Ain Shams University
Faculty of Women for Arts, Science& Education
Department of Zoology

Possible Protective Effect of Certain Flavonoid against Flutamide-Induced Hepatotoxicity in Adult Male Rats.

A Thesis submitted For (Ph.D.) Degree in Zoology By

Aml Salem Saleh Ahmed
Assist. Lecturer in Zoology Department
Faculty of Women for Arts, Science and
Education-Ain Shams University.

Supervisors

Prof. Dr. Fatma Mohamed Mazhar
Prof. of Comparative Anatomy and Embryology.
Department of Zoology,
Faculty of Women for Arts, Science and
Education-Ain Shams University.

Prof. Dr. Shadia Mohamed Kadry
Prof. of Histology& Histochemistry.
Department of Zoology,
Faculty of Women for Arts, Science
and Education-Ain Shams University.

Assist.Prof. Hala Fahmy Abd-Ellah Sayed
Assist. Prof. of Histology& Cytology.
Department of Zoology,
Faculty of Women for Arts, Science and
Education-Ain Shams University.

Dr. Hend Ahmed Sabry
Lecturer of Zoology.
Department of Zoology,
Faculty of Women for Arts, Science and Education
Ain Shams University.

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جامعة عين شمس
كلية البنات للآداب والعلوم والتربية
قسم علم الحيوان

التأثير الوقائى المحتمل للفلافونويد ضد التسمم الكبدى المستحدث بمادة فلوتاميد فى ذكور الجرذان البالغة.

رسالــة مقدمة للحصـول على درجة دكتوراه الفلسفة في العلوم (علم الحيوان)

مقدمة من

أمل سالم صالح أحمد

(ماجستير في العلوم)

مدرس مساعد بقسم علم الحيوان

تحت إشراف

أ.د/ فاطمة محمد مظهر

أستاذ التشريح المقارن والأجنة

قسم علم الحيوان

كلية البنات للآداب والعلوم والتربية- جامعة عين شمس

أ.د شادية محمد قدرى

أستاذ الأنسجة وكيمياء الأنسجة

قسم علم الحيوان

كلية البنات للآداب والعلوم والتربية- جامعة عين شمس

أ.م.د. هالة فهمى عبد اللاه سيد

أستاذ مساعد علم الأنسجة و الخلايا

قسم علم الحيوان

كلية البنات للآداب والعلوم والتربية- جامعة عين شمس

د. هند أحمد صبرى

مدرس فى علم الحيوان

قسم علم الحيوان

كلية البنات للآداب والعلوم والتربية-جامعة عين شمس

٢٠١٥

بسم الله الرحمن الرحيم

قَالُوا سُبْحَانَكَ لاَ عِلْمَ
لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ
أَنتَ الْعَلِيمُ الْحَكِيمُ"

صدق الله العظيم
سورة البقرة آية (32)

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Qualifications

Name: Aml Salem Saleh Ahmed.

Scientific Degree: M.Sc.

Department: Zoology

College: Faculty of Women for Arts, Science and Education

University: Ain Shams University.

B.Sc. Graduation Year: 2007

M.Sc. Graduation Year: 2012

Approval Sheet

Name: Aml Salem Saleh Ahmed

Title : **Possible Protective Effect of Certain Flavonoid against Flutamide-Induced Hepatotoxicity in Adult Male Rats.**

Scientific Degree: M.Sc.

Supervisors

Prof. Dr. Fatma Mohamed Mazhar
Prof. of Comparative Anatomy and Embryology.
Department of Zoology
Faculty of Women for Arts, Science and Education–Ain Shams University

Prof. Dr. Shadia Mohamed Kadry
Prof. of Histology & Histochemistry
Department of Zoology
Faculty of Women for Arts, Science and Educatio–Ain Shams University

Assist. Prof. Hala Fahmy Abd-Ellah
Assist.Prof. of Histology& Cytology
Department of Zoology
Faculty of Women for Arts, Science and Education–Ain Shams University

Dr. Hend Ahmed Sabry
Dr. of Zoology
Department of Zoology
Faculty of Women for Arts, Science and Education–Ain Shams University

ABSTRACT

Flutamide, a non-steroidal anti-androgenic anilide compound, is widely used for the treatment of prostatic cancer. It is an idiosyncratic hepatotoxicant that sometimes results in severe liver toxicity. It shows a photo hemolytic effect on human erythrocytes and photo induces lipid peroxidation. Plant flavonoids are emerging as potent therapeutic drugs effective against a wide range of free radical mediated diseases. Morin, a member of flavonols, exerts antioxidant potential and offers protection against the oxidative stress induced by hydrogen peroxide. The present study aimed to investigate the protective effect of morin against flutamide on the rat liver. Seventy male albino rats weighing 190-200 g were used to study the biochemical analysis of liver function parameters, including ALT, AST, direct and total bilirubin activities in the blood sera and MDA, SOD, GSH and GST in liver tissue.

In addition, the histological alterations and histochemical changes, including polysaccharides and total proteins in liver tissues were investigated, as well as studying the electron microscopic alterations. The experimental animals were divided into seven groups, 10 rats each, and treated as follows: 1) rats did not receive any treatment (control group); 2) rats received 0.5 ml of carboxy methyl cellulose (CMC, 0.5%) for 8 weeks (vehicle group); 3) rats received CMC for 4 weeks then 50 mg morin / kg b.w. for other 4 weeks (CM group); 4) rats

received 50 mg morin / kg b.w. for 8 weeks (morin group); 5) rats received CMC for 4 weeks followed by treatment with 100 mg flutamide/ kg b.w. for additional 4 weeks (CFgroup); 6) rats received CMC for 4 weeks then received 50 mg morin/ kg b.w.+ 100 mg flutamide/ kg b.w. for another 4 weeks (CMF group) and 7) rats received 50 mg morin/ kg b.w. for 4 weeks then received 50 mg morin/ kg b.w.+ 100 mg flutamide/ kg b.w. for extra 4 weeks (MMFgroup). Rats received their respective doses daily by oral gavage.

The results of the present study in flutamide group revealed that the mean final body weight decreased; meanwhile, the absolute and relative liver weights increased. There was a very highly significant increase in ALT, AST, direct and total bilirubin activities in serum while, in the hepatic tissue there was an increase in MDA and a decrease in SOD, GSH and GST levels.

The histopathological studies displayed deleterious alterations in the liver tissue where flutamide caused distortion of hepatic architecture with swollen vacuolar hyaline degeneration, atrophy and necrosis of hepatocytes. Some nuclei of the degenerated cells showed pyknosis and the other showed karyolysis. Inflammatory cellular infiltration in addition to congestion and dilatation of the blood vessels were also detected. Histochemical studies revealed that flutamide alone decreased polysaccharides and total proteins in the liver tissue. These changes were confirmed at ultrastructural level, including pyknotic nuclei as well as irregular nuclear envelopes, swollen

mitochondria with ill-differentiated cristae, fragmented rough endoplasmic reticulum and increased collagenous fibrils manifesting early sign of fibrosis.

In CMF group, morin showed significant improvement in the levels of ALT, AST, direct and total bilirubin and MDA, SOD, GSH, GST and the mean final body weight and absolute and relative liver weights. Also, the histological, histochemical and electron microscopic alterations were improved.

On the other hand, MMF group showed marked recovery in all these changes induced by flutamide. The results of this study indicated that pre- and co-administration of morin was found to be more effective in restoring flutamide-induced biochemical, histological, histochemical and electron microscopic alterations.

CONTENTS

LIST OF ABBREVIATIONS

ABT	Aminobenzotriazole
AC	Ammnium chloride
ALF	Acute liver failure
ALP	Alkaline phosphatase
Alpha-SMA	Alpha-smooth muscle actin
ALT	Alanine aminotransferase
APAP	Acetaminophen
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BSEP	Bile salt export pump
CAT	Catalase
CMC	Carboxy methyl cellulose
Cox-2	Cyclooxygenase
CP	Cisplatin
CPA	Cyproterone acetate
CPR	Cytochrome P450 reductase
CPX	Cyclophosphamide
DEN	Diethylnitrosamine
DMH	Dimethylhydrazine
DOCA	Deoxycorticosterone acetate
DMN	Dimethylnitrosamine
DPPH	Diphenylpicrylhydrazyl
ETC	Electron transport chain
GGT	Gamma-glutamyl transpeptidase
GLDH	Glutamate dehydrogenase
GPx	Glutathione peroxidase
GSH	Reduced glutathione
GSSG	Glutathione disulfide
GR	Glutathione reductase
HP	Hydroperoxide

Il- 1 beta	Interleukin-1 beta
Il-6	Interleukin-6
LDH	Lactate dehydrogenase
LFTs	Liver function tests
LHRH	Luteinizing hormone releasing hormone
LPO	Lipidperoxidation
LPS	Lipopolysaccharide
MC- LR	Microcystin- LR
MDA	Malondialdehyde
Mor	Morin
NaMSA	Morin-5'-sulfonic acid sodium salt
NaQSA	Quercetin-5'-sulfonic acid sodium salt
NF- $_K$B	Nuclear factor Kappa of activated B cells
PAB	Partial androgen blockage
PB	Phenobaribital
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RT	Radiation therapy
RT-PCR	Reverse transcriptase polymerase chain reaction
SOD	Superoxide dismutase
TAB	Total androgen blockage
TAS	Total androgen suppression regimen
TBARS	Thiobarbituric acid reactive substances
TGF- beta1	Transforming growth factor beta (1)
TNF	Tumor necrosis factor
UDCA	Ursodeoxycholic acid