The side effects on the kidneys after Treated with Warfarin in male mice *Musculus domesticus*

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Abstract: The warfarin drug is an oral anticoagulant have prominent side effects on the body organs when it is used for treatment of the cardiovascular system diseases, the current study focused to investigation the histo logical and bio chemical change in the kidney of male white mice after being treated with warfarin for 30 day. The present work carryout 40 males white mice, all laboratory animals were housed in the animal house of the sciences college / AL-Muthanna University. The experimental animals were divided into two groups which including group A was consider as control group, group B was treated with warfarin. The results after treated time with warfarin, showed prominent damage in renal corpuscle, the mechanism leading to renal damage is glomerular and tubular hemorrhage, spot of hemorrhage in parenchyma of the kidney and cystic dilation filled with blood. The biochemical results noted significantly increase in the levels of urea and crietinine comparation with control group. In conclusion, the warfarin was effective in treating blood clots, but it has side effects on the kidneys.

***Keywords*:** warfarin, kidney, blood hemorrhage.

1. Introduction

Cardiovascular disease (CVD) is the world's largest cause of mortality, and preventing it is critical to halting the global epidemic[1].

Blood clots in the arteries can lead to heart attacks, strokes, severe leg pain, and difficulties walking, whereas blood clots in the veins or venous systems can lead to deep venous thrombosis. As these DVTs break, they cause deep vein thrombosis (DVT) in the pelvic, leg, and upper extremity veins.

They go through the bloodstream to the heart and then to the blood arteries in the lungs, causing acute pulmonary embolism. [2].

Warfarin is a 4-hydroxycoumarin analog that was presented as a rodenticides in the 1940s. Warfarin has been extensively used as an anticoagulant for the prevention and

treatment of thrombotic and thromboembolic diseases in the world since the 1950s [3]. Warfarin is a vitamin K antagonist that works by preventing vitamin K-dependent coagulation factors. It has recently become apparent that warfarin also affects vascular calcification by inactivating the Gla protein matrix [4]. Warfarin dosing is complex between patients and must to be individualized normal doses are about 5 mg per day, but can be as low as 0.5 mg per day in some patients or as low as 50 mg per day in others, factors such as gender, race , age, anticoagulation indication, albumin body weight, vitamin K intake and interactive medication may be all contribute to this variability [5].

Patients undergoing warfarin treatment require routine blood checks to assess how long blood clots take, called the International Normalised Ratio (INR), vegetables such as broccoli , spinach, parsley, kale, etc. are rich sources of vitamin K and consuming large amounts or making sudden changes in the consumption of these vegetables can interfere with the effectiveness and safety of warfarin therapy [6].

According to numerous studies, the annual incidence of severe bleeding in warfarin patients ranges from 0.4 percent to 7.2 percent. Minor bleeding can occur at a rate of up to 15.4 percent per year. [7]. The international normalized ratio (INR) is the most important parameter used to monitor its effect on the clotting system during patient follow-up. Typically, the dose of warfarin is frequently adjusted to maintain the INR level between 2 and 3.5 depending on the underlying condition. Because of its narrow therapeutic index, patients taking warfarin may experience minor and major bleeding, especially in those with poor drug compliance[8]. The kidney is bean-shaped, reddish - brown in color, and is located in the posterior cavity of the abdomen, it begins in most animals almost symmetrically one on each side of the vertebral column and dominantly in the lumbar region, although often extending forward under the last ribs [9]. The parenchyma of the kidney is enclosed in a hard fibrous and thin, strong capsule, the kidney consists of two regions, the outer cortex of a dark region under the capsule, a cortex that is granular, reddish-brown in color like most nephrons, the inner medulla of the light area under the cortex, which was very dark in color [10]. The role of the kidney in the purification of blood,

electrolyte balance and regulate fluids of the body and the introduction of harmful substances such as food products (urea, creatinine and toxins) that the body and the site of producing hormones such as erythropoietin and rennin [11]. Warfarin-related nephropathy, in which acute kidney damage is caused by glomerular hemorrhage and renal tubular blockade by red blood cells, warfarin therapy for chronic kidney failure, macroscopic hematuria and acute kidney damage, a renal biopsy revealed a large occlusion of red blood cells and casts in the renal tubules, the potential association between warfarin treatment, intratubular hemorrhage and acute kidney injury is explored [12]. Renal biopsies revealed significant blockage of renal tubules by red blood cells due to tubular cell destruction compatible with acute tubular necrosis, and the patient was on warfarin because of a history of deep venous thrombosis[13]. During warfarin drug pathological observations in renal biopsy samples of 9 patients with warfarin overdose, each biopsy sample showed acute glomerular hemorrhage and tubular damage , red blood cells RBCs in the Bowman area, and multiple occlusive RBCs in the tubules, each biopsy noted chronic kidney damage, and in most cases of warfarin-related nephropathy, red blood cells in the renal tubules block the flow of urine, resulting in acute kidney injury[14].

2. Methods and Materials (Experimental animals)

Fourty adult male white mice, the average weight was 28-30gm, the animals housed at 25-28ºC and humidity about 40 to 45% with feeding by using standard pellets and water. Mice were acclimated to the laboratory environment for two weeks before the experiment began.

Experimental design: The mice were divided into two groups, each group composed of twenty adult male mice. Group A: twenty animals were served as control group. Group B: twenty animals treated with 0.3 ml of warfarin solution for thirty days.

Preparation of histological slide: For 48 hours, the tissues samples were fixed in formalin solution. After that, the samples were dehydrated in graduated degrees of ethanol, cleaned in xylene, and embedded in paraffin wax for cutting, with 5-µm tissue sections mounted on glass slides and stained with hematoxyline and eosin stain for light microscopic examination. [15].

3. Results and Discussion

3.1 The histological results:

The tissue section of the kidney (fig.1) noted the kidney surrounded by a thin layer of connective tissue capsule. This capsule is consisted of two layers, an outer layer which composed of irregular dense connective tissue mostly collagenous fibers and a small amount of elastic fiber, the inner layer is consist of mainly smooth muscle cells, also, noted the cortical region of the kidney consists of spherical structures called the renal corpuscles and tubular structures called renal tubules. In tissue section (fig.2) showed prominent damage in renal corpuscle, prominent degeneration in bowman space, so, noted abnormal cystic dilation filled with secretion and blood, so, noted spot of hemorrhage in parenchyma of the kidney, this may be cause according to [16], who demonstrated the over-anti coagulation due to warfarin overdose and INR greater than 3 may be found to cause bleeding in glomerular of the kidney and this results agree with [13], who noted high INR levels were an significant risk factor for hemorrhage associated with the use of warfarin. Tissue section showed a prominent defect in the partial layer of bowman's capsule, so noted parenchymal degenerated of the kidney with prominent hemorrhage, the hemorrhage noted may be due to the side effect of warfarin on the tissue structures of the kidney, aggregation of inflammatory cells around blood hemorrhage and cystic dilation (fig.3). These results coincided with [17], were explained that warfarin cause acute kidney damage and hemorrhage in the kidney.

The tissue section showed acute degeneration in proximal convoluted tubules wall, the prominent isolated epithelial layer of the P.C.T without brush border (fig.4), this acute degeneration in tubules may be due to the high toxicity of warfarin, this result agreement with [18], which noted that warfarin cause degeneration in renal tubules and tubules toxicity that lead to worsening kidney damage.

In (fig.5), showed Henley loop arms have destroyed epithelial layers that lining both branches of Henley loop, both branches were filled with a thick fluid, the tissue section noted prominent blood congestion and hemorrhage between the arms of Henley loop and disappeared cells from epithelial layers that lining both arms of Henley loop, this histopathological change

in the tissue section of the kidney may be due to an increase in the rate of excretion or elimination the toxic material through the kidney which increased the blood congestion in the renal tubules which lead to these changes, this result agreement with [19], who explained that tubular cell injury consistent with acute tubular necrosis resulted in extensive blockage of renal tubules by red blood cells.

Tissue section noted abnormal dilated of D.C.T filled with secretion and aggregation of inflammatory cells around D.C.T. Tissue section showed irregular wide cystic dilation in parenchyma of kidney (fig.6). This may be due to warfarin's toxicity. the after the treated time that leads to degeneration some renal tubules. This histopathological change constant with [20], which explained warfarin caused glomerular bleeding with degeneration tubular red blood cell (RBC) casts and severe kidneys damage.

3.2 The biochemical results:

The result appeared in (Table-1) the value of urea in the serum of a control group was (34.69±0.100 mg/dl), and value of urea in treated mice with warfarin after 30 days was (135.17±0.136 mg/dl), the statistical study noted the level of urea in the treated group with warfarin have significant increased compared with the control. This increase in urea level of group that treated with warfarin which may be due to the warfarin toxicity that leads to these physiological deference's in the urea values which may be causes kidney dysfunction (injury in the kidney), this result constant with [21], which noted blood urea increase as a result of receiving warfarin treatment.

The level of creatinine serum in the control group (Table-1) was (0.362±0.0076 mg/dl), while the level of creatinine in the warfarin-treated group was (0.457±0074 mg/dl), the statistical study noted the level of creatinine in the treated group with warfarin has significantly increased compared with control group. A high level of creatinine in the treated group with warfarin may be due to the toxicity of warfarin that leads to damage in renal corpuscle that effected on the infiltration rate, or the degeneration in tubules that leads to an increase in the reabsorption of creatinine from renal tubules. This physiological change after treated with warfarin was similar to [22], which explained the increase in the concentration creatinine level in the rats with increasing the dose of warfarin.

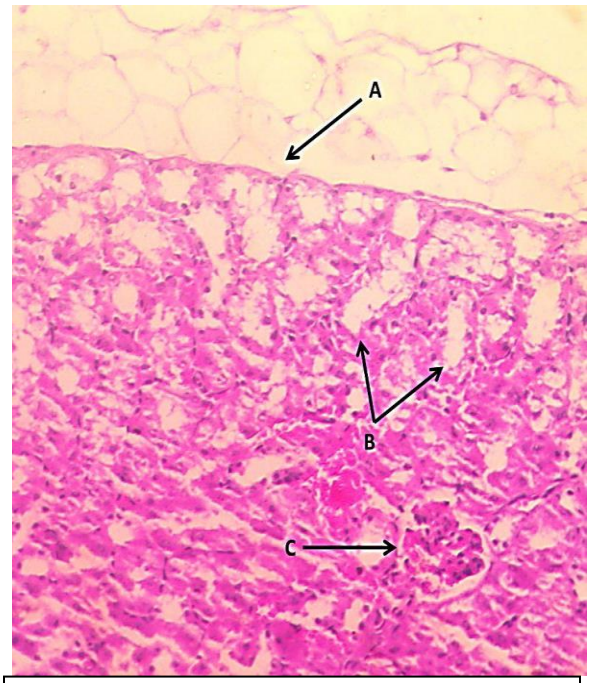

fig.1: section of kidney in the control group which showed A-kidney capsule, B-renal tubule, C-renal corpuscle. **(H and E)** stain 20X.

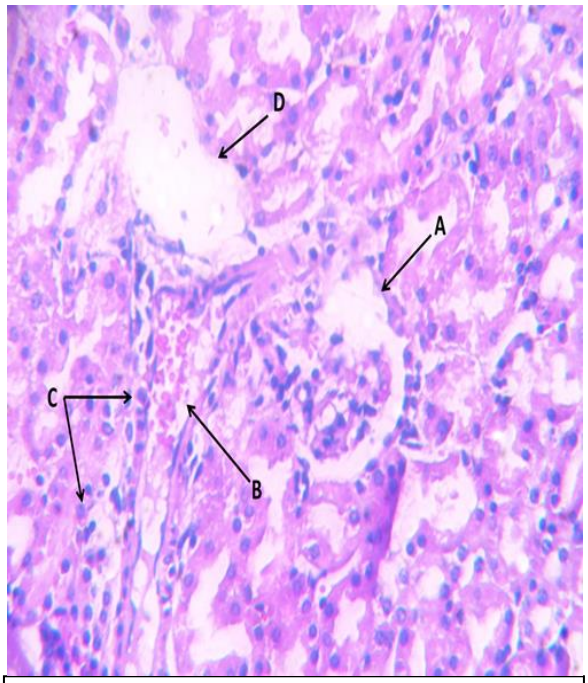

fig.2 section of kidney in treated group with warfarin which showed A-damage in renal corpuscle, B cystic dilation filled with blood, C- spot of hemorrhage. **(H and E)** stain X20.

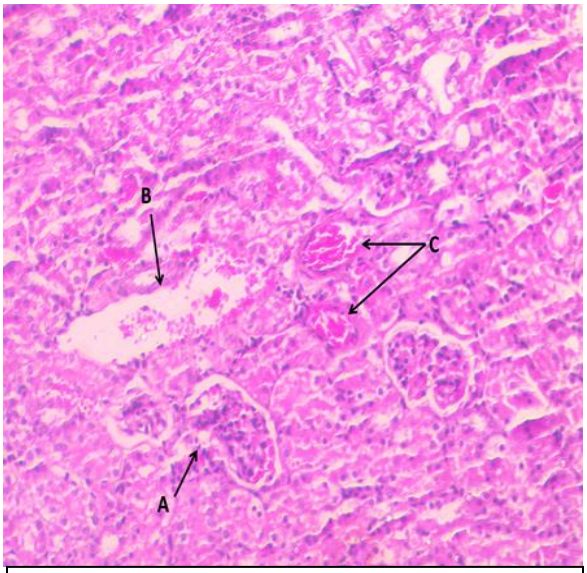

fig.3: section of kidney in treated group with warfarin which showed, A-degeneration of bowman's space, B-hemorrhage, C--inflammatory cells., D-cystic dilation. **(H and E)** stain X40.

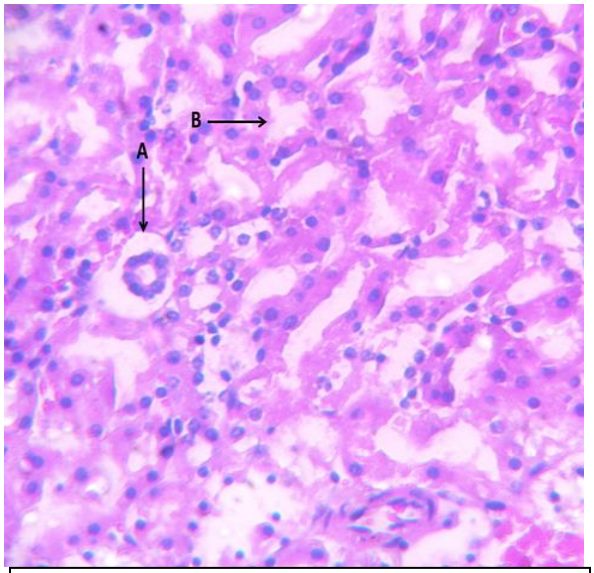

fig.4 : section of kidney in treated group with warfarin which showed, A- isolated epithelial layer of the P.C.T, B-disappeared brush border. **(H and E)** stain X40.

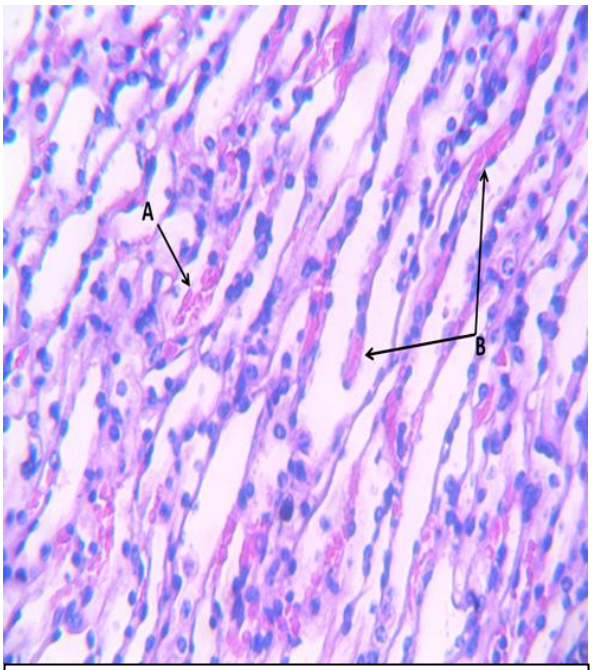

fig.5 : section of kidney in treated group with warfarin which showed A- hemorrhage, B- blood congestion beside Henley arms. (**H and E)** stain X40.

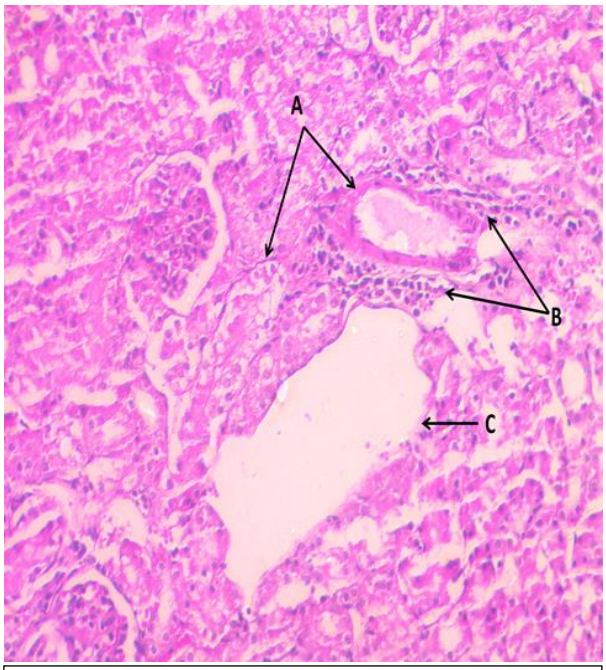

fig.6 : section of kidney in treated group with warfarin which showed A- Distal convoluted tubules, B- inflammatory cells, C- irregular wide cystic dilation. **(H and E)** stain X40.

4. Conclusion

Warfarin effect on the kidneys which leads to injery in the renal corpuscle, acute hemorrhage, cystic dilation filled with blood in the parenchyma of kidney, sever degeneration between renal tubules.

Table.1: Levels of serum urea and creatinine in mice (mg/dl).

	N	Mean	Std. Error	N	Mean	Std. Error	
Urea	20	34.6950	.10065	20	135.1750	.13667	.000
creatinine	20	.3625	.00767	20	.4570	.00744	.000

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Refrences

[1] Huffman, M. D., & Bhatnagar, D. (2012). Novel treatments for cardiovascular disease prevention.

Cardiovascular therapeutics, 30(5), 257-263.

[2] Goldhaber, S. Z., & Morrison, R. B. (2002). Pulmonary embolism and deep vein thrombosis. Circulation, 106(12), 1436-1438.

[3] Holbrook, A. M., Pereira, J. A., Labiris, R., McDonald, H., Douketis, J. D., Crowther, M., & Wells, P. S. (2005). Systematic overview of warfarin and its drug and food interactions. Archives of internal medicine, 165(10), 1095-1106.

[4] Krüger, T., Oelenberg, S., Kaesler, N., Schurgers, L. J., van de Sandt, A. M., Boor, P., & Ketteler, M. (2013). Warfarin induces cardiovascular damage in mice. Arteriosclerosis, thrombosis, and vascular biology, 33(11), 2618-2624.

[5] Ansell, J., Hirsh, J., Poller, L., Bussey, H., Jacobson, A., & Hylek, E. (2004). The pharmacology and management of the vitamin K antagonists: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest, 126(3), 204S-233S.

[6] Hebert, M. F., Park, J. M., Chen, Y. L., Akhtar, S., & Larson, A. M. (2004). Effects of St. John's wort (Hypericum perforatum) on tacrolimus pharmacokinetics in healthy volunteers. The Journal of Clinical Pharmacology, 44(1), 89-94.

[7] DiMarco, J. P., Flaker, G., Waldo, A. L., Corley, S. D., Greene, H. L., Safford, R. E., & AFFIRM Investigators. (2005). Factors affecting bleeding risk during anticoagulant therapy in patients with atrial fibrillation: observations from the Atrial Fibrillation Follow-up Investigation of Rhythm Management (AFFIRM) study. American heart journal, 149(4), 650-656.

[8] Witt, D. M., Delate, T., Clark, N. P., Martell, C., Tran, T., Crowther, M. A., & Hylek, E. M. (2009). Outcomes and predictors of very stable INR control during chronic anticoagulation therapy. Blood, The Journal of the American Society of Hematology, 114(5), 952-956.

[9] Goldny, B., UnterholznerV., Taferner B., Hofmann K.J., Rehder P., Strasak A. and Petersen J.(2009). ″Normal Kidney size and its influencing factors. A64 slice MDCT study of 1.040 a symptomatic patients″. BMC. Urology. 9(1)P:19.

[10] Dunnill, M. S., & Halley, W. (1973). Some observations on the quantitative anatomy of the kidney. The Journal of pathology, 110(2), 113-121.

[11] Rayner, H., Thomas, M., & Milford, D. (2016). Kidney anatomy and

physiology. In Understanding Kidney Diseases (pp. 1-10). Springer, Cham.

[12] Santos, C., Gomes, A. M., Gomes, A. M., Ventura, A., Almeida, C., & Seabra, J. (2013). An unusual cause of glomerular hematuria and acute kidney injury in a chronic kidney disease patient during warfarin therapy. Nefrología (English Edition), 33(3), 400-403.

[13] Abt, A. B., Carroll, L. E., & Mohler, J. H. (2000). Thin basement membrane disease and acute renal failure secondary to gross hematuria and tubular necrosis. American journal of kidney diseases, 35(3), 533-536.

[14] Brodsky, S. V., Satoskar, A., Chen, J., Nadasdy, G., Eagen, J. W., Hamirani, M., ... & Nadasdy, T. (2009). Acute kidney injury during warfarin therapy associated with obstructive tubular red blood cell casts: a report of 9 cases. American Journal of Kidney Diseases, 54(6), 1121-1126.

[15] Luna, G. (1968). Manual of histological staining methods of the armed forced institute of pathology. 3rd.MCRW hill book Co. New York.

[16] Brodsky, S. V., Collins, M., Park, E., Rovin, B. H., Satoskar, A. A., Nadasdy, G., & Hebert, L. A. (2010). Warfarin therapy that results in an International Normalization Ratio above the therapeutic range is associated with accelerated progression of chronic kidney disease. Nephron Clinical Practice, 115(2), c142-c146.

[17] Garcia, D. A., Regan, S., Crowther, M., & Hylek, E. M. (2006). The risk of hemorrhage among patients with warfarin-associated coagulopathy. Journal of the American College of Cardiology, 47(4), 804-808.

[18] Lim, A. K. (2013). Haematuria and acute kidney injury associated with warfarin anticoagulation. General Med, 1(105), 2.

[19] Conjeevaram, A., Lohia, P., GS, R., & Vankalakunti, M. (2019). Double whammy: Pigment nephropathy and warfarin-related nephropathy as aetiology for acute kidney injury in a patient with mechanical heart valves. The Open Urology & Nephrology Journal, 12(1).

[20] Ware, K., Qamri, Z., Ozcan, A., Satoskar, A. A., Nadasdy, G., Rovin, B. H., ... & Brodsky, S. V. (2013). N-acetylcysteine ameliorates acute kidney injury but not glomerular hemorrhage in an animal model of warfarin-related nephropathy. American Journal of physiology-Renal physiology, 304(12), F1421-F1427.

[21] Ishii, H., Hirai, K., Yanai, K., Kitano, T., Shindo, M., Miyazawa, H., ... & Mori, H. (2018). Warfarin-related nephropathy with acute kidney injury in a patient with immunoglobulin A nephropathy. CEN Case Reports, 7(2), 198-203.

[22] Ozcan, A., Ware, K., Calomeni, E., Nadasdy, T., Forbes, R., Satoskar, A. A., ... & Brodsky, S. V. (2012). 5/6 nephrectomy as a validated rat model mimicking human warfarin-related nephropathy. American journal of nephrology, 35(4), 356-364.