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

Submitted to Zoology Department, Faculty of Science, New Valley University.

For

Partial fulfillment the degree of Master of Science (M.SC.) in Zoology (Animal Physiology)

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(2021-1443)

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List of abbreviations

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

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

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

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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 $\mathbf{LD_{50}}$ and 1/2 $\mathbf{LD_{50}}$ 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 jagular 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.