, the meningeal blood vessel was obvious which was thick wall and empty from blood, the whole molecular layer appeared as spongy layer appearance, and the internal pyramidal layer and polymorphic layer of brain cortex had different sizes of pyramidal cells, the

nuclei of most cells not clear, the glial cells were a abundant and present individually or groups with vacuolar zone around most of the nerve and glial cells. The micro blood vessels were engorged with hemolyzed blood with great cavities around each one. And showed macrophage in the brain sulci and the molecular layer was seen as spongy layer of nerve fibers dissociation the pyramidal cells were scanty of even atrophied, also glial cells were rarely seen, and demonstrated around the glial cells and around the apoptotic cells. The white matter was engorged with many glial cells and few degraded pyramidal cells, the myelinated nerve fibers around it was degraded also and many sinuses, The internal pyramidal layer of brain cortex was containing pyramidal nervous cells of medium size and small size which were surrounded by vacuolar zone. Apoptotic glial cells were indicated in certain place (fig2; 3:1-3:2).

In the liver's tissue the results appeared that the parenchyma of the liver lobule in control group had central vein which enclosed by groups and normal columns of liver cells, each cell was polygonal in shape and had spherical nucleus, in between the liver cells there was blood sinusoid with kupffer cells inside blood channels of sinusoid, and the liver cells in the form of cords were arranged in radial pattern around the central vein (fig 3;1). Whereas in the second experimental group showed that the parenchyma of liver tissue was hypertrophy associated with pyknotic nuclei and vacuolar cytoplasm. The blood sinusoids were narrow and had blood with kupffer cells. The portal vein in the portal area of the liver was engorged with hemolyzed blood and the wall of vein was infiltrated by white blood cells and macrophages. The portal area was surrounded from outside by crowded degraded and hypertrophic liver cells and certain number of those cells had pyknotic nuclei, others had karyorrahexis nuclei (fig 3;2). While the central vein in third group was showed wide and engorged by the blood, Its periphery was continuous with blood sinusoid which appeared narrow channels with kupffer cells and surrounded by crowded degraded liver cells, and hypertrophic liver cells and certain number of those cells had pyknotic nuclei, others had karyorrahexis nuclei (fig 3;3).

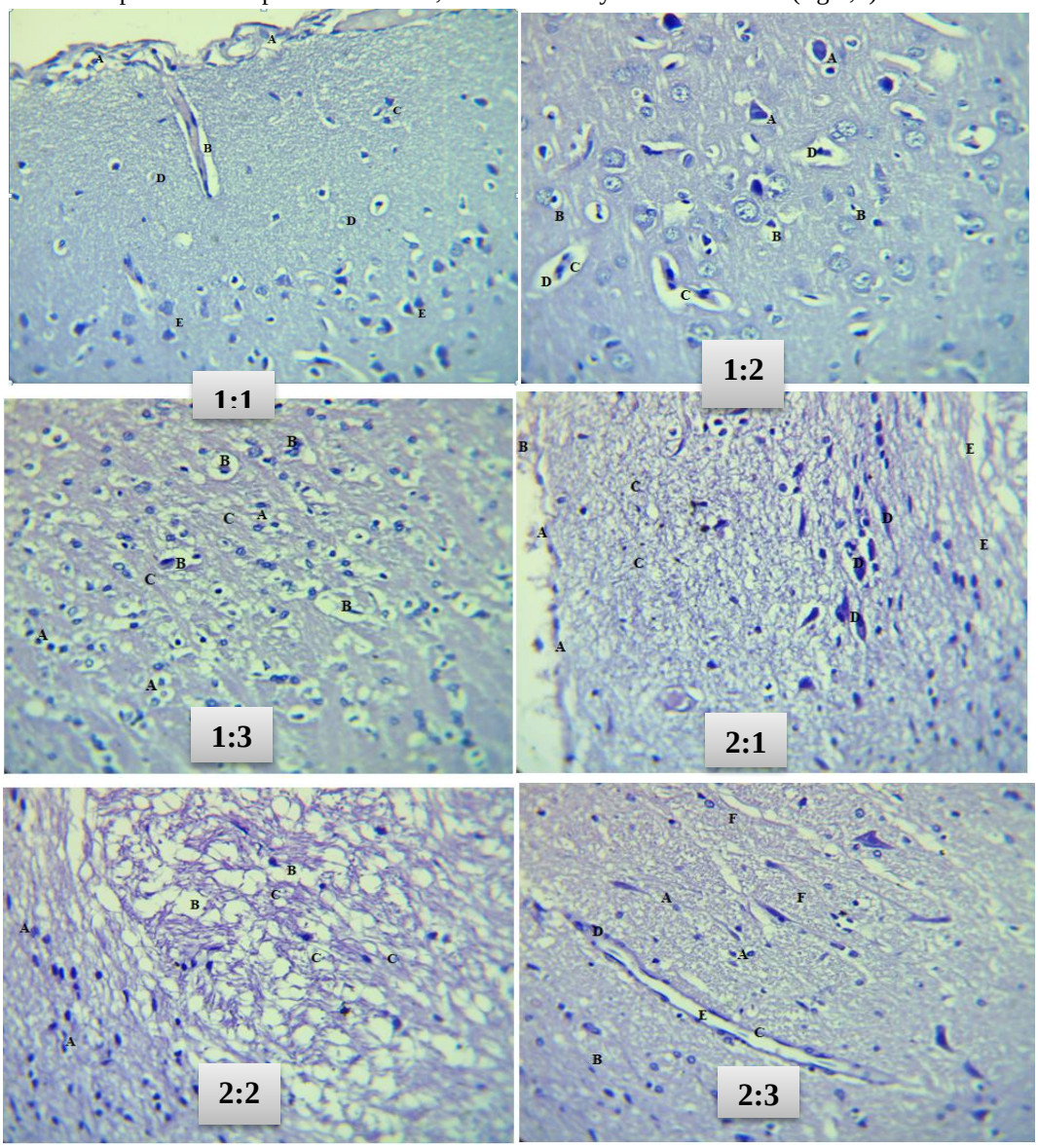

Fig (1). Effects of pretreatment with Clopidogrel on brain tissue damage. Group (1): (1:1): brain cortex, meningeal membrane (A), meningeal blood vessels (B), molecular layer with small pyramidal cells (C), glial cells (D), external granular layer with pyramidal

cells (E). (1:2): polymorphic layer of brain cortex. Pyramidal cells (A), glial cells (B), blood vessels (C), surrounded by vacuolar zone (D).(1:3): White matter of brain. Multiple glial cells (A), vacuolar zone (B), myelinated nerve fibers (C). Group (2): (2:1); Detachment of pail membrane (A) from the surface of brain cortex. Macrophage (B), molecular layer as spongy-like pattern (C) with absent many pyramidal cells. Atrophy of pyramidal cells of external pyramidal layer (D), degradation of nerve fibers (E). (2:2); Atrophic pyramidal cells (A) of polymorphic layer of brain cortex. Degradation and cavitation of white matter (B), scattered glial cells (C).(2:3) Molecular layer with small pyramidal cells (A), external granular layer (B), microblood vessels (C) with protoplasmic astrocytes (D) around it. Great cavities (E) , Foamy appearance of nerve fibers (F),(H&E, X40).

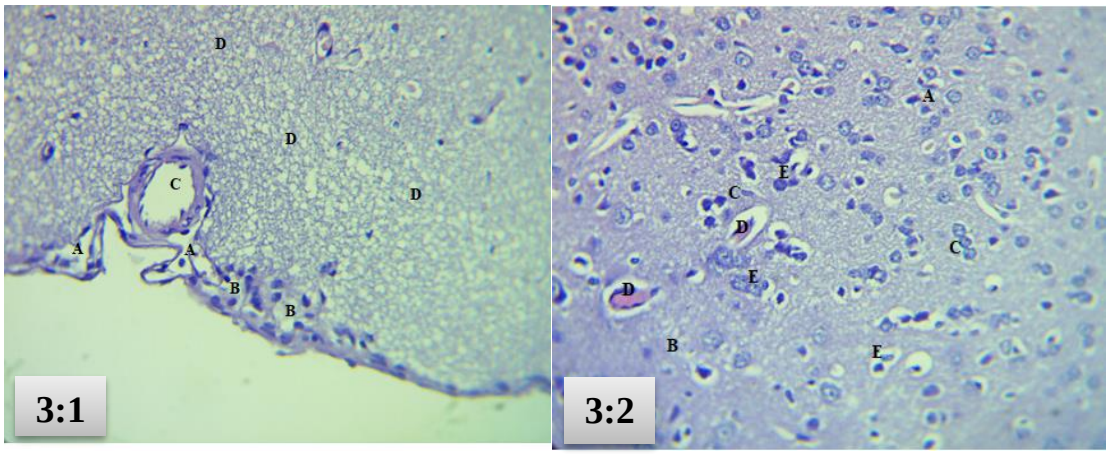

Fig (2). Effects of pretreatment with Clopidogrel on brain tissue damage. Group (3): (3:1); Detachment of thick pail membrane (A). White blood cells and Macrophage (B), meningeal blood vessel(thick and empty from blood) (C) spongy-like molecular layer (D). (3:2); internal pyramidal cells (A), polymorphic layer (B), hyperplasia of glial cells (C), hemolyzed blood of microblood vessels (D) Apoptotic cells(E),(H&E, X40).

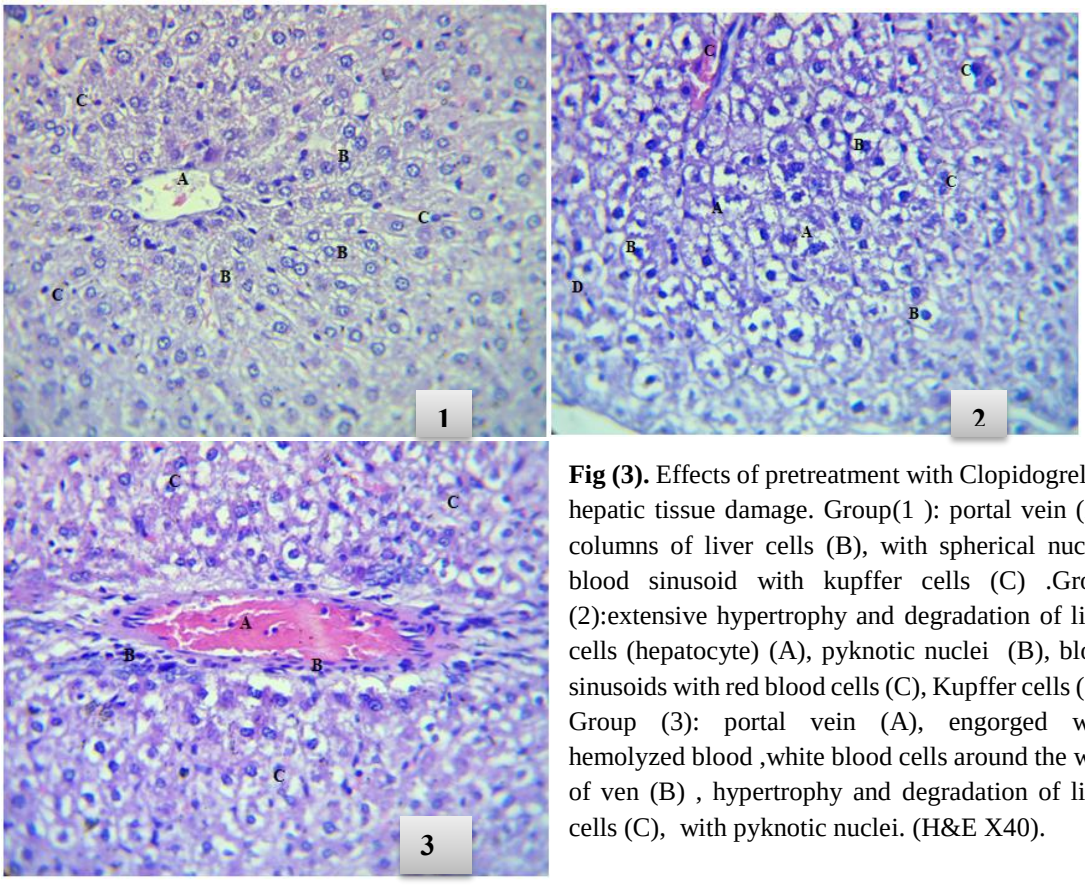

Fig (3). Effects of pretreatment with Clopidogrel on hepatic tissue damage. Group(1): portal vein (A), columns of liver cells (B), with spherical nuclei, blood sinusoid with kupffer cells (C) .Group (2):extensive hypertrophy and degradation of liver cells (hepatocyte) (A), pyknotic nuclei (B), blood sinusoids with red blood cells (C), Kupffer cells (D). Group (3): portal vein (A), engorged with hemolyzed blood ,white blood cells around the wall of ven (B) , hypertrophy and degradation of liver cells (C), with pyknotic nuclei. (H&E X40).

The results showed that the control group appeared a renal cortex had glomeruli which were partially segmented and surrounded by capsular space and Bowman's capsule, the proximal convoluted tubule were around the Bowman's capsule which were lined by pyramidal epithelial cells and it's lumens were narrow, the distal convoluted tubules were lined with simple cuboidal cells with wide lumen (fig 4;1).while the results of second group showed that the renal cortex was invested by thin capsule. The glomeruli were formed by tuft of blood capillaries enclosed by capsular space and Bowman's capsule. White blood cells were present on the surface of glomeruli. The lumen of renal tubule had filaments of glomerular filtrate and other renal convoluted tubule appeared normal. (fig 4;2). Whereas the results of third group showed certain epithelial cells were desquamated in the lumen of tubules and separated from its basement membrane. The interstitial connective tissue had macrophage and white blood cells infiltration (fig 4;3).

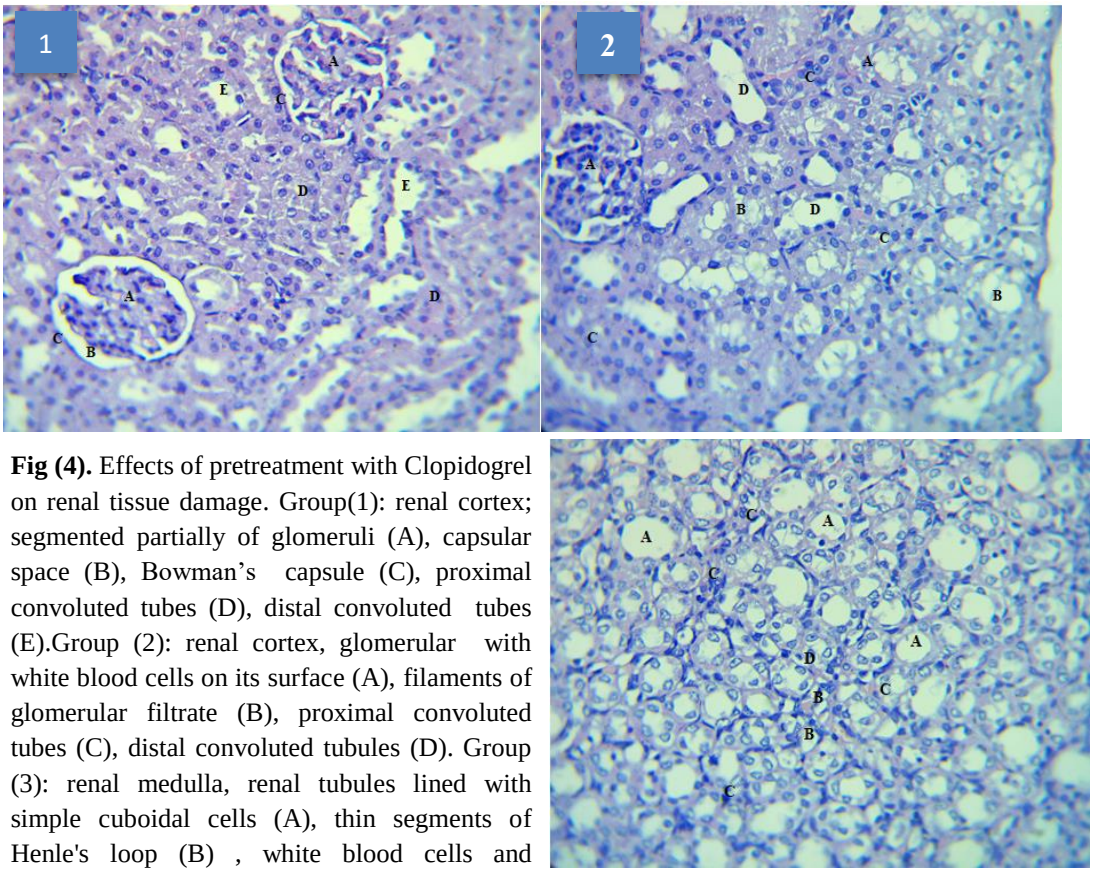

Fig (4). Effects of pretreatment with Clopidogrel on renal tissue damage. Group(1): renal cortex; segmented partially of glomeruli (A), capsular space (B), Bowman's capsule (C), proximal convoluted tubes (D), distal convoluted tubes (E).Group (2): renal cortex, glomerular with white blood cells on its surface (A), filaments of glomerular filtrate (B), proximal convoluted tubes (C), distal convoluted tubules (D). Group (3): renal medulla, renal tubules lined with simple cuboidal cells (A), thin segments of Henle's loop (B) , white blood cells and macrophages in the interstitial (C), (H&E X40).

DISCUSSION

The risk associated with Clopidogrel includes overall recurrent ischemic event, stent thrombosis, or bleeding in preserved patient [8]. Additionally, chronic kidney disease has been linked to an augmented risk of recurring cardiovascular and bleeding proceedings in treated patient [12,13]. Clopidogrel can also lead to associated toxicity [14]. In this study, we aimed to investigate the relationship between Clopidogrel and tissue damage in albino rats.

The results revealed significant histological changes in the brain cortex. Many macrophages, along with other white blood cells, were observed on the outer surface of the brain and beneath the pia mater. The molecular layer exhibited a spongy appearance with the absence of most pyramidal cells, and a few scattered glial supporting cells were observed in different regions of this layer. The external pyramidal cells were present in small groups and showed signs of atrophy surrounded by wide vacuoles. The myelinated nerve fibers were degraded, with significant spaces in between. The polymorphic layer of the deeper layer of the brain cortex showed atrophic pyramidal nerve cells, and the adjacent white matter contained degraded nerve fibers with scattered glial cells appearing with dark bluish color stain of pyknotic nuclei, surrounded by a foamy-appearing nerve fiber plexus and large vacuoles around the glial cells and apoptotic cells. Our findings demonstrated that tissue damage increased with higher doses of Clopidogrel compared to the control group, These changes suggest potential damage to brain tissue caused by Clopidogrel administration. This contradicts the findings of Hu *et al* in 20011, who reported that Clopidogrel increased the total antioxidant capacity of the kidney. Also, pretreatment with Clopidogrel reduced the number of CD41-positive cells, indicating its defensive belongings on renal ischemia-reperfusion injury in mice by preventing renal cell apoptosis through improved renal antioxidant capacity. Severe tubular dilatation, necrosis, and histological damage were also observed [18].

In the liver tissue, significant histological changes were observed. The liver parenchyma showed hypertrophy of liver cells with pyknotic nuclei and vacuolar cytoplasm. The blood sinusoids were narrow and contained kupffer cells, as well as infiltrating white blood cells and macrophages. The portal area was surrounded by degraded and hypertrophic liver cells, with some exhibiting pyknotic nuclei and others displaying karyorrahexis nuclei and degraded liver cells. Our findings demonstrated that tissue damage increased with higher doses of Clopidogrel compared to the control group, These changes suggest potential damage to brain tissue caused by Clopidogrel administration. This contrasts with the findings of Zahno *et al*, in 2013, who reported that Clopidogrel incubated with CYP3A4 leads to the formation of hepatocyte-toxic metabolites that can be stuck by glutathione. High CYP3A4 action and low cellular glutathione supplies may be risk issues for Clopidogrel-associate hepatocellular toxicity [19]. Fascinatingly, Clopidogrel alone too did not reduction intimal hyperplasia [15,16].

In the kidney, significant histological changes were observed in the interstitial connective tissue and Bowman's capsule, which showed infiltration of macrophages and white blood cells, These changes suggest potential damage to brain tissue caused by Clopidogrel administration. Clopidogrel result in an augmented total antioxidant volume of the kidney. Also, pretreatment with Clopidogrel reduced the amount of CD41-positive cell, indicating its defensive belongings on renal IRI in mice by preventing renal cell apoptosis through improved renal antioxidant capacity. Severe tubular dilatation, necrosis, and histological damage were also observed [17,18,20].

CONCLUSION

In conclusion, our study investigated the histopathological effects of Clopidogrel on various tissues, including the brain, liver, and kidneys, in albino rats. The findings demonstrated significant histological changes in these organs, indicating the potential adverse effects of Clopidogrel. In the brain, we observed the presence of macrophages and other white blood cells on the outer surface and beneath the pia mater, along with spongy changes in the molecular layer and atrophy of pyramidal cells. Similarly, the liver tissue exhibited hypertrophy of liver cells with pyknotic nuclei and vacuolar cytoplasm. Narrowed blood sinusoids, infiltrated by white blood cells and macrophages, were observed, indicating potential liver damage associated with Clopidogrel. In the kidneys, we observed infiltration of macrophages and white blood cells in the interstitial connective tissue and Bowman's capsule. These changes suggest potential renal tissue injury due to Clopidogrel. It is significant to note that this study was lead in rats and might not unavoidably reflect the effects of Clopidogrel (Plavix) in humans. Further research is needed to fully comprehend the potential impact of this medication on brain , liver and kidney tissues.

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