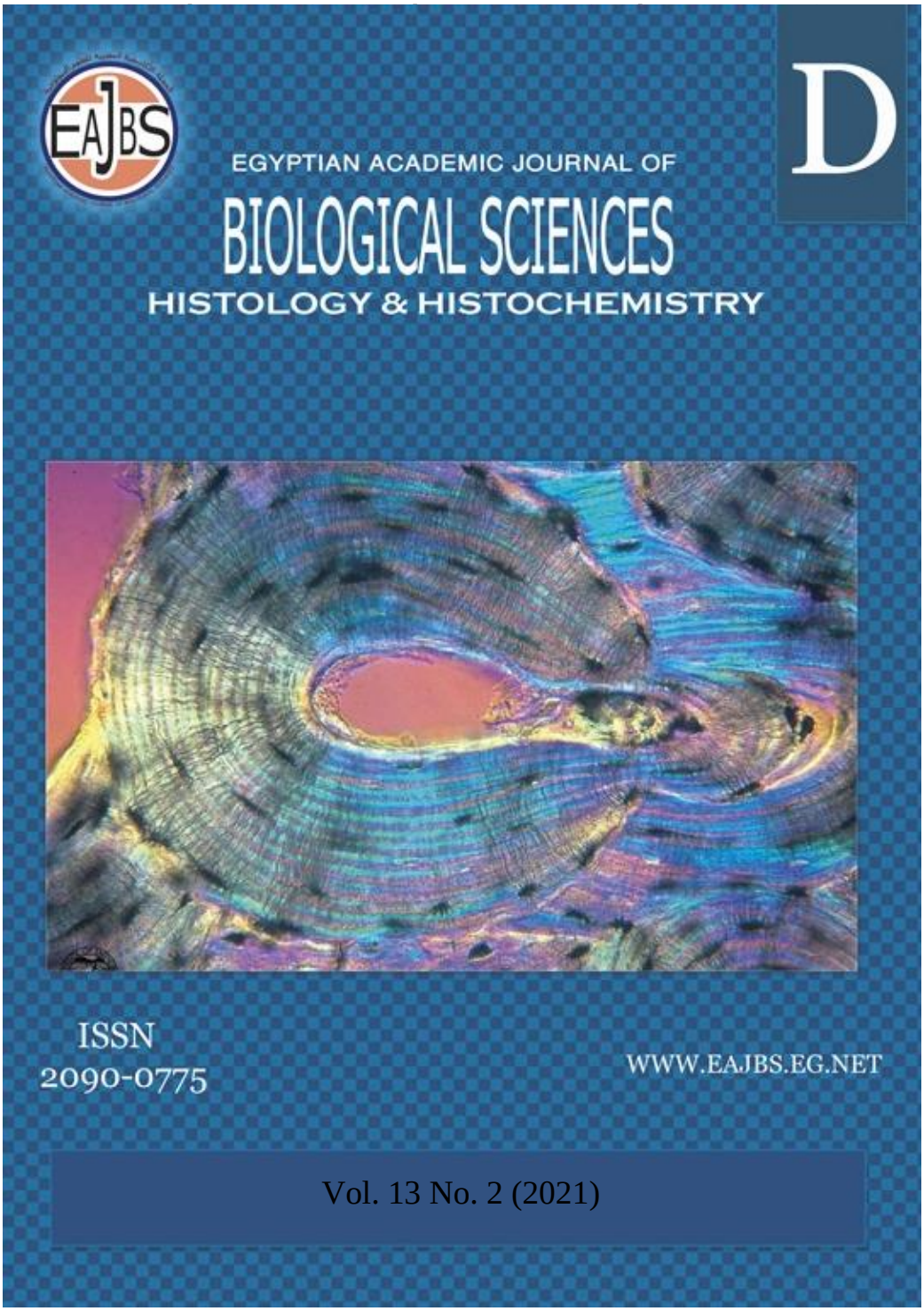

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Warfarin-Triggered Renal Toxicity: A Biochemical and Histopathological Study

Elham A. Abd-Allah*[1], Heba Allah Ahmed Mahmoud[2], Magdy Welson[2] and Mona M. Atia[3]

1-Laboratory of Physiology, Department of Zoology, Faculty of Science, New Valley University, EL-kharga, Egypt.
2-Plant Protection Research Institute (PPRI), Department of Animal pests, Egypt.
3-Laboratory of Molecular Cell Biology, Department of Zoology, Faculty of Science, Assiut University, Egypt.
E.Mail: elham_abdelsabour@aun.edu.eg - heba8587@gmail.com - magdy129@yahoo.com - monatia@aun.edu.eg

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ABSTRACT

Several alternative ways for dealing with rodents or correcting mouse damage have been passed down through the generations. Anticoagulant rodenticides were developed in the late 1940s, and they were a significant step forward in rodent management. In many locations, warfarin and its sodium counterpart are used to control rodents. Warfarin is a human drug that is used to treat blood clotting problems. These anticoagulants are administered as rodenticides in rats and mice, causing hemorrhaging and ultimately death. In this study, the acute oral warfarin $LD_{50}$of wild rats was determined. The effects of different doses of warfarin (¼ LD_{50} 27.5, 9 mg/kg.b.w and ½ LD_{50}55, 18 mg/kg.b.w) on rat kidneys of males and females respectively, were examined. These effects included considerable elevation of kidney functions, remarkable raise in LPO level with a significant decrease in GSH, SOD, CAT, and NO activities. Concerning histopathological changes, there were changes in glomeruli and renal tubules represented by expanding in the lining epithelium of the renal tubules with vascular degeneration of the epithelial cells covering the tubules, throughout the inner cavities of tubules there were wide areas of necrosis and appearance of inflammatory cellular infiltration, lymphocytes, and monocytes in interstitial tissues. It was concluded that warfarin oral intake of wild rats produced clear dose-related renal toxicity with different pathological effects.

INTRODUCTION

Rodenticides (chemicals being used to control rodents) have been used for nearly a century and are still widely used today. Anticoagulants, which prevent blood clotting, are the most often used rodenticides today (Rattner *et al.*, 2014). Warfarin, sold under the commercial name Coumadin. It's a highly efficient anticoagulant that is being used to treat a variety of conditions, including the risk of venous thromboembolism.

The indicators of intoxication are bleeding beneath the skin, around the neck, as well as the nose, eyes, and mouth. All of the remaining animals consumed far less food than the controls. Rats were discovered dead between days 4 and 6 after being given warfarin. (Muktha Bai *et al.*, 1992).

According to histological examination, the number of nephrons in the kidneys of warfarin-treated rats was reduced, there was dilatation of the renal tubules with infiltration of the inflammatory cells in all treated groups. Squamous metaplasia occurred in certain tubular epithelial cells, with the appearance of necrotic cells. The degree of tissue fibrosis was determined using Masson trichrome staining. (Lou *et al.*, 2019). The kidney can bleed retroperitoneal, intraluminal, or intrarenal (Vitellas *et al.*, 2000). Kidney damage and eosinophilia were among the side effects of warfarin therapy. (Goudarzipour *et al.*, 2015; Teragaki *et al.*, 2012). The kidney tissue reveals significant glomerular bleeding as well as red blood cell tubular blockage. Animals given warfarin had an elevated serum creatinine and morphologic changes in the kidneys that were identical to all those seen in humans taking the drug (Brodsky, 2014).

SOD and CAT are the primary defense line versus oxidative harm among antioxidant enzymes. Warfarin doses may influence SOD and CAT activities varies based on the tissue kind. The need to activate defense mechanisms required for removing the produced ROS may have caused changes in CAT and SOD activity. (Oishi *et al.*, 1999; Toth *et al.*, 1984). Because warfarin has no significant impact on SOD activity, a decline in SOD activity in warfarin-treated rats indicates enzyme expenditure (in transforming O2 to H2O). (Oishi *et al.*, 1999; Toth *et al.*, 1984).

In warfarin-related nephropathy (WRN), increased oxidative stress in the kidney causes: (1) RBCs release free hemoglobin into the tubular lumen, which impacts tubular epithelial cells by creating ROS and increasing lipid peroxidation. (Patel *et al.*, 1996); (2) Several surface receptors, such as megalin-cubilin receptors, allow free hemoglobin to enter tubular epithelial cells (Tracz *et al.*, 2007); Unbound hemoglobin stimulates caspases and causing apoptosis within cells (Homsi *et al.*, 2006); (3) heme, a powerful oxidant that activates proinflammatory pathways, dissociates from intracellular hemoglobin (Tracz *et al.*, 2007; Tsiftsoglou *et al.*, 2006). The most common cause of WRN is tubular blockage caused by RBC casts, which causes an increase in oxidative stress in the kidney (Ware *et al.*, 2013).

The aim of the present study was to investigate the acute toxicity of warfarin on oxidative stress, antioxidants and histopathological changes in kidneys of wild rats.

MATERIALS AND METHODS

Materials;

Warfarin tablets 5mg from Zentiva, Istanbul were purchased from a local pharmacy, superoxide dismutase, epinephrine, and 5,5'-dithio-bis-(2-nitrobenzoic acid; DTNB), naphthylethylene diamine dihydrochloride, sulfanilamide, thiobarbituric (TBA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other materials were sourced with the highest analytical grade from local suppliers.

Animals and experimental design

Collection of Animals:

Sixty mature male and female wild rats of species *Rattus rattus* were trapped alive from poultry farms in Assiut valley, weighing 150-200 g used in this experiment. The trapped rats were classified according to their morphological characteristics. They were collected and kept in separate traps in the animal house, Zoology Department, Faculty of Science, Assiut University at room temperature (25±5°C) with a normal 12 h light/12 h dark cycle for 2 weeks to be acclimatized before starting of the

experiment. All experimental protocols were carried out in accordance with the National Institutes of Health guidelines. The committee for medical ethics of the Faculty of Medicine at Assiut University reviewed and approved the research procedures (IRB no: 17300533). All animals were feed on *ad libitum food intake* on wet bread, tomatoes and drunk from tap water. Every day, food and water were replenished. Under each cage, metallic trays were kept to collect faces and spilled food, and these were cleaned regularly.

Experimental Design:

Trapped rats (n=60) were divided into 6 groups of 10 animals each for both sexes as the following:

- The first group: 10 adults of male rats *Rattus rattus* served as control were kept untreated.
- The second group: 10 adults of female rats *Rattus rattus* served as control were kept untreated.
- The third group: 10 adults of female rats *Rattus rattus* were treated orally with (9 mg/kg.b.w) which is 1/4 $\mathbf{LD_{50}}$of Warfarin for 18 days.
- The fourth group: 10 adults of female rats *Rattus rattus* were treated orally with (18 mg/ kg.b.w.), which is 1/2 $\mathbf{LD_{50}}$of Warfarin for 18 days.
- The fifth group: 10 adults of male rats *Rattus rattus* were had (27.5 mg/ kg.b.w.) ich were 1/4 $\mathbf{LD_{50}}$ of Warfarin for 18 days.
- The sixth group: 10 adults of male rats were had (55 mg/ kg.b.w.) which were 1/2 $\mathbf{LD_{50}}$of Warfarin for 18 days.

Sample Collection:

Rats were dissected on a regular basis, and specimens from the kidney were promptly taken and chopped into small pieces for use in biochemical measurements and histological preparations.

Determination of $\mathbf{LD_{50}}$:

The acute oral warfarin LD_{50} of rats was determined. Doses of warfarin were prepared as 60, 70, 80, and 90 mg/kg for males, and 15, 20, 30, and 40 mg/kg for females. Five adult rats of each sex, caged individually, were administered each dose, and the mortality and time to death were recorded up to four days after treatment. The LD_{50} values were calculated after 96 h using the tables published by (Horn, 1956) and according to the "Probit analysis" technique described by (Finney, 1971).

Kidney Functions Tests:

Determination of Creatinine, Urea and Uric Acid:

Plasma **c**reatinine, urea and uric acid were determined by using commercial kits (Spectrum Diagnostics Company, Egypt) according to the method of (Tietz, 1990).

Total protein concentration in plasma and tissue cytosols of the studied targeted organs were determined by the method of (Lowry *et al.*, 1951) and expressed as mg/ml.

Estimation of superoxide dismutase, catalase, and activity reduced glutathione

SOD activity was measured by its inhibitory effect on epinephrine oxidation (Misra & Fridovich, 1972). CAT activity was measured as described by (Beers & Sizer, 1952). The concentration of GSH was estimated as described by (Ravi *et al.*, 2004). Aliquots of 50 µl of tissue homogenate were added to 14.5 mg EDTA at 4000 rpm for 10 minutes. A mixture of 1 ml PBS at pH 8 and 100 µl DTNB to 100 µl was added to the supernatant, which was kept at room temperature for 5 minutes, before being used for the estimation of reduced glutathione at 412 nm.

Estimation of lipid peroxidation and nitric oxide

The thiobarbituric acid reaction was used to calculate lipid peroxidation (LPO) in the liver, as described by (Ohkawa *et al.*, 1979). To prevent additional oxidation, 1 percent v/v DMSO was added after homogenization. The reaction buffer was added to 0.2 ml aliquots of tissue homogenates and subjected to

spectrophotometric measurement. Nitric oxide was calculated as the concentration of nitrite in the tissue cytosols of the organs using the method of (Ding *et al.*, 1988).

Histopathological Examination:

For histological studies, small sections of kidney tissues were fixed in 10% neutral formalin (pH 7.2), dried in an escalating sequence of alcohols. After producing paraffin slices with a thickness of 5 micrometers were stained by Hematoxylin and eosin, and Masson's trichrome (Gabe, 1976). Stained sections were examined by a light microscope.

Histopathology Score and Fibrosis Percentage for The Kidney:

Pyknosis, region of degeneration, fibrosis, nuclear fragmentation, and inflammation were graded as five histopathological parameters (Heijnen *et al.*, 2003). In a nutshell, the Masson's trichrome positive regions in the kidney portions of at least six individual animals of each animal group were quantified using Image J software to determine the content of collagen in kidney slices stained with Masson's trichrome (Bataller *et al.*, 2003; Wang *et al.*, 2007), The percentage of fibrosis was estimated as follows:

% fibrosis = Masson's trichrome positive area / Total section area- vascular lumen area X 100.

Statistical Analysis:

All data were introduced as mean ± SD. Measurable examinations were performed by utilizing an ANOVA with treatment and time of study included as components. P-value <0.05 was showed as significant. These studies were carried out by means of the Prism 3.0 software package for computer statistics (Graph and Software, Ink, San Diego, USA).

RESULTS

Biochemical Results:

Kidney Function Tests:

Table (1) revealed that the level of urea pronounced decreased in both doses of female rats that were treated with ¼ and ½ $LD_{50}$by 16 % and 8.7 % respectively (P > 0.05), while the elevation of urea levels was significant in both doses of male rats by 94.6 % (P < 0.01) and 156.6 % (P < 0.001) respectively, compared with control. Concerning creatinine, there was an insignificant increase in case of female rats with a dose of ¼ LD_{50} by 52.1 %, while the increment was significant in the second dose by 220.7 % (P<0.001), the elevation of creatinine levels was significant in both doses of males (¼ LD_{50} and ½ LD_{50}) by 613.7 % (P < 0.001) and 799.4 % (P < 0.001) respectively, when compared with control. Regarding uric acid level there was significant elevation in both doses of females by 792.4 % (P < 0.001) and 530% (P < 0.001) respectively. However uric acid levels were decreased significantly in both doses of males by 62.2% (P < 0.001) and 61.6% (P < 0.001) respectively.

Table 1: Effect of ¼ and ½ LD $_{50}$ of Warfarin on some plasma activities of kidney functions, Urea, Creatinine and Uric acid in male and female of wild rats *(Rattus rattus*).

	Male *Rattus rattus*					Famle *Rattus rattus*				
	Control	27.5 mg/kg.b. w (1/4 LD_{50})		55 mg/kg.b. w (1/2 LD_{50})		Control	9 mg/kg.b. w (1/4 LD_{50})		18mg/kg.b. w (1/2 LD_{50})	
	Mean±SE	Mean±SE	% Of change	Mean±SE	% Of change	Mean±SE	Mean±SE	% Of change	Mean±SE	% Of change
Plasma Urea (mg/L)	20.29 ± 2.116	39.48± 2.608**↑	94.6%	52.06± 3.300***↑	156.6%	18.53 ± 1.352	21.5± 3.987↑	16%	20.15± 5.405↑	8.7%
Plasma Creatinine (mg/L)	1.750 ±0.6423	12.49± 0.558***↑	613.7%	15.74± 1.192**↑	799.4%	2.400 ±0.872	3.650± 0.510↑	52.1%	7.696 ± 0.852***↑	220.7%
Plasma Uric acid (mg/dL)	6.456 ±0.5415	2.442± 0.247***↓	62.2%	2.482± 0.648***↓	61.6%	1.701 ±0.255	15.18± 0.788***↑	792.4%	10.73 ± 1.031***↑	530%

Data are represented as mean ± SE. Number of rats (n) = 5. *= significant difference from control at P<0.05, **= significant difference from control at P<0.01, ***= significant difference from control at P<0.001.

Estimation of Superoxide Dismutase, Catalase, and Activity Reduced Glutathione:

Table (2) showed that SOD level showed an insignificant decrease of females with a dose of ¼ LD_{50} by 5.68%, while a significant decrease was found in females with a dose of ½ LD_{50} by 27.11% ($P < 0.01$). On the other hand, the decrease was significant in both doses of males by 23.74% and 25.04% ($P < 0.05$) respectively. CAT activity indicated a significant elevation in both doses of female rats by 37.81% and 47.05% ($P < 0.001$) respectively, Also, a significant elevation in CAT activity in both doses of male rats by 53.37% ($P<0.001$) and 43.19 % ($P < 0.001$) were respectively observed. GSH content significantly decremented in both doses of female rats by 24.65% ($P < 0.05$) and 54.22% ($P< 0.05$) respectively, the changes were insignificant in both doses of male rats by 2.94% and 2.34% respectively, when compared with control male rats.

Estimation of Lipid Peroxidation and Nitric Oxide:

Table (2) showed significant elevation of LPO levels in both doses of female rats by 67.56 % and 81.3 % ($P < 0.001$) respectively. Similarly, in both doses of male rats, the elevation of LPO level was significant by 84.76 % and 76.57 % ($P < 0.01$) respectively. NO levels were significantly decreased in females treated with ¼ LD_{50} by 33.13 % ($P < 0.05$), while, the decrement was insignificant in females treated with ½ LD_{50} 15.62 %. The decrement of NO levels was significant in male rats treated with the two doses by 27.35% and 26.09 % ($P < 0.01$) respectively, in comparison with control male rats.

Table 2: Effect of ¼ and ½ LD 50 of Warfarin on renal activities of GSH, SOD, CAT, LPO and NO of kidney tissues in male and female of wild rats (*Rattus rattus*).

	Male *Rattus rattus*					Famle *Rattus rattus*				
	Control	27.5 mg/kg.b. w (1/4 LD_{50})		55 mg/kg.b. w (1/2 LD_{50})		Control	9 mg/kg.b. w (1/4 LD_{50})		18mg/kg.b. w (1/2 LD_{50})	
Antioxidant & Oxidative stress Parameters	Mean±SE	Mean± SE	% Of change	Mean± SE	% Of change	Mean± SE	Mean± SE	% Of change	Mean± SE	% Of change
Renal GSH (ng/mg protein)	5.424 ±0.328	5.584↑ ±0.459	2.94%	5.297↓ ±0.255	2.34%	6.554 ±0.3091	4.938↓ ±0.317*	24.65%	3.000↓ ±0.254*	2.34%
Renal SOD (ng/mg protein)	5.202 ±0.488	3.967↓ ±0.157*	23.74%	3.899↓ ±0.368*	25.04%	7.528 ±0.3267	7.100↓ ±0.436	5.68%	5.487↓ ±0.270**	27.11%
Renal CAT activity (U/min/mg protein)	388 ±7.100	180.9↓ ±9.944***	53.37%	220.4↓ ±13.39***	43.19%	361.5 ±17.23	224.8↓ ±23.72***	37.81%	191.4↓ ±18.94***	47.05%
Renal LPO (nmol/ mg protein)	4.55 ±0.4712	8.407↑ ±0.817**	84.76%	8.034↑ ±0.985**	76.57%	5.968 ±0.3610	10.69↑ ±0.728***	67.56%	10.82↑ ±0.704***	81.3%
Renal NO (nmol/mg protein)	2.062 ±0.1354	1.498↓ ±0.096**	27.35%	1.524↓ ±0.091**	26.09%	1.651 ±1107	1.104↓ ±0.136*	33.13%	1.393↓ ±0.085	15.620%

Data are represented as mean ± SE. Number of rats (n) = 5. *= significant difference from control at P<0.05, **= significant difference from control at P<0.01, ***= significant difference from control at P<0.001.

Histopathological Results:

Microscopic examination of kidney sections of female and male wild rats staining with H &E and Masson's trichrome Figs (**1** & 2). The control group showed normal architecture of renal sections Fig.1 (a.1), & Fig.2 (d.1) and also, rats possessed a minimal amount of collagen fibers around the renal tubules, and Bowman's capsules Fig.1 (a.2) & Fig. 2 (d.2). The group treated with 9 mg/kg Fig.1 had disappearance of the normal structure of the kidney, a notable expansion of glomeruli, broadened urinary space of Bowman's capsule, and severe degeneration in the epithelial lining of tubule with hyperemia Figs (b.1 & b.2). The increase of collagen fibers around renal corpuscle and tubules Fig. (b.3). The group treated with 18 mg/kg Fig.1 had severe disorganization, and acute warfarin-related nephropathy, an atrophied glomerulus, the hypertrophied renal corpuscle and severe pyknotic nuclei in renal tubules Fig. (c.1). Also, expansion of the renal tubules, inflammation, significant fibrosis, and

wide degenerative areas were shown Fig. (c.2). the huge amount of fiber hyperplasia had instead of renal corpuscle and tubules also appeared Fig. (c.3). The 27.5 group Fig.**2** had degeneration of the epithelial cells covering the renal tubules and acute abnormality renal corpuscle, atrophied and hyperplasia of glomeruli, outrageous expanded urinary space of Bowman's capsule, and the warfarin-related nephropathy symptom Figs (e.1, e.2). Masson's trichrome stain revealed an intensive increase in the massive collagenous fibers between the renal cortical tubules, and hypertrophied corpuscles Fig. (e.3). The 55 mg/kg group Fig.**2** had fatal injury renal cortex, losses its normal structure, giant wipers of the degenerative lumen by hyperemia, highly shrunken renal corpuscle, and other glomeruli with swelling tuft and renal tubular with pyknotic nuclei Fig. (f.1). Wide degeneration areas, many inflammations, and severe warfarin-related nephropathy that causes destructive tubular injury were observed Fig.**2** (f.1, f.2). Markedly sharped increase of collagen fibers in tissue and many renal corpuscle and l tubules were replaced by fibrosis Fig.**2** (f.3).

Estimation of Heijnen's Scores and The Percentage of Fibrosis in Kidney:

Experimental groups of warfarin in female and male rats (9, 18, 27.5 & 55 mg/kg) showed a significant increase (1222.0, 1523.4, 1049.2, & 1485.7 %) of the Heijnen's score respectively, compared with controls groups. The female rat exposure to 9 & 18 mg /kg doses of warfarin induces more symptoms and degree of the toxicity in kidney than male rats. It was found that the small doses given to females have the same devastating effect as the high doses male Fig. 3 (a). Morphometric analysis of kidney fibrosis in (9, 18, 27.5 &55 mg/kg) groups showed increasing (209.8, 346.3, 146.1 & 432.5 %) respectively, compared with control rat Fig. 3 (b).

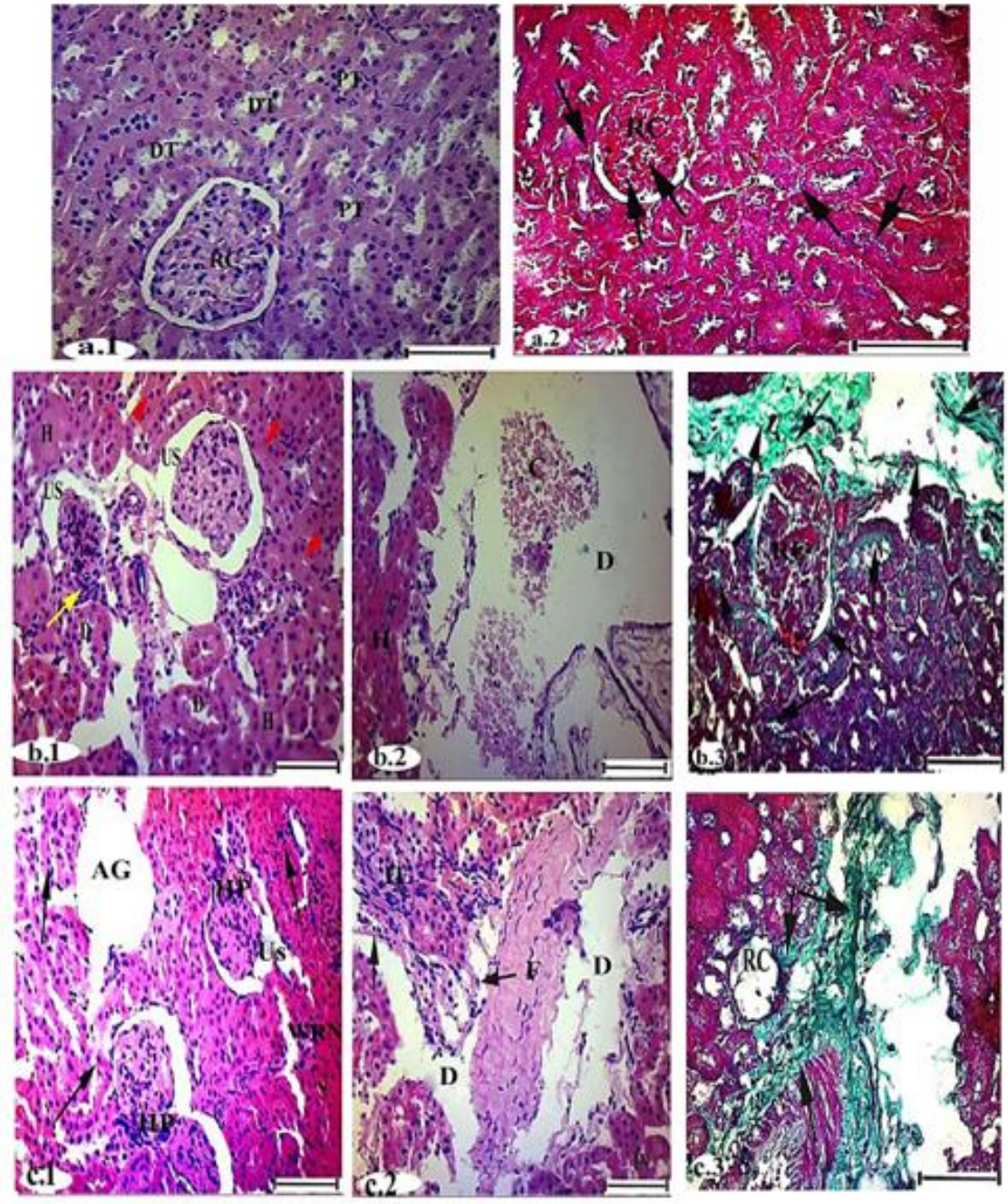

Fig. 1. Histopathological examination of control female rats demonstrating ordinary appearance of the renal structures and moderate of collagenous fibers around renal corpuscle (RC) (arrows) **Figs (a.1 & a.2).** In 9 mg/kg group, showing expansion of glomeruli (yellow arrow), broadened urinary space (US) of capsule, congestion (C), sever hyperemia (H) and wide degenerative (D) area **Figs (b.1 & b.2)** and collagen fibers (arrows) **Fig. (b.3).** In 18 mg/kg group, appearing hypertrophied renal corpuscle (HP), necrotic regions (N), pyknotic nuclei (black arrow), and atrophied glomeruli (AG) **Fig. (c.1).** Notably degeneration (D), warfarin-related nephropathy (WRN), inflammation (IL), and bands of fibrosis (F) in renal section **Fig. (c.2).** Huge amounts of collagenous fibers mainly in almost all the cortical tissues (arrows) were shown **Fig. (c.3)** (scale bar = 50 µm).

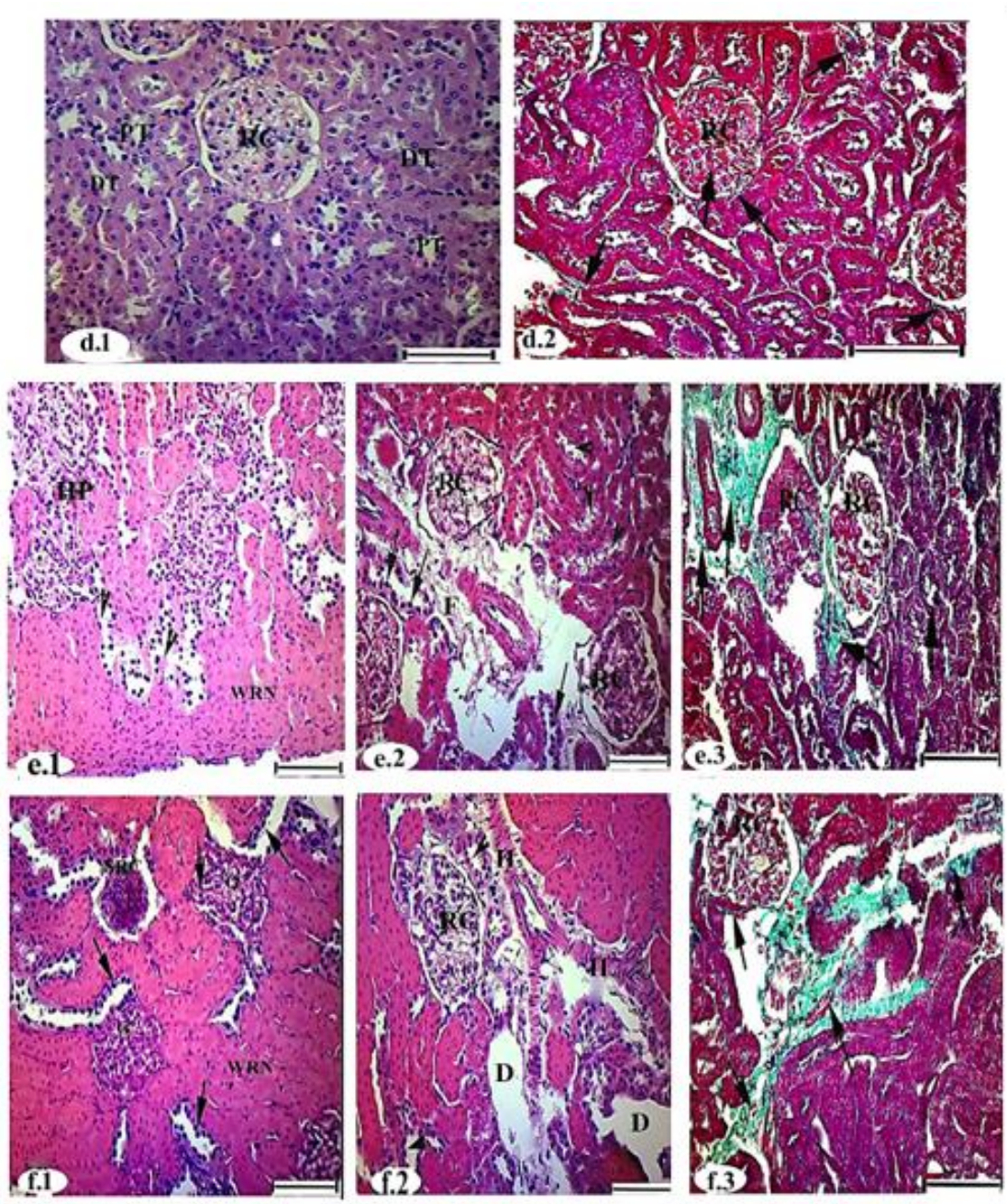

Fig. 2. Histopathological examination of control male rats demonstrating normal structure of renal sections and collagenous fibers (arrows) **Figs (d.1 & d.2).** In 27.5 mg/kg group, showing hypertrophied glomeruli (HP), losses of epithelial linings renal tubules (arrow head), and warfarin-related nephropathy (WRN), atrophied glomeruli (AG), degeneration of the renal tubules (D), bands of fibrosis (F), and numerous of pyknotic nuclei (black arrow) of tubules **Figs (e.1 & e.2).** Also, an increase in the amounts of fibers around RC (arrows) were observed **Fig. (e.3).** In 55 mg/kg group, appearing a huge shrunken renal corpuscle (SRC), hemolyzed blood (H), and giant glomeruli with no urinary space (G) **Fig. (f.1).** Wide spaces of degeneration areas (D), pyknotic nuclei (arrow head), many inflammatory cells (IL) **Fig. (f.2)**, and massive amount of fibrosis (arrows) in renal sections were appeared **Fig. (f.3)** (scale bar = 50 µm).

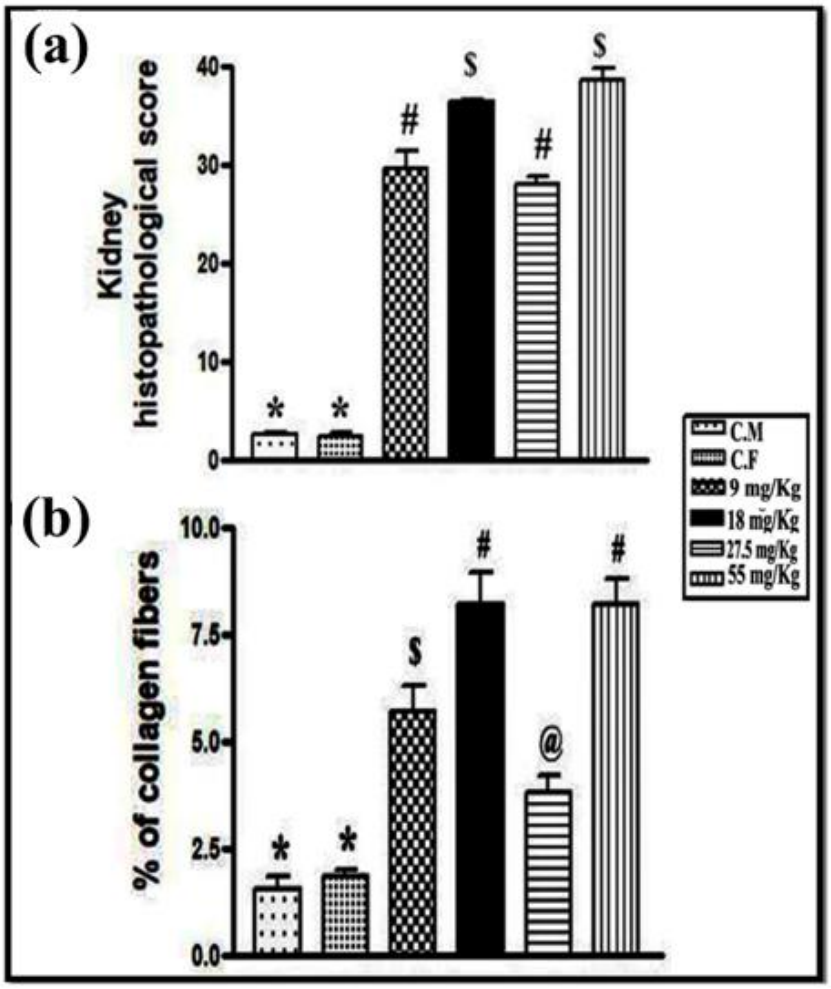

Fig. 3. (a & b) Heijnen's scores and the percentage of kidney fibrosis of groups, data with different signs are significantly different ($P < 0.05$).

DISCUSSION

The results of this study found that oral administration of different doses of warfarin (low and high) for 18 days enhanced Reactive oxygen species (ROS) and oxidative stress in male and female wild rats. Anticoagulant rodenticides are the most powerful way to get rid of rodents (Lund, 1988). Warfarin (4-OH coumarin), a first-generation anticoagulant rodenticide, and its coumarin analogues are Vitamin K (VK) antagonists, and their use is based on the inhibition of the vitamin K-dependent (VKD) step in the synthesis of many biologically active blood coagulation factors (Shearer, 1990) in addition to causing damage to small blood vessels, ultimately leads to death from internal hemorrhage (Lund, 1988).

Warfarin has a relatively narrow therapeutic window so hemorrhage and severe hypoxia complicate this treatment (Mendonca *et al.,* 2017). Our study confirmed that point and was identified the side effects of warfarin therapy, as well as bleeding that resulted in the death of the wild rat. Our study confirmed the previous study of (Mikhail & Abdel-Hamid, 2007) which revealed that the LD_{50} in female and male rats were calculated at 35.97, and 110.16 mg/kg bw. A close relation between oxidative stress, hemorrhage and apoptosis has been discovered in recent research, so after an intracerebral hemorrhage, apoptosis has long been connected to signaling via different cytokines. (MacManus & Linnik, 1997).

Serum creatinine levels were the best predictors of impaired anticoagulation regulation and hemorrhages in patients with the liver disease taking warfarin, according to (Efird *et al.,* 2014).

18 and 55 mg/kg groups had an extensive amount of fiber hyperplasia, leading to collagen deposition in the kidney. These data are in agreement with (Canbay *et al.*, 2002; Faouzi *et al.*, 2001). The fibrosis-promoting conditions have elevated iron levels, this indicates that iron overload could hasten disease progression kidney pathology. This result confirms our study in increasing kidney pathology in rats induced by iron and increase in serum transferrin after exposed warfarin led to the accumulation of Hemosiderin. Since there is no physiological mechanism for the removal of excess iron from the body, maintaining body iron homeostasis is critical. (Almhanna & Philip, 2009; Gardi *et al.,* 2002) approved that hemosiderin is often formed after bleeding, as the red blood cells die, and release hemoglobin is into the extracellular environment (Cheng *et al.*, 2017).

Our result revealed that warfarin oral intake causes severe toxicity in the kidney concluded by many biochemical parameters in plasma and Antioxidants and oxidative stress biomarkers of the kidney such as uric acid and urea. It was a decrease in renal GSH, SOD, CAT, and NO. Many studies back up our findings, ROS can oxidize proteins, and nucleic acids, facilitating the initiation of fibrosis, apoptosis, and necrosis. ROS-induced malondialdehyde, acts as a profibrogenic stimuli (Mehta *et al.,* 2019) by-product of lipid peroxidation of cellular organelle membranes, stimulated the expression of COL1A1 and TGF- in iron-loaded rats (Greuter & Shah, 2016). The previous researches appeared the relation between the increase of lipid peroxidation and COL1A1 which is compatible with our result. A high positive correlation was observed between the deposition of fibronectin and collagen I, and a decrease in NO and kidney injury (Langer *et al.,* 2008).

The decrease-in activity of renal SOD, one of the basic oxygen free radical enzyme scavengers, could have resulted from the host tissue's attempt to counteract ROS species and limit the damage by consuming the enzyme as intestinal homogenates of rats given warfarin had higher levels of proinflammatory cytokines IFN-g and IL-17. (Mowat, 2003; Sarra *et al.,* 2010; Yen *et al.,* 2006) in rats. Because cytokines like IL-17 are known inducers of a range of leukocyte effector actions, they may have exacerbated inflammatory processes in the tissue (Jovanovic *et al.,* 1998; Weaver *et al.,* 2007). Upregulation of inflammatory activity may aid in the protection of the kidney, gut and duodenum, as well as the induction of local reparative processes in this area (Mirkov *et al.*, 2016).

Organisms have evolved multiple systems of antioxidant defense, non-enzymatic antioxidants include metabolic low molecular weight antioxidants like glutathione, ascorbic acid, tocopherol, uric acid, and others, whereas high molecular weight defenses (enzymatic antioxidants) include enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione-s-transferase (GST) (Droge, 2002; Halliwell & Gutteridge, 2015; Hermes-Lima & Storey, 1993; Lushchak, 2002). These enzymes were the first line of antioxidant enzymatic defense since they dealt directly with radical species and the harm they caused to macromolecules.

Warfarin use was linked to higher levels of IL-6, fibrinogen, and haptoglobin in the blood, as well as increases in the function of erythrocyte antioxidant, enzymes superoxide dismutase and catalase (Kataranovski *et al.,* 2008). There was a strong positive association found between these

granulocyte activities and decreased NO development and expression (Brovkovych *et al.,* 2008).

Depletion of glutathione (GSH) is a frequent biological model of oxidative stress because of its defensive and potent efficacy (Kane *et al.,* 1993; Murphy *et al.,* 1990; Ratan *et al.,* 1994) and this is in agreement with our result in decreasing GSH after exposure to warfarin. The slow and progressive accumulation of ROS within the cells causes a loss of GSH and oxidative cell death, resulting in stress (Wallin *et al.,* 2000). GSH depletion is a prerequisite for cell death caused by glutamate or cystine deficiency (Back *et al.,* 1998; Murphy *et al.,* 1989). Another explanation for vitamin K's role in preventing GSH depletion-induced cell death is that low-dose vitamin K interacts with early signaling events that lead to ROS development. In several organs and models, several lines of evidence indicate that GSH depletion-induced oxidative death is mediated through a particular cell death signaling pathway (Stanciu *et al.,* 2000; Xiao *et al.,* 1999).

Our result is in agreement with some research as, Hypoproteinemia, hypoalbuminemia, hyperglycemia, bilirubinemia, increased urea concentration, and elevated activities of alanine aminotransferase, alkaline phosphatase, and gamma-glutamyltransferase are among the findings. (Binev *et al.,* 2005). Proteinuria, hematuria, and a large number of erythrocytes in the sediment are also present. Animals given warfarin had higher blood creatinine levels and morphologic changes in the kidneys that were similar to those seen in humans with Warfarin-related Nephropathy (WRN) (Radi & Thompson, 2004). Evidence has recently been published that warfarin-related coagulopathy can cause renal complications, including acute kidney injury (AKI) was reported (Brodsky *et al.,* 2010). The finding of anticoagulant-related kidney injury describes an animal model for studying it and offers advice to nephrologists and renal pathologists who may come across the condition (Ryan *et al.,* 2014).

Our result about increasing uric acid after exposure to warfarin in agreement with (Menon et al., 1986) who reported, the impact of warfarin on plasma uric acid levels were examined. Changes in antioxidant enzyme activity in tissues of warfarin-treated rats could be used as an indicator for systemic inflammation in these animals., given the interrelation of oxidative activity and inflammation (Aktop *et al.,* 2017; Hong *et al.,* 2009).

Our result explained the relationship between a decrease in kidney function enzymes and chronic kidney disease. As a result, more research is required to prevent chronic kidney disease (CKD) and clinical care. Changes in coagulation and fibrinolysis are frequently related to CKD, resulting in hypercoagulability, bleeding, and aberrant coagulation factors (Irish & Green, 1998).

In the present study, warfarin revealed many severe morphological changes in the kidneys known as chronic kidney diseases (CKD) such as changes in glomeruli and wide areas of the urinary tubules, high count of red blood cells which is called congestion, wide degenerative lumen, fractional loss of brush fringe of proximal convoluted tubules, hypertrophied renal corpuscle with urinary space of Bowman's capsule. Also, highly pathologically affected such as expanding in the lining epithelium of the renal tubules with vascular degeneration of the epithelial cells covering the renal tubules, wide areas of necrosis. lots of inflammatory leucocytic infiltration, pyknotic and fragmented nuclei in the tubular epithelial cells, and Renal fibrosis is the result of the unnecessary gathering of extracellular matrix and indicates of the failed wound healing process of the kidney tissue, both tubules and

glomeruli are dead and degeneration of epithelial lining.

Our findings revealed that glomerular bleeding and tubular RBC casting produce blockage and inflammatory and oxidative damage to tubular epithelial cells, resulting in renal injury. Our findings revealed that glomerular bleeding and tubular RBC casting produce blockage and inflammatory and oxidative damage to tubular epithelial cells, resulting in renal injury. In the mouse kidney, (Ge *et al.*, 2011) discovered aberrant glomerular, tubulointerstitial, and glycogen depletion, as well as activation of glycogen synthase kinase (GSK).

As a result, new medications are required for patients who need anticoagulation. In the warfarin group, histology of the kidney revealed a reduction in nephron numbers, dilated renal tubules, and inflammatory cell infiltrates. Squamous metaplasia occurred in certain tubular epithelial cells, and necrotic cells were seen. The degree of tissue fibrosis of the kidney fibrosis in the warfarin group was revealed by Masson trichrome staining. However, it has a number of negative side effects, including gastrointestinal bleeding, subcutaneous hemorrhage, and cerebral hemorrhage, which limit its use (Robertson *et al.*, 2017; Weber *et al.*, 2019) Previous research has shown that Indobufen is an effective antiplatelet medication.

We could conclude that Warfarin oral intake causes severe toxicity in the kidney which may cause a decrease in vitamin K leading to change in many biochemical parameters in plasma, Antioxidants, and oxidative stress biomarkers of the kidney as an increase in LPO, creatinine, uric acid, and urea. Also, it was a decrease in GSH, SOD, CAT, and NO. Warfarin produced clear dose-related renotoxicity, and many pathological effects in the renal tissues.

Ethical Approval:

All applicable international, national, and institutional guidelines for the care and use of animals were followed. We respected the welfare of animals and excluded situations when animals were in pain.

REFERENCES

Aktop, S., Emekli-Alturfan, E., Gönül, O., Göçmen, G., Garip, H., Yarat, A., & Göker, K. (2017). Effect of Ankaferd Blood Stopper on Skin Superoxide Dismutase and Catalase Activities in Warfarin-Treated Rats. *Clinical and Applied Thrombosis/Hemostasis*, *23*(2), 168–174. https://doi.org/10.1177/1076029615604049

Almhanna, K., & Philip, P. A. (2009). Safety and efficacy of sorafenib in the treatment of hepatocellular carcinoma. *OncoTargets and Therapy*, *2*, 261.

Back, S. A., Gan, X., Li, Y., Rosenberg, P. A., & Volpe, J. J. (1998). Maturation-dependent vulnerability of oligodendrocytes to oxidative stress-induced death caused by glutathione depletion. *Journal of Neuroscience*, *18*(16), 6241–6253.

Bataller, R., Schwabe, R. F., Choi, Y. H., Yang, L., Paik, Y. H., Lindquist, J., Qian, T., Schoonhoven, R., Hagedorn, C. H., & Lemasters, J. J. (2003). NADPH oxidase signal transduces angiotensin II in hepatic stellate cells and is critical in hepatic fibrosis. *The Journal of Clinical Investigation*, *112*(9), 1383–1394.

Beers, R. F., & Sizer, I. W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry,* 195(1), 133–140.

Binev, R., Petkov, P., & Rusenov, A. (2005). Intoxication with anticoagulant rodenticide bromadiolone in a dog.

Veterinarski Arhiv, *75*(3), 273–282.

Brodsky, S. V. (2014). Anticoagulants and acute kidney injury: clinical and pathology considerations. *Kidney Research and Clinical Practice*, *33*(4), 174–180.

Brodsky, S. V, Collins, M., Park, E., Rovin, B. H., Satoskar, A. A., Nadasdy, G., Wu, H., Bhatt, U., Nadasdy, T., & 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.

Brovkovych, V., Gao, X.-P., Ong, E., Brovkovych, S., Brennan, M.-L., Su, X., Hazen, S. L., Malik, A. B., & Skidgel, R. A. (2008). Augmented inducible nitric oxide synthase expression and increased NO production reduce sepsis-induced lung injury and mortality in myeloperoxidase-null mice. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *295*(1), L96–L103.

Canbay, A., Higuchi, H., Bronk, S. F., Taniai, M., Sebo, T. J., & Gores, G. J. (2002). Fas enhances fibrogenesis in the bile duct ligated mouse: a link between apoptosis and fibrosis. *Gastroenterology*, *123*(4), 1323–1330.

Cheng, Y., Luo, R., Zheng, H., Wang, B., Liu, Y., Liu, D., Chen, J., Xu, W., Li, A., & Zhu, Y. (2017). Synergistic anti-tumor efficacy of sorafenib and fluvastatin in hepatocellular carcinoma. *Oncotarget*, *8*(14), 23265.

Ding, A. H., Nathan, C. F., & Stuehr, D. J. (1988). Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. *The Journal of Immunology*, *141*(7), 2407–2412.

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Reviews*, *82*(1), 47–95.

Efird, L. M., Mishkin, D. S., Berlowitz, D. R., Ash, A. S., Hylek, E. M., Ozonoff, A., Reisman, J. I., Zhao, S., Jasuja, G. K., & Rose, A. J. (2014). Stratifying the risks of oral anticoagulation in patients with liver disease. *Circulation: Cardiovascular Quality and Outcomes*, *7*(3), 461–467.

Faouzi, S., Burckhardt, B. E., Hanson, J. C., Campe, C. B., Schrum, L. W., Rippe, R. A., & Maher, J. J. (2001). Anti-Fas induces hepatic chemokines and promotes inflammation by an NF-κB-independent, caspase-3-dependent pathway. *Journal of Biological Chemistry*, *276*(52), 49077–49082.

Finney, D. J. (1971). Probit analysis, Cambridge University Press. *Cambridge, UK*.

Gabe, M. (1976). *Histological techniques*. Springer.

Gardi, C., Arezzini, B., Fortino, V., & Comporti, M. (2002). Effect of free iron on collagen synthesis, cell proliferation and MMP-2 expression in rat hepatic stellate cells. *Biochemical Pharmacology*, *64*(7), 1139–1145.

Ge, Y., Si, J., Tian, L., Zhuang, S., Dworkin, L. D., & Gong, R. (2011). Conditional ablation of glycogen synthase kinase 3 β in postnatal mouse kidney. *Laboratory Investigation*, *91*(1), 85–96.

Goudarzipour, K., Ghazizadeh, F.,

Tavassol, H. H., & Behnam, B. (2015). Warfarin-induced eosinophilia in a child with urkitt Lymphoma: a case report. *Iranian Journal of Pharmaceutical Research: IJPR*, *14*(3), 887.

Greuter, T., & Shah, V. H. (2016). Hepatic sinusoids in liver injury, inflammation, and fibrosis: new pathophysiological insights. *Journal of Gastroenterology*, *51*(6), 511–519.

Halliwell, B., & Gutteridge, J. M. C. (2015). *Free radicals in biology and medicine*. Oxford university press, USA.

Heijnen, B. H. M., Straatsburg, I. H., Gouma, D. J., & van Gulik, T. M. (2003). Decrease in core liver temperature with 10 C by in situ hypothermic perfusion under total hepatic vascular exclusion reduces liver ischemia and reperfusion injury during partial hepatectomy in pigs. *Surgery*, *134*(5), 806–817.

Hermes-Lima, M., & Storey, K. B. (1993). In vitro oxidative inactivation of glutathione S-transferase from a freeze tolerant reptile. *Molecular and Cellular Biochemistry*, *124*(2), 149–158.

Homsi, E., Janino, P., & De Faria, J. B. L. (2006). Role of caspases on cell death, inflammation, and cell cycle in glycerol-induced acute renal failure. *Kidney International*, *69*(8), 1385–1392.

Hong, Y.-K., Wu, H.-T., Ma, T., Liu, W.-J., & He, X.-J. (2009). Effects of Glycyrrhiza glabra polysaccharides on immune and antioxidant activities in high-fat mice. *International Journal of Biological Macromolecules*, *45*(1), 61–64.

Horn, H. J. (1956). Simplified LD 50 (or ED 50) calculations. *Biometrics*, *12*(3), 311–322.

Irish, A. B., & Green, F. R. (1998). Factor VII coagulant activity (VIIc) and hypercoagulability in chronic renal disease and dialysis: relationship with dyslipidaemia, inflammation, and factor VII genotype. *Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association-European Renal Association*, *13*(3), 679–684.

Jovanovic, D. V, Di Battista, J. A., Martel-Pelletier, J., Jolicoeur, F. C., He, Y., Zhang, M., Mineau, F., & Pelletier, J.-P. (1998). IL-17 stimulates the production and expression of proinflammatory cytokines, IL-β and TNF-α, by human macrophages. *The Journal of Immunology*, *160*(7), 3513–3521.

Kane, D. J., Sarafian, T. A., Anton, R., Hahn, H., Gralla, E. B., Valentine, J. S., Ord, T., & Bredesen, D. E. (1993). Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species. *Science*, *262*(5137), 1274–1277.

Kataranovski, M., Mirkov, I., Vrankovic, J., Kataranovski, D., & Subota, V. (2008). Percutaneous toxicity of anticoagulant warfarin in rats. *Cutaneous and Ocular Toxicology*, *27*(1), 29–40.

Langer, D. A., Das, A., Semela, D., Kang-Decker, N., Hendrickson, H., Bronk, S. F., Katusic, Z. S., Gores, G. J., & Shah, V. H. (2008). Nitric oxide promotes caspase-independent hepatic stellate cell apoptosis through the generation of reactive oxygen species. *Hepatology*, *47*(6), 1983–1993.

Lou, X., Jin, J., Gong, J., Zhao, L., Li,

Y., & He, Q. (2019). Comparison of the Effects of Indobufen and Warfarin in a Rat Model of Adenine-Induced Chronic Kidney Disease. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, *25*, 3566.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–275.

Lund, M. (1988). Selection of baits and their distribution. *Rodent Pest Management*, 261–268.

Lushchak, V. I. (2002). Oxidative stress in bacteria. *P. Johnston, AA Boldyrev (Eds.), Oxidative Stress at Molecular, Cellular and Organ Levels, Research Signpost, Trivandrum (India); 2000, Pg. 45, 65*.

MacManus, J. P., & Linnik, M. D. (1997). Gene expression induced by cerebral ischemia: an apoptotic perspective. *Journal of Cerebral Blood Flow & Metabolism*, *17*(8), 815–832.

Mehta, K. J., Farnaud, S. J., & Sharp, P. A. (2019). Iron and liver fibrosis: Mechanistic and clinical aspects. *World Journal of Gastroenterology*, *25*(5), 521.

Mendonca, S., Gupta, D., Valsan, A., & Tewari, R. (2017). Warfarin related acute kidney injury: A case report. *Indian Journal of Nephrology*, *27*(1), 78.

Menon, R. K., Mikhailidis, D. P., Bell, J. L., Kernoff, P. B., & Dandona, P. (1986). Warfarin administration increases uric acid concentrations in plasma. *Clinical Chemistry*, *32*(8), 1557–1559.

Mikhail, M. W., & Abdel-Hamid, Y. M. (2007). Effect of warfarin anticoagulant rodenticide on the blood cell counts of Rattus norvegicus and Rattus rattus. *Journal of the Egyptian Society of Parasitology*, *37*(3), 853–861.

Mirkov, I., Popov Aleksandrov, A., Demenesku, J., Ninkov, M., Mileusnic, D., Zolotarevski, L., Subota, V., Kataranovski, D., & Kataranovski, M. (2016). Intestinal toxicity of oral warfarin intake in rats. *Food and Chemical Toxicology*, *94*, 11–18. https://doi.org/10.1016/j.fct.2016.05.007

Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, *247*(10), 3170–3175.

Mowat, A. M. (2003). Anatomical basis of tolerance and immunity to intestinal antigens. *Nature Reviews Immunology*, *3*(4), 331–341.

Muktha Bai, K., Krishnakumari, M. K., & Majumder, S. K. (1992). Single dose toxicity (oral) of a chronic anticoagulant rodenticide warfarin in albino rats. *Comparative Physiology and Ecology*, *17*(2), 75–82.

Murphy, T. H., Miyamoto, M., Sastre, A., Schnaar, R. L., & Coyle, J. T. (1989). Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. *Neuron*, *2*(6), 1547–1558.

Murphy, T. H., Schnaar, R. L., & Coyle, J. T. (1990). Immature cortical neurons are uniquely sensitive to glutamate toxicity by inhibition of cystine uptake. *The FASEB Journal*, *4*(6), 1624–1633.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction.

Analytical Biochemistry, *95*(2), 351–358.

Oishi, K., Yokoi, M., Maekawa, S., Sodeyama, C., Shiraishi, T., Kondo, R., Kuriyama, T., & Machida, K. (1999). Oxidative stress and haematological changes in immobilized rats. *Acta Physiologica Scandinavica*, *165*(1), 65–69.

Patel, R. P., Svistunenko, D. A., Darley-usmar, V. M., Symons, M. C. R., & Wilson, M. T. (1996). Redox cycling of human methaemoglobin by H2O2 yields persistent ferryl iron and protein based radicals. *Free Radical Research*, *25*(2), 117–123.

Radi, Z. A., & Thompson, L. J. (2004). Renal subcapsular hematoma associated with brodifacoum toxicosis in a dog. *Veterinary and Human Toxicology*, *46*(2), 83–84.

Ratan, R. R., Murphy, T. H., & Baraban, J. M. (1994). Rapid communication: oxidative stress induces apoptosis in embryonic cortical neurons. *Journal of Neurochemistry*, *62*(1), 376–379.

Rattner, B. A., Lazarus, R. S., Elliott, J. E., Shore, R. F., & van den Brink, N. (2014). Adverse outcome pathway and risks of anticoagulant rodenticides to predatory wildlife. *Environmental Science & Technology*, *48*(15), 8433–8445.

Ravi, K., Ramachandran, B., & Subramanian, S. (2004). Protective effect of Eugenia jambolana seed kernel on tissue antioxidants in streptozotocin-induced diabetic rats. *Biological and Pharmaceutical Bulletin*, *27*(8), 1212–1217.

Robertson, L., Yeoh, S. E., & Ramli, A. (2017). Secondary prevention of recurrent venous thromboembolism after initial oral anticoagulation therapy in patients with unprovoked venous thromboembolism. *Cochrane Database of Systematic Reviews*, 12. Art. No.: CD011088, Published by John Wiley & Sons, Ltd.

Ryan, M., Ware, K., Qamri, Z., Satoskar, A., Wu, H., Nadasdy, G., Rovin, B., Hebert, L., Nadasdy, T., & Brodsky, S. V. (2014). Warfarin-related nephropathy is the tip of the iceberg: direct thrombin inhibitor dabigatran induces glomerular hemorrhage with acute kidney injury in rats. *Nephrology Dialysis Transplantation*, *29*(12), 2228–2234.

Sarra, M., Pallone, F., MacDonald, T. T., & Monteleone, G. (2010). Il-23/Il-17 Axis in IBD. *Inflammatory Bowel Diseases*, *16*(10), 1808–1813.

Shearer, M. J. (1990). Vitamin K and vitamin K-dependent proteins. *British Journal of Haematology*, *75*(2), 156–162.

Stanciu, M., Wang, Y., Kentor, R., Burke, N., Watkins, S., Kress, G., Reynolds, I., Klann, E., Angiolieri, M. R., & Johnson, J. W. (2000). Persistent activation of ERK contributes to glutamate-induced oxidative toxicity in a neuronal cell line and primary cortical neuron cultures. *Journal of Biological Chemistry*, *275*(16), 12200–12206.

Teragaki, M., Kawano, H., Makino, R., Inoue, K., Sai, Y., Hosono, M., Suehiro, S., & Okamura, M. (2012). A case of warfarin-induced eosinophilia. *Internal Medicine*, *51*(12), 1627–1629.

Tietz, N. W. (1990). *Clinical guide to Laboratory tests. 2nd, edn*. Philadelphia: Saunders.

Toth, K. M., Clifford, D. P., Berger, E. M., White, C. W., & Repine, J.

E. (1984). Intact human erythrocytes prevent hydrogen peroxide-mediated damage to isolated perfused rat lungs and cultured bovine pulmonary artery endothelial cells. *The Journal of Clinical Investigation*, *74*(1), 292–295.

Tracz, M. J., Alam, J., & Nath, K. A. (2007). Physiology and pathophysiology of heme: implications for kidney disease. *Journal of the American Society of Nephrology*, *18*(2), 414–420.

Tsiftsoglou, A. S., Tsamadou, A. I., & Papadopoulou, L. C. (2006). Heme as key regulator of major mammalian cellular functions: molecular, cellular, and pharmacological aspects. *Pharmacology & Therapeutics*, *111*(2), 327–345.

Vitellas, K. M., Vaswani, K., & Bova, J. G. (2000). Case 3. Spontaneous uroepithelial hemorrhage caused by warfarin overdose. *AJR. American Journal of Roentgenology*, *175*(3), 881–885.

Wallin, C., Puka-Sundvall, M., Hagberg, H., Weber, S. G., & Sandberg, M. (2000). Alterations in glutathione and amino acid concentrations after hypoxia–ischemia in the immature rat brain. *Developmental Brain Research*, *125*(1–2), 51–60.

Wang, H., Zhang, Y., & Heuckeroth, R. O. (2007). PAI-1 deficiency reduces liver fibrosis after bile duct ligation in mice through activation of tPA. *FEBS Letters*, *581*(16), 3098–3104.

Ware, K., Qamri, Z., Ozcan, A., Satoskar, A. A., Nadasdy, G., Rovin, B. H., Hebert, L. A., Nadasdy, T., & 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.

Weaver, C. T., Hatton, R. D., Mangan, P. R., & Harrington, L. E. (2007). IL-17 family cytokines and the expanding diversity of effector T cell lineages. *The Annual Review of Immunology.*, *25*, 821–852.

Weber, J., Olyaei, A., & Shatzel, J. (2019). The efficacy and safety of direct oral anticoagulants in patients with chronic renal insufficiency: a review of the literature. *European Journal of Haematology*, *102*(4), 312–318.

Xiao, N., Callaway, C. W., Lipinski, C. A., Hicks, S. D., & DeFranco, D. B. (1999). Geldanamycin provides posttreatment protection against glutamate-induced oxidative toxicity in a mouse hippocampal cell line. *Journal of Neurochemistry*, *72*(1), 95–101.

Yen, D., Cheung, J., Scheerens, H., Poulet, F., McClanahan, T., McKenzie, B., Kleinschek, M. A., Owyang, A., Mattson, J., & Blumenschein, W. (2006). IL-23 is essential for T cell–mediated colitis and promotes inflammation via IL-17 and IL-6. *The Journal of Clinical Investigation*, *116*(5), 1310–1316.

ARABIC SUMMARY

السمية الكلوية التي يسببها الوارفارين: دراسة كيميائية حيوية ونسيجية

إلهام عبد الصبورعبد الله[1]؛ هبه الله أحمد محمود[2]؛ مجدي ويلسون[2]؛ منى محمد عطيه[3]
1 معمل الفسيولوجى- قسم علم الحيوان- كلية العلوم- جامعة الوادى الجديد- الخارجة - مصر
2 معمل بحوث وقاية النبات – قسم الآفات الحيوانية – مصر
3 معمل بيولوجيا الخلية الجزيئية- قسم علم الحيوان- كلية العلوم- جامعة أسيوط – مصر.

تم تداول العديد من الطرق البديلة للتعامل مع القوارض أو تصحيح تلف الفئران عبر الأجيال. تم تطوير مبيدات القوارض المضادة للتخثر في أواخر الأربعينيات من القرن الماضي، وكانت خطوة مهمة إلى الأمام في التعامل مع القوارض. في المواقع يتم استخدام الوارفارين ونظيره من الصوديوم للسيطرة على القوارض. الوارفارين هو دواء بشري يستخدم لعلاج مشاكل تخثر الدم وتُعطى مضادات التخثر هذه كمبيدات للقوارض في الجرذان والفئران، مما يسبب النزيف والموت في النهاية. في هذه الدراسة، تم تحديد الوارفارين الفموي الحاد من LD50 (نصف الجرعة المميتة) للجرذان البرية. تم فحص تأثيرات الجرعات المختلفة من الوارفارين (ربع نصف الجرعة المميتة وهي 27.5 و9 ملجرام/ كلجم) و(نصف نصف الجرعة المميتة وهي 55 و18 ملجرام/ كلجم) وذلك على كلي ذكور وإناث الجرذان على التوالي. تضمنت هذه الآثار ارتفاعًا ملحوظًا في وظائف الكلى، وارتفاعًا ملحوظًا في مستوى أكسدة الدهون مع انخفاض كبير في أنشطة الجلوتاثيون والسوبر اوكسيد ديزميوتيز واكسيد النيتريك . فيما يتعلق بالتغيرات النسيجية المرضية، كانت هناك تغييرات في الكبيبات والانيبيبات الكلوية تتمثل في التمدد في الظهارة المبطنة للانيبيبات الكلوية مع تنكس الأوعية الدموية للخلايا الظهارية التي تغطي الأنابيب، وفي جميع أنحاء التجاويف الداخلية للأنابيب كانت هناك مناطق واسعة من النخر وظهور التهابات وتسلل للخلايا الالتهابية، الخلايا الليمفاوية، ووحيدات الأنوية في الأنسجة البينية. وقد خلصت الدراسة إلى أن تناول الوارفارين عن طريق الفم للجرذان البرية أدى إلى سمية كلوية واضحة مرتبطة بالجرعة مع تأثيرات مرضية مختلفة.