



New Valley University



Faculty of Science

***Estimation of devastating effect of Warfarin on  
liver, kidney and immune system in male and  
female wild rats***

**A THESIS**

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## **List of abbreviations**

<b>IPM</b>	Integrated pest management.
<b>ARs</b>	Anticoagulant Rodenticides.
<b>FGARs</b>	First generation anticoagulant rodenticides.
<b>SGARs</b>	Second generation anticoagulant rodenticides.
<b>EU</b>	European Union.
<b>INR</b>	International normalized ratio.
<b>CYP2C9</b>	Cytochrome P450 family 2 subfamily C member 9.
<b>CYP1A1</b>	Cytochrome P450 family 1 subfamily A member 1.
<b>CYP1A2</b>	Cytochrome P450 family 1 subfamily A member 2.
<b>CYP3A4</b>	Cytochrome P450 family 3 subfamily A member 4.
<b>CYP2C9</b>	Cytochrome P450 family 2 subfamily C member 9.
<b>DOACs</b>	Direct- acting oral anticoagulants.
<b>PCC</b>	Prothrombin complex concentrate.
<b>WRN</b>	Warfarin Related nephron-pathy.
<b>AKI</b>	Acute Kidney Injury.
<b>EPA</b>	Environmental Protection Agency's.
<b>RBCs</b>	Red blood cells
<b>PLT</b>	Platelets
<b>WBCs</b>	White blood cells
<b>HB</b>	Hemoglobin
<b>HCT</b>	Hematocrit
<b>PBMC</b>	Peripheral blood mononuclear cells.
<b>BBB</b>	Blood brain barrier.
<b>MMPS</b>	Matrix metalloproteinases.
<b>HT</b>	Hemorrhagic transformation.

## *Abbreviations*

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<b>IRI</b>	Ischemic reperfusion injury.
<b>ROS</b>	Reactive oxygen species.
<b>Gpx</b>	Glutathione peroxidase.
<b>GSH</b>	Glutathione.
<b>Nrf2</b>	Nuclear factor erythroid-related factor 2.
<b>VKOR</b>	Vitamin K epoxide reductase.
<b>AIB</b>	Serum albumin level.
<b>PT-INR</b>	Prothrombin time.
<b>AF</b>	Arterial Fibrillation. Arterial fibrosis.
<b>MB</b>	Major bleeding events.
<b>BSA</b>	Bovine serum albumin.
<b>NAR</b>	Nagase an albuminemic rats.
<b>TIBC</b>	Total iron binding capacity.
<b>IDA</b>	Iron deficiency anemia.
<b>IgM</b>	Immunoglobulin M.
<b>IgG</b>	Immunoglobulin G.
<b>IgA</b>	Immunoglobulin A.
<b>IL</b>	Interleukins.
<b>ALT</b>	Alanine aminotransferase activity.
<b>AST</b>	Aspartate aminotransferase activity.
<b>ALP</b>	Alkaline phosphatase.
<b>CKD</b>	Chronic kidney disease.
<b>SOD</b>	Superoxide dismutase.
<b>CAT</b>	Catalase.
<b>GST</b>	Glutathione s-transferase.
<b>NO</b>	Nitric oxide.
<b>iNOS</b>	Inducible nitric oxide stimulator.

## *Abbreviations*

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<b>LPS</b>	Lipopolysaccharide.
<b>NOACs</b>	Non –vitamin k oral anticoagulants.
<b>SDS-PAGE</b>	Sodium dodecyl sulphate–polyacrylamide gel electrophoresis.
<b>RIPA Lysis buffer</b>	Radio-immunoprecipitation assay buffer.
<b>APS</b>	Amonium persulfate.
<b>ECL</b>	Enhanced Chemiluminescence Luminal.
<b>DAB</b>	3, 3'-diaminobenzidine.
<b>PBS</b>	Phosphate- buffered saline solution.
<b>POD</b>	Peroxidase.
<b>LPO</b>	Lipid peroxidation.
<b>GOD/PAP</b>	Colorimetric method with glucose oxidase, and 4-aminoantipyrine.
<b>TBA</b>	Thiobarbituric acid.
<b>VKD</b>	Vitamin K-dependent.
<b>CYP2</b>	Cytochrome P450 Family 2 (hemoprotein)
<b>PRE</b>	Particular responsive element in nuclear DNA.
<b>HSC</b>	Hepatic sinusoidal endothelial cells.
<b>SMC</b>	Smooth muscle cells.
<b>GSK</b>	Glycogen synthase kinase

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**Fig. 42:** (a) kidney histopathology score (Heijnen's scores), and (b) the percentage of kidney fibrosis score were assessed of kidney in female and male wild rats (*Rattus rattus*)

## 5. Summary

Sixty adult female and male wild rats of the species *Rattus rattus*, weighing 150–200 g, were captured alive from Poultry farm in the Assiut valley for the experiment. They were housed in separate traps for one week at room temperature (25°C), with a typical 12 h light/12 h dark cycle in the animal house of the Zoology Department, Faculty of Science, Assiut University. All animals were fed on wet bread, tomatoes, and tap water. Trapped rats were divided into six groups of ten animals each, including both sexes.

Group (I) was the male control, group II was the female control, groups III and IV were female rats treated orally with warfarin for 18 days  $1/4 \text{ LD}_{50}$  and  $1/2 \text{ LD}_{50}$  at 9 and 18 mg/kg, respectively. Groups V and VI were male rats treated orally with warfarin for 18 days at 27.5 and 55 mg/kg, respectively. Different leukocyte, red blood cell count, serum protein fraction, total bilirubin, direct bilirubin, alkaline phosphatase, glucose, albumin, and total protein and ALT and AST activity in blood plasma were estimated using blood samples taken from the jugular vein during rats' scarification in clean, dry, sterile tubes containing heparin. After centrifugation at 5000 rpm for 5 minutes, serum samples were collected from clotted blood without

anticoagulant using anticoagulant buffer. The liver and kidney were diced for further examination such as Western blotting, Immunohistochemistry, Estimation of superoxide dismutase, catalase, activity reduced glutathione, lipid peroxidation and nitric oxide in liver and kidney.

**The results can be summarized in the following points:**

The effects of warfarin on liver, kidney, and blood of female & male wild rat have been studied. The administration of warfarin induced a marked oxidative stress. Antigens of different related functions as the level of CYP2C9, NRF2 and the level of P450 were investigated by western blot analysis or the level of casp.3 was observed by immunohistochemistry. Total blood serum protein fraction, white blood cell differential count and red blood cell and body weight were studied to clarify the effect of warfarin at serological and immunological levels.

Accordingly, warfarin oral intake cause sever toxicity in plasma, liver and kidney concluded by biochemical parameters in plasma and Antioxidants and oxidative stress biomarkers of liver and kidney. The increasing oxidative stress in the liver and kidney due to warfarin orally intake in wild rat cause by the following mechanisms:

- 1- Pathomorphological changes of wild rats various as dormancy, dull eyes, and hemorrhages of many regions of the body. And the regulation of CYP2C9 appears to be dependent on NRf2 and the level of P450, and affects the amount of bleeding.
- 2- A decrease in NRf2 is considered to be a non-defense system against oxidative stress, decreasing the endogenous antioxidant defense system and accelerating hemorrhage.
- 3- Free hemoglobin and decreased serum albumin and antitrypsin activate cleaved caspases-3, inducing apoptosis and fibrosis after liver hemorrhage and bleeding.
- 4- Intracellularly, free hemoglobin caused by warfarin activates cleaved caspases-3 and induces apoptosis after liver hemorrhage. The intracellular hemoglobin activates proinflammatory pathways a significant role in the pathogenesis of acute liver injury.
- 5- The by-products of lipid peroxidation such as malondialdehyde (MDA) detected in the liver of iron-loaded rats, act as profibrogenic stimuli. free hemoglobin released by RBCs in the liver lumen and increased lipid peroxidation.

- 6- Warfarin increases serum transferrin and lipid peroxidation, which are pro-fibrogenic stimuli, by elevating iron levels and hemosiderin, promoting fibrosis and the pathogenesis of acute liver and kidney injury.
- 7- warfarin effect on NRF2 path way and inhibit it to bind antioxidant response elements lead to decreasing in endogenous antioxidant defense system (SOD, CAT & GSH).
- 8- Since warfarin is primarily bound to serum albumin, hypoalbuminemia is likely to increase the free fraction of warfarin and to increase the risk of bleeding & hemorrhagic.
- 9- Warfarin reduces antitrypsin which lead to decrease modulate systemic inflammatory responses, stimulate apoptosis and fibrosis by deposition of collagen fibers in liver cell. Also, antitrypsin has affecting a wide range of inflammatory cells such as neutrophils, monocytes, lymphocytes, RBC, and eosinophil cells.
- 10- Elevated iron level is a common feature of all these fibrosis-promoting warfarin exposures led to increase in serum transferrin and accumulation of Hemosiderin after bleeding.

- 11- Warfarin-related nephropathy (WRN) refers to glomerular leaking of red blood cells that causes tubular injury and chronic kidney diseases.
- 12- Warfarin oral intake cause sever toxicity in plasma, liver and kidney and decreased of vitamin K which led to change in many Biochemical parameters in plasma and Antioxidants and oxidative stress biomarkers of liver and kidney as an increase on LPO, ALT, AST, ALP, creatinine, plasma glucose, direct bilirubin, total bilirubin, uric acid and urea. Also, it was decrease of GSH, SOD, CAT, and NO. Warfarin produced clear dose-related hepatotoxic, reno toxicity and pathological effects in the rat.