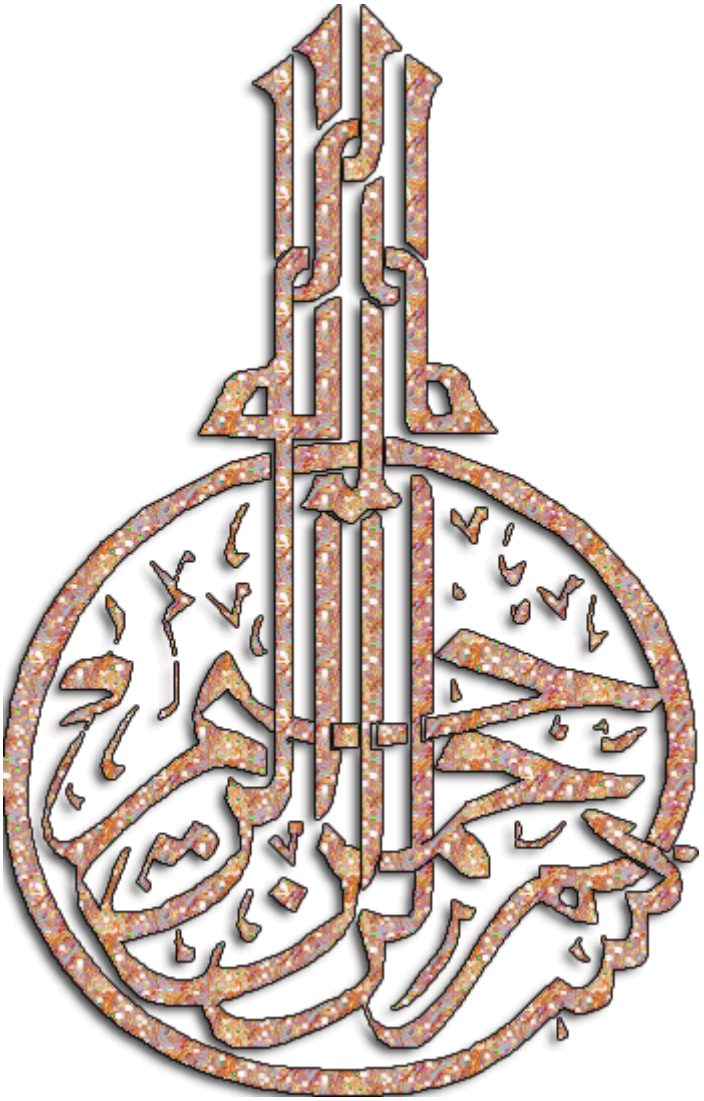


Hepatoprotective Effect of Chamomile Capitula Extract against 2, 4-Dichlorophenoxyacetic Acid- Induced Hepatotoxicity in Rats

By
Dalal Abdul Aziz Al baroudi

A thesis submitted for the requirements of the degree of Master of Home Economics
[Food and Nutrition]

Supervised By
Dr. Reham Abu El mahasen Arafat
Dr. Thanaa Abdel Fattah Ali El-Kholy

FACULTY OF HOME ECONOMICS
KING ABDULAZIZ UNIVERSITY
JEDDAH – SAUDI ARABIA
Rajab 1434H – May 2013G

Hepatoprotective Effect of Chamomile Capitula Extract against 2, 4-Dichlorophenoxyacetic Acid- Induced Hepatotoxicity in Rats

By
Dalal Abdul Aziz Al baroudi

This thesis has been approved and accepted in partial fulfillment of the requirements for the degree of Master of Home Economics [Food and Nutrition]

EXAMINATION COMMITTEE

	Name	Rank	Field	Signature
Internal Examiner	Dr. Hala Abd El-Rahman Khattab	Associate professor	Food and Nutrition	
External Examiner	Prof. Jalalueddin A. Jalal Khan	professor	Biochemistry	
Co-Advisor	Dr. Thanaa Abdel Fattah ElKholy	Associate professor	Clinical Nutrition	
Advisor	Dr. Reham Abu Elmahasen Arafat	Associate professor	Food and Nutrition	

KING ABDULAZIZ UNIVERSITY
1434H- 2013G

إهداء

إلى من كلّل العرق جبينه وشققت الأيام يديه..

إلى من جرع الكأس فارغاً ليسقيني قطرة حب..

إلى من حصد الأشواك عن دربي ليمهد لي طريق العلم..

إلى من علمني العطاء بدون انتظار..وعلمني أن الأعمال الكبيرة لاتتم إلا بالصبروالإصرار..

كم تمنيت أن يمد الله في عمرك لترى ثماراً قد حان قطافها بعد طول انتظار..

الى من أودعني لله... ستبقى كلماتك نجوم أهتدي بها اليوم وفي الغد وإلى الأبد..

أبي الحبيب.. أهديك ثمرة من ثمار غرسك..

إلى من نذرت عمرها في أداء رسالة صنعتها من أوراق الصبر..وطرزتها في ظلام الدهر..

رسالة تعلم العطاء والوفاء كيف يكون..

أمي الحبيبة.. إليك أهدي هذه الرسالة.. وشتان بين رسالة ورسالة..

أمد الله في عمرك بالصالحات.. فأنت زهرة الحياة ونورها..

إلى القلوب الطاهرة الرقيقة والنفوس البريئة إلى رياحين حياتي إخـــــوتي..

ACKNOWLEDGEMENT

In the name of Allah most gracious, most merciful.

All praise and glory to Allah the almighty who made this small work to be accomplished and I am asking him to accept my efforts.

My deep appreciation goes to my principle supervisor Dr. Reham Abu Elmahasen Arafat, Associate Professor in the Food and Nutrition Department, Faculty of Home Economics, King Abdul Aziz University, for her constant help, valuable guidance, constructive criticism and continual interest over all the period of this course of work.

I also give my thanks to my co-supervisor Dr. Thanaa ElKholy Assistant Professor in the Clinical Nutrition Department, Faculty of Applied Medical Science, King Abdul Aziz University, for her guidance and valuable recommendations throughout my work.

I am especially thankful and grateful for my dear friends Tahani Dakhakhni and Noura Al-Otaibi for their great efforts in helping me in the fulfillment of this work.

Finally, a lot of thanks also to my precious parents, sisters and brothers for their continuous encouragement and support for me through my hard times.

أتقدم بخالص الشكر والتقدير لمدينة الملك عبد العزيز للعلوم والتكنولوجيا لدعمها المالي لمشروع البحث رقم (أ-ط-١٢-٠٦٦٦) مما مكني من توفير اللوازم والمواد الكيميائية الضرورية للبحث.

I wish to express my thanks and appreciation to King Abdul Aziz City for Science and Technology for its financial support to the project of the research number (P-S-12-6660) and which enabled me in the provision of requirements and necessary chemical substances for the research.

Hepatoprotective Effect of Chamomile Capitula Extract against 2, 4-Dichlorophenoxyacetic Acid- Induced Hepatotoxicity in Rats

By
Dalal Abdul Aziz Al baroudi

Abstract

Objective: The present study was carried out to investigate the effect of oral administration of aqueous extract of Chamomile capitula extract for 4 weeks on hepatotoxicity induced to rats by herbicide 2, 4-Dichlorophenoxy acetic acid (2, 4 D). These effects could be explored by measuring body weight gain, feed efficiency ratio and relative weight of the liver. Serum levels of liver enzymes; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP); albumin, total protein, total bilirubin, lactate dehydrogenase (LDH) enzyme and antioxidant enzymes; glutathione reductase (GR) and superoxide dismutase (SOD) were determined. Histopathological examination of liver was also performed.

Methods: Thirty six male Wistar rats were allocated into six groups as follows: - Group1: negative (normal rats) control; Group 2: positive (hepatotoxic) control given accumulative dose of 75 mg/kg b.wt., of 2, 4- D; Group 3: Positive (hepatotoxic) control given accumulative dose of 150 mg/kg b.wt., of 2, 4-D; Groups 4: given orally Chamomile capitula extract in a dose of 500 mg/kg b.wt., alone. Groups 5 and 6: given combination of Chamomile capitula extract with either the accumulative doses of the 75 mg/kg b.wt., or the 150 mg/kg b.wt., of 2, 4-D.

Results: The results showed that oral administration of Chamomile capitula extract to hepatotoxic rats for 28 days significantly decreased the elevated serum levels of liver enzymes (AST, ALT and ALP), total bilirubin and lactate dehydrogenase enzyme and increased serum total protein, albumin, when compared to the corresponding control positive groups. Levels of antioxidant enzymes glutathione reductase and superoxide dismutase were significantly increased as compared to the control positive groups. Histopathological examination of liver sections of rats administered Chamomile capitula extract showed alleviation of histological degenerative changes caused by 2, 4-D herbicide.

Conclusion: The results suggest that Chamomile capitula aqueous extract induces potent hepatoprotective and antioxidant effects in 2, 4 D - hepatotoxic rats. This study recommends that intake of Chamomile capitula extract as a herbal tea may be beneficial for patients who suffer from liver diseases and oxidative stress.

التأثير الوقائي الكبدي لمستخلص الكاموميل على الفئران المصابة بالتسمم الكبدي بـــ٢و٤ داي كلورو فينوكسي استيك اسيد

دلال عبدالعزيز البارودي

المستخلص

الهدف: استهدفت هذه الدراسة ايضاح تأثير الإعطاء الفموى للمستخلص المائي لأزهار نبات البابونج لمدة أربعة أسابيع على الفئران المصابة بتسمم الكبد المحدث بـــــ٢و٤ داي كلوروفينوكسى حمض الأستيك وذلك من خلال دراسة تأثير ذلك على كلاً من وزن الجسم، معدل التحويل الغذائى، الوزن النسبي للكبد، مستوى إنزيمات الكبد والألبيومين والبروتين الكلى والبيليروبين وانزيم لاكتات ديهيدروجينيز، ومستوى الإنزيمات المضادة للأكسدة فى مصل الدم. كما تم إجراء الفحص الهستوباثولوجى لأنسجة الكبد.

الطرق: استخدم فى هذه الدراسة عدد ٣٦ فأر ذكر من فصيلة ويستر وتم توزيعهم على ست مجموعات وكانت المجموعة الأولى ضابطة سالبة. أما المجموعة الثانية فكانت ضابطة موجبة تم إعطاؤها جرعة تراكمية بمقدار ٧٥ مجم/ كجم من وزن الجسم من ٢و٤ داي كلوروفينوكسى حمض الاستيك لإحداث تسمم كبدى بها. المجموعة الثالثة ضابطة موجبة تم إعطاؤها جرعة تراكمية بمقدار ١٥٠ مجم/ كجم من وزن الجسم من ٢و٤ داي كلوروفينوكسى حمض الاستيك. المجموعة الرابعة اعطيت مستخلص البابونج منفردا عن طريق الفم بجرعة ٥٠٠ مجم/كجم من وزن الجسم. المجموعة الخامسة والسادسة أعطيت كلاً من مستخلص البابونج مع جرعات تراكمية من ٢، ٤–د بالجرعتين ٧٥ مجم/ كجم من وزن الجسم أو ١٥٠ مجم/ كجم من وزن الجسم.

النتائج: أظهرت النتائج أن الإعطاء الفموي لمستخلص نبات البابونج للفئران المصابة بتسمم الكبد لمدة ٢٨ يوم قد أدى إلى انخفاض معنوي في المستويات المرتفعة في مصل كلاً من أنزيمات الكبد و البيليروبين وإنزيم لاكتات ديهيدروجينيز. كذلك أدى إلى ارتفاع في البروتين الكلي والألبيومين بالمقارنة مع المجموعة الضابطة الموجبة. بالإضافة إلى الإرتفاع المعنوي في مستويات الإنزيمات المضادة للأكسدة (جلوتاثيون ريدكتيز، سوبر أكسيد دسميوتيز)

بالمقارنة مع المجموعة الضابطة الموجبة. كما أن الفحص الهستوباثولوجي لأنسجة الكبد في الفئران التي اعطيت مستخلص البابونج أظهر تحسن واضح في التغيرات المرضية التي احدثها المبيد ٢، ٤-د.

الاستنتاج: تشير النتائج إلى أن المستخلص المائي للبابونج له تأثير وقائي للكبد ومضاد للأكسدة في الفئران المصابة بتسمم الكبد المحدث بـــ ٢و٤-د. توصي هذه الدراسة بأن تناول البابونج كمشروب عشبي قد يكون مفيدا للمرضى الذين يعانون من أمراض الكبد والإجهاد التأكسدي.

TABLE OF CONTENTS

Chapter IV: Results

LIST OF FIGURES

Figure **Page**

LIST OF TABLES

Table **Page**

LIST OF SYMBOLS AND TERMINOLOGY

2, 4-D	2, 4-Dichlorophenoxyacetic acid
2, 4-DB	2, 4-Dichlorophenoxybutyric acid
2, 4-D-CoA	2, 4-Dichlorophenoxyacetol-S-acetyl-CoA
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransaminase
AMP	2-amino-2methyle-1-propanol
AST	Aspartate aminotransferase
Bcp	Bromocresol purple
b.w.	Body weight
BWG	Body weight gain
Ca^{2+}	Calcium ion
CAT	Catalase
CCl_4	Carbon tetrachloride
cm	Centimeter
Cu^{++}	Cupric ion
Cu/Zn	Copper/zink
DH	Dark hepatocytes
EDTA	Ethylene diamine tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and drug administration
Fe	Iron
FER	Feed efficiency ratio
FSH	Follicle-stimulating hormone

g	Gram
GGT	Gamma-glutamyl transpeptidase
g/l	Gram per liter
GOT	Glutamic-oxaloacetic transaminase
GPX	Glutathione peroxidase
GR	Glutathione reductase
GRAS	Generally regarded as safe
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione S-transferase
H	Hepatocytes
hr	Hour
HepG2	Hepatoma cell line
H_2O_2	Hydrogen peroxide
HPLC	High performance liquid chromatography
HTC	Hepatoma tissue culture
IP	Intraperitoneal
IU/g	International unit per gram
L	Lipid droplets
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LH	Luteinizing hormone
LPO	Lipid peroxidation
M	Mitochondria
MDA	Malondialdehyde
MDH	Malate dehydrogenase
mg/kg b.wt.	Milligram per kilogram body weight
mg/kg b.wt./day	Milligram per kilogram body weight per day
μm	Micromiter

mm	Millimiter
mM	Millimolar
μ mol/l	Micromol per liter
Mn	Manganese
N	Nucleus
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NADP	Nicotinamide-adenine dinucleotide phosphate
NADPH	Reduced nicotinamide-adenine dinucleotide phosphate
nm	Nanometers
NOEL	No-observed effect level
NTB	Nitro tetrazolium blue
Nu	Nucleolus
O_2	Oxgyen molecule
OH	Hydroxyl group
OsO_4	Osmium tetra oxide
PH	Pale hepatocytes
pH	Potential of hydrogen
p-NP	p-nitrophenol
p-NPP	p-nitrophenylphosphate
PO_4	Phosphate functional group
ppm	Part per million
redox	Reduction oxidation balance
RER	Rough endoplasmic reticulum
rpm	Revolutions per minutes
SD	Standard deviation
SHE	Syrian hamster embryo
SOD	Superoxide dismutase

STZ	Streptozotocin
TBIL	Total bilirubin
TEM	Transmission electron microscope
TP	Total protein
VCE	Vitamin C equivalent
U/l	Unit per liter
U/ml	Unit per milliliter
w/v	Wight per volume

Chapter I

Introduction

Chapter I

Introduction

Since the early 1990s, it has been argued that man-made chemicals used for agricultural, industrial or domestic purposes which can be released in the environment, enter the food chain and produce a number of disorders in animals and possibly in man **(Raseir *et al.* 2006).**

Hepatotoxic, genotoxic and neurotoxic effects of different pesticides have been evaluated in many *in vivo* and *in vitro* studies. The 2, 4-Dichlorophenoxyacetc acid (2, 4-D) is a herbicide widely used to control the growth of broadleaf plants **(Tuschl and Schwab 2003)**. It is chemically derived from phenoxyacetic acid **(Tayeb *et al.* 2010)**. The major uses of 2, 4-D are on cereal crops such as wheat, corn, oats, rye, barley and the cane crops **(U.S. Environmental Protection Agency 2002).**

The Toxicity of 2, 4-Dichlorophenoxyacetic acid has been shown to produce oxidative stress and/or depleted antioxidant enzymes both *in vitro* and *in vivo* **(Tayeb *et al.* 2010)**. Rats subjected to acute intoxication showed ataxia, central nervous system depression, muscular weakness and gasping for breaths **(Rosso *et al.* 2000)**. The subchronic toxicity affected the kidneys (increased kidney weight and histopathological lesions) and the liver (increased liver weight and increased liver enzymes levels). Chronic toxicity of 2, 4-D in rats was manifested by decreased body

weight gain, altered organ weights and hematological parameters and other biochemical changes as explored by **Timchalk (2004).**

The use of traditional medicine is widespread and plants still represent a large source of natural antioxidants that might serve as leads for the development of novel drugs **(Conforti *et al.* 2009)**. The herbal based preparations were effective for the treatment of liver disorders. Therefore, several herbal medicines are experimented for their possible antioxidant and hepatoprotective effects against various chemical induced liver damages in animals. Chamomile is one of these medicinal herbs **(Merlin and Parthasarathy 2011).**

Chamomile contains a large number of therapeutically interesting and active compound classes. The most important ones are the components of the essential oil and the flavonoids fraction. The Chamomile is used as a drug and is included in the pharmacopoeia of 26 countries. It is used mainly as an anti-inflammatory, antiseptic and antispasmodic **(Singh *et al.* 2011).** Traditionally, the essential oil obtained from Chamomile flowers has been used to treat inflammations of the skin and mucosa. It is also inhaled to treat nasal catarrh, inflammation and irritation of the respiratory tract. The tea is drunk to treat flatulent nervous dyspepsia, gastritis, diarrhea, travel sickness and mild anxiety **(Wang *et al.* 2005).**

There are few scientific investigations have so far been reported in literature regarding action of Chamomile on the liver. Therefore, the present study was designed to investigate hepatoprotective effect of Chamomile aqueous extract, on some serum biochemical parameters and liver histopathological changes against 2, 4-Dichlorophenoxyacetic acid (2, 4-D)-induced hepatotoxicity in male rats.

1.1 Aims of the Study

The present study was carried out to investigate the hepatoprotective effect of Chamomile capitula aqueous extract against 2, 4-Dichlorophenoxyacetic acid – induced hepatotoxicity in rats.

The tested parameters were as follows:

1. Chemical analysis of active constituents of Chamomile capitula.
2. Feed intake, body weight gain, feed efficiency ratio and relative liver weight in normal and hepatotoxic rats.
3. Biochemical analysis of serum samples (liver functions and some antioxidant enzymes) in normal and hepatotoxic rats.
4. Histopathological examination of liver in normal and hepatotoxic rats.

Chapter II

Review of Relevant Literature

Chapter II

Review of Relevant Literature

2.1 The herbicide 2, 4-Dichlorophenoxyacetic acid (2, 4-D®)

Herbicides, commonly known as weed killers, are substances used to kill the unwanted weeds. Selective herbicides kill specific target weeds while leaving the desired crop relatively unharmed. The herbicide 2, 4-Dichlorophenoxyacetic acid (2, 4-D) is a selective systemic phenoxy herbicide **(Hassanein 2012)**. The herbicide 2, 4-D is chemically derived from phenoxyacetic acid (Figure 2.1) that acts as a plant growth regulator **(Tayeb *et al.* 2010)**. The main agricultural use of 2, 4-D includes its applications to agricultural crops, forests and recreational areas. Animals or humans intoxications with 2, 4-D can occur upon exposure to contaminated soil, air, water or cereal crops such as wheat, corn, oats, rye, barley and cane crops **(U.S. Environmental Protection Agency, 2002).**

The 2, 4-D is easily absorbed from the alimentary tract and skin and subsequently excreted in the urine in nearly unchanged form in humans. The 2, 4-D is detected in stomach, blood, brain and kidney tissues. The production and degradation of 2, 4-D leads to the creation of many compounds including chlorophenols or dioxins that exert high toxicity **(Bukowska 2006)**.

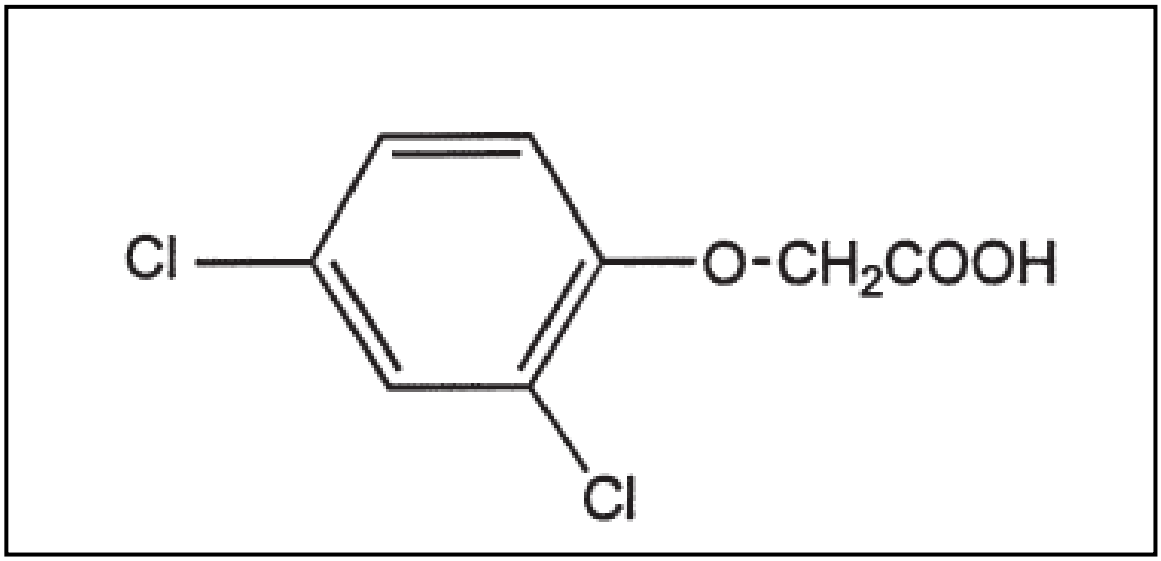


Figure 2.1: 2, 4-Dichlorophenoxyacetic acid chemical structure (Garabrant and Philbert 2002).

2.1.1 Toxicity of 2, 4-Dichlorophenoxyacetic acid

It is well known that some herbicides have many cytotoxic effects on various organs. Teratogenic, genotoxic, neurotoxic, immunosuppressive and hepatotoxic effects of 2, 4-D have been well documented. The herbicide 2, 4-D causes an array of adverse effects to the nervous system such as disruption of the activity of nervous system neurotransmitters and behavioral changes. The researchers also postulated a possible positive association between exposure to 2, 4-D and cancer in humans. The exposure to phenoxyherbicides was associated with an increased risk of Non-Hodgkin's lymphoma. Herbicides represent a double-edged weapon as from one hand we gain great benefits from application of herbicides on crops, from the other we have environmental pollution problems due to their accumulation in animals and humans, so they become hazardous to the public health **(Bukowska 2006).**

Many studies have demonstrated the cytotoxic effect of herbicide 2, 4-D. In this concern, **Charles *et al.* (1996)** studied the subchronic toxicity of three forms of 2, 4-D: the parent form, 2, 4-D acid; 2, 4-D dimethylamine salt and 2, 4-D 2-ethylhexyl ester in dogs. The three studies were designed to allow comparison of the toxicity of the three forms. The results revealed that doses of the subchronic studies ranged from 0.5 to 7.5 mg/kg b.wt./day. Treatment-related findings in the three studies included reductions in body weight gain, food consumption, and minor increases in blood urea nitrogen, creatinine, and alanine aminotransferase. Data of the three subchronic studies demonstrated that the comparable toxicity of the three forms and supported a subchronic no-observed effect level (NOEL) of 1.0 mg/kg b.wt./day for all the three forms.

Due to the similarity in toxicity of the three forms of 2, 4-D, a one -year chronic toxicity study was performed on the parent acid to fully characterize the potential toxicity of 2, 4-D in the male and female dogs. The clinical pathological alterations were similar to those seen in the subchronic studies and were not progressive. The observed histopathological alterations were not severe in nature, and the NOEL in the chronic study was determined to be 1.0 mg/kg b.wt./day. Major histological findings included active hepatic inflammation in both sexes at 5 and 7.5 mg/kg b.wt./day, and mildly elevated sinusoidal pigment in females. Increased pigmentation of the renal tubular epithelium was found in both sexes at the tested doses.

Sulik *et al.* (1998) studied the morphological changes in mitochondria and lysosome of hepatocytes in acute intoxication with 2, 4-D by using transmission electron microscope. The experiment was conducted by using 60 male Wistar rats received 2, 4-D acid by gastric gavage in a dose of 200 mg/kg b.wt. The animals were sacrificed after 12, 24, 48 hours and 4, 10 and 30 days of the experiment. The results obtained indicated that the administration of 2,4-D acid to rats in a dose inducing acute intoxication leads to ultrastructural changes in the liver, which suggested nonspecific reversible adaptative-type damage to parenchymal cells. The authors concluded that the changes observed indicated disorders in energetic processes in hepatocytes and are morphological exponents of detoxicative processes.

Tuschl and Schwab (2003) investigated the cytotoxic effects of the herbicide 2, 4-D on human hepatoma cell line (HepG2) cells. The induction of apoptosis by 2, 4-D was accompanied by a disruption of the mitochondrial membrane potential. The authors concluded that 2, 4-D exerts its cytotoxic effect by the induction of apoptosis (programmed cell death) via a direct potential effect on the mitochondrial membrane.

Maire *et al*. (2007) studied the effect of low and non cytotoxic concentrations of 2, 4-D on Syrian hamster embryo (SHE).The results indicated that the active ingredients of 2, 4-D biotransformation can induce genotoxicity in mammalian cells and should be considered as potentially hazardous for human.

Hassanein (2012) investigated the toxic effects of 2, 4-D on male Sprague-Dawley rats. In this study, 30 rats were divided into three equal groups. The first group received orally normal saline (3 ml/kg b.wt.) and kept as normal control in parallel to the treated groups (5 rats each), the second group received orally 2, 4-D (30 mg/kg b.wt.) for 2 months and the third group received orally 2, 4-D (30 mg/kg b.wt.) for 6 months. At the end of each treatment, specimens from the liver, kidneys, lungs, testicles, epididymis and brain were obtained and subjected for the histopathological examination. After 2 months of treatment with 2, 4-D, the overall changes were milder and ranged from vascular to necrobiotic findings. On the other hand, changes seen after 6 months were severe particularly in the liver and kidneys. In the liver, there were focal areas of necrosis and pre-neoplastic changes in the form eosinophilic pre-neoplastic foci, oval cell proliferation and spongiotic peri-cytoma. Kidneys showed glomerular swelling, thickening of the glomerular basement membrane and proliferation of the renal tubular epithelium. Lesions in the other organs included necrosis and sloughing of bronchiolar epithelial lining and mononuclear cellular infiltration in the lungs, necrosis of seminiferous tubular epithelium in the testicles and neuronal degeneration in the brain. The author concluded that 2, 4-D induces deleterious pathological effects on the vital organs including pre-neoplastic changes in the liver of Sprague-Dawley rats.

Furthermore, **Joshi *et al.* (2012)** investigated the toxic effect of 2, 4-D on fertility and biochemical parameters of male albino rats reproductive system. The herbicides 2, 4-D was dissolved in olive oil and administered orally to 24 male rats weighing 150-200 g at dosage levels 50, 100 and 150 mg/kg b.wt./day for 30 and 45 days. The reproductive toxicity of 2, 4-D was evaluated on the basis of fertility indexes, hormonal analysis and biochemical parameters. The results showed that there was a significant decrease in the weight of testes and reduction in sperm counts in treated animals. Histological examination of testes showed degenerative changes in seminiferous tubules. Testicular glycogen and sialic acid were reduced, whereas testicular protein and cholesterol were increased. In addition, a significant decrease in serum testosterone, Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) level were also observed. This study indicated that 2, 4-D exhibit toxic effects on the male reproductive functions.

Mazhar *et al.* (2012) studied the potential role of 2, 4-D in inducing developmental toxicity in pregnant rats and their fetuses as well as to assess the efficacy of vitamin E to prevent or alleviate such defects. Pregnant rats received 2, 4-D (100 mg/kg b.wt.) alone or in combination with vitamin E (100 mg/kg b.wt.) daily from gestation day 1 to day19. The results showed that fetuses from maternally treated rats with 2, 4-D were characterized by decreased body weight and high rate of morphologic and skeletal defects. The authors concluded that coadministration of vitamin E can counteract the deleterious effects of 2, 4-D.

2.1.2 Effect of 2, 4-Dichlorophenoxyacetic acid on biological evaluations

Charles and Leeming (1998) performed a one-year chronic toxicity study on 2, 4- dichlorophenoxybutyric acid (2, 4-DB) in the dog. Doses of 0, 75, 225, and 450

(ppm) were administered to six animals/sexes/groups. The top dose was reduced from 675 ppm to 450 ppm during week 7 of the study due to body weight loss and decreased food consumption. Four animals/sexes/groups were euthanized after 52 weeks of treatment and two animals/sexes/groups were euthanized at week 56. Treatment-related findings included reductions in body weight gain and food consumption, minor increases in inorganic phosphorus, blood urea nitrogen and creatinine. Gross pathology evaluation revealed distended gallbladders and decreased organ weights were noted in females for the adrenal, spleen, and ovaries. Histologically, the liver and kidney were the target organs. Data from this study support a chronic no observed adverse effect level of 75 ppm (2.39 and 2.15 mg/kg b.wt./day for males and females, respectively) for 2, 4-DB.

Morgulis *et al.* (1998) reported the effect of 2, 4 –D in chicks dosed with 100, 300, 500, or 600 mg 2, 4-D/kg b.wt., by the oral route. Clinical, laboratory, and histopathological methods were used. After acute exposure, this herbicide decreased motor activity and induced muscular weakness and motor incoordination; decreased weight gain; increased serum creatine kinase, serum uric acid and creatinine levels. These changes were time- and dose-dependent and of reversible manner. Chromatographic analysis of serum of the intoxicated chicks showed presence of the herbicide; the amount found was dose- and time-dependent, increasing from 2 to 8 h after exposure and decreasing afterwards. Histopathological post-mortem studies conducted on intoxicated chicks showed hepatic (vacuolar degeneration of the hepatocytes), renal (tubular nephrosis), and intestinal (hemorrhagic spots) lesions. Taken together, the observed alterations mainly reflected kidney and muscle tissue damages, although hepatic toxicity may also occur after acute 2, 4-D intoxication.

Charles *et al.* (2001) investigated the potential effect of 2, 4-D and its salts and esters to induce developmental toxicity in rats and rabbits. Maternal toxicity associated with exposure was dependent on the dose level of 2, 4-D. The severity of the maternal effects was correlated to the 2, 4-D dose, with increasing dose levels that exceeded renal clearance causing increasingly more severe maternal effects. In both species, maternal body weight effects began to be manifested at a dose level of 30 mg /kg b.wt./day. At higher dose levels (50 to 75 mg/kg b.wt./day in rats and 75 to 90 mg/kg b.wt./day in rabbits), body weights and feed consumption were more severely affected. The NOEL for maternal toxicity in both species across the family of 2, 4-D salts and esters was approximately 10 mg/kg b.wt./day. Significant decrease in fetal body weight and increased fetal variations were seen in rats only at maternally toxic dose levels in excess of 90 mg/kg b.wt./day. At maternally toxic doses in rabbits, embryonic and fetal development was essentially unaffected. The authors concluded that no adverse fetal effects were noted at dose levels that did not also produce evidence of maternal toxicity or exceed renal clearance of 2, 4-D, indicating that the developing rat and rabbit fetus were not uniquely sensitive to 2, 4-D and its forms.

Uyanikgil *et al.* (2009) examined the effect of 2, 4- D on rat kidney cortex tissue. Oral administration of 2, 4-D to 40 adult male Wistar albino rats weighing (170–180 g) for 28 days resulted in decreases in body weight gain and kidney weight. Histological examination showed vacuolization in glumeruli with disintegration of the basal membrane; tissue edema, cystic dilation and invagination of the basal laminae in the tubular structures; dilation and congestion in renal corpuscular vessels as well as marked decrease in glomerular and stromal fibronectin reaction. The

authors concluded that subacute 2, 4-D administration induces dose-dependent histopathological degenerative effects in rat kidney cortex.

Moreover, **Tayeb *et al.* (2010)** investigated the effect of oral administration of 2, 4-D on body weight and liver weight of 40 male Wistar rats, weighing from 150 to 180 g. Animals given 2, 4-D in doses of 15, 75, 150 mg/kg b.wt., via oral gavage during 4 weeks. At the end of experimental period the results showed that when rats orally given 2, 4-D at different doses there was a significant decrease in body weight while there was a significant increase in the liver weight compared to control group.

Troudi *et al.* (2012) tested the effects of 2, 4-D on body weight of adult female rats and their progeny. Female Wistar rats were divided into two groups: the controls and the treated rats which received 600 ppm of 2, 4-D in drinking water from the 14^{th} day of pregnancy until day 14 after delivery. The authors found that in 2, 4-D treated-group, a significant decrease in body weight of pups was noted, when compared to the control groups.

2.1.3 Effect of 2, 4-Dichlorophenoxyacetic acid on liver enzymes

Yilmaz and Yuksel (2005) investigated the effect of 2, 4-D, on mouse liver, hexokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, lactate dehydrogenase (LDH) and malate dehydrogenase which are required for the generation of the pyridine nucleotide pool. The experiments were carried out with a 2, 4-D group, an ethanol control for 2, 4-D and saline group for ethanol control group on three generations of mice. Only female parents were given 2, 4-D during the gestation period, lactation period and for 33 days following the

lactation period. The results showed that in females of the first cross, 2, 4-D caused a significant increase in the activity of LDH, and ethanol alone caused a significant increase in the activities of hexokinase and LDH. In the male offsprings of the first cross maternal, 2, 4-D caused a significant increase in the activity of LDH, and ethanol alone caused a significant decrease in the activity of 6-phosphogluconate dehydrogenase. In the female offsprings of the first cross maternal, ethanol caused a significant increase in the activities of glucose-6-phosphate dehydrogenase and malate dehydrogenase. In the female offsprings of the third cross maternal, 2, 4-D caused a significant increase in the activity of malate dehydrogenase. The authors concluded that 2, 4-D had an effect on the first cross maternal and their offsprings while it did not affect the studied parameters except malate dehydrogenase enzyme activity in the second and third generation of mice.

Nakbi *et al.* (2010b) assessed the effects of virgin olive oil and its fractions on 2, 4-D-induced oxidative damage in the liver of rats. Male Wistar rats were randomly divided into eight groups of ten each. The rats were daily administered (5 mg/kg b.wt.) by gavage for 4 weeks. The results showed that there was a significant liver damage in rats treated with 2, 4-D as evident from increased serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and hepatic lipid peroxidation. The liver's fatty acid composition was also significantly modified with 2, 4-D exposure. The authors concluded that the protective effect of olive oil against oxidative damage induced by 2, 4-D is mainly related to the antioxidant effec of its hydrophilic fraction.

Tayeb *et al.* (2010) studied the hepatotoxic effect of oral administration of 2, 4-D on serum enzymes markers of hepatotoxicity (ALT, AST and ALP) and other

parameters of 40 male Wistar rats, weighing from 150 to 180 g. Animals given 2, 4-D in doses of 15, 75, 150 mg/kg b.wt., via oral gavage during 4 weeks. At the end of experimental period the results demonstrated that after 2, 4- D administration, AST, ALT, ALP, LDH, gamma-glutamyl transpeptidase (GGT) and total bilirubin (TBIL) activities significantly increased when compared to the control group. Moreover, a significant decrease in total protein (TP) and albumin (ALB) levels was shown in plasma concentration of rats treated with 2, 4-D. The authors concluded that 2, 4-D induces hepatotoxicity in rats.

Troudi *et al.* (2012) evaluated the effect of 2, 4-D (600 ppm) on liver function of female Wistar rats weighing 160- 180g and their progeny. The results showed that plasma aminotransferases (ALT, AST), gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), total bilirubin (TBIL) and albumin (ALB) levels were increased significantly. The serum biochemical alterations were positively correlated with the reported histopathological findings. The authors concluded that 2, 4-D induces hepatotoxicity in adult rats and their suckling pups.

2.1.4 Effect of 2, 4-Dichlorophenoxyacetic acid on antioxidant enzymes

Celik, Tuluce and Isik (2006) investigated the effect of 2, 4-D on serum marker enzymes, antioxidant defense systems reduced glutathione (GSH), glutathione reductase (GR), superoxide dismutase (SOD), glutathione-S-transferase (GST), catalase (CAT) and lipid peroxidation content malondialdehyde (MDA) in various tissues of rats. Addition of 50 and 100 ppm of 2, 4-D to drinking water of Sprague–Dawley rats was done for 25 days continuously. The 2, 4-D treatment caused different effects on the serum marker enzymes, antioxidant defense systems and the MDA content in treated rats when compared to the control group. The authors

concluded that 2, 4-D produces substantial systemic organ toxicity in the erythrocytes, liver, brain, heart, and kidneys.

Bongiovanni *et al.* (2007) assessed the effect of 2, 4-D on reduction / oxidation balance (redox) of rat cerebellar granule cells and melatonin antioxidant effect *in vitro*. The cellular viability, reduced glutathione (GSH) levels and the activities of the antioxidant enzymes SOD, selenium-glutathione peroxidase and CAT in cerebellar granule cells exposed to 2, 4-D and/or melatonin for 48 h were studied. The results showed that in cerebellar granule cells cultures exposed to 2, 4-D, the cell viability, GSH levels and CAT activity decreased significantly. Except for selenium-glutathione peroxidase activity, all these changes were observed by the associated addition of 0.1 or 0.5 mM melatonin. In addition, incubation of cerebellar granule cells with melatonin alone resulted in an augmentation of cell viability, GSH levels and selenium-glutathione peroxidase activity.The SOD activity remained unaffected by either treatment. The authors concluded that since melatonin was able to counteract most of redox changes produced by 2, 4-D in cerebellar granule cells in culture, the experimental evidence reported further support the efficacy of melatonin to act as a neuroprotective.

Mountassif *et al.* (2007) studied the effect of 2, 4-D on jerboa (*Jaculus orientalis*). The Jerboas were daily given intraperitoneally 2, 4-D in a dose of 3 mg/kg b.wt., for 4 weeks. Plasmatic markers and antioxidant defenses systems were assessed and histological alterations were evaluated. The *in vivo* and *in vitro* oxidative stress effects of 2, 4-D on the mitochondrial D-3-hydroxybutyrate dehydrogenase, which play an essential role in the redox balance, were also determined. The results showed an increase in Glutamic-Oxaloacetic Transaminase (GOT) level and low-density lipoprotein (LDL) cholesterol. The microscopic

evaluation showed that 2, 4-D induced necrosis of cells in testis, hyperplasia of hepatocytes in liver and presence of multinucleated giant cells in brain. The results showed also an inhibitory effect on D-3-hydroxybutyrate dehydrogenase in terms of activity and kinetic parameters. The authors concluded that 2, 4-D induces toxicity which affect energy metabolism, morphological perturbation and oxidative stress.

Nakbi *et al.* (2010a) examined the possible protective effect of extra virgin olive oil, olive oil lipophilic fraction and olive oil hydrophilic fraction on oxidative stress and fatty acid profile of erythrocytes in 2, 4-D treated rats. Male Wistar rats were divided randomly into eight groups. The rats were daily administered 2, 4-D in a dose of 5 mg/kg b.wt., by gavages for 4 weeks. The authors found that 2, 4-D treatment caused a significant decrease in antioxidant enzyme activities, namely, SOD, CAT, GR and GPx associated with a higher amount of malondialdehyde level. Fatty acid composition of the erythrocyte membranes was also modified with 2, 4-D exposure. Extra virgin olive oil and hydrophilic fraction of olive oil supplemented to rats with or without 2, 4-D treatment showed an improvement in both the antioxidant enzymes activity and in the malondialdehyde level. Lipophilic fraction did not show any improvement in oxidative damage induced by 2, 4-D in spite its richness in monounsaturated fatty acid and vitamins. The authors concluded that extra virgin olive oil administered to 2, 4-D-treated rats protected erythrocyte membranes against oxidative damage by preventing excessive lipid peroxidation to increase the monounsaturated fatty acid composition and to normalize antioxidant enzymes to the normal levels.

Nakbi *et al.* (2010b) reported the effects of olive oil and its fractions on oxidative stress in the liver of 2, 4-D treated male Wistar rats. The herbicide 2, 4-D

was daily administered in a dose of 5 mg/kg b.wt., by gavage for 4 weeks. The authors observed a significant liver damage in rats treated with 2, 4-D via decreased hepatic antioxidant enzyme activities, namely, SOD, CAT, GPX and GR. Extra virgin olive oil and its fraction intake during 2, 4-D treatment induced a significant increase in the antioxidant enzyme activities.

Tayeb *et al.* (2010) studied the oxidative stress induced by 2, 4-D exposure in doses of 75 and 150 mg/kg/b.w., to male Wistar rats. During 4 weeks, the oxidative stress markers catalase and glutathione reductase (CAT and GR), were analyzed in the liver. They also examined liver tissues histopathologically. The results showed that there was a significant reduction in the hepatic antioxidant enzymes activity in rats of 2, 4-D treated groups when compared with the control group. Histological effects were found in all treated groups and their severity was dose dependent. The authors concluded that 2, 4-D induces hepatotoxicity and cellular alterations in the rat at the doses of 75 and 150 mg/kg b.wt.

Troudi *et al.* (2012) investigated the effect of 2, 4-D on liver antioxidant enzymes activity of twelve pregnant female rats that were randomly divided into a control group and 2, 4-D (126 mg/kg b.wt.) treated group, of six each. The authors found that there were decreases in the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) while malondialdehyde (MDA) levels increased in dams and pups compared to the control group.

Mazhar *et al.* (2012) studied the oxidative damage induced by 2, 4-D in the liver of pregnant rats and their fetuses. The results showed that there was a

significant elevation in malondialdehyde level associated with reduction in catalase activity and total antioxidant capacity in 2, 4-D group. The authors suggested that 2, 4-D induced oxidative stress in pregnant rats and their fetuses.

2.2 Chamomile recutita

Chamomile recutita, (family Asteraceae) popularly known as Chamomile, is an annual plant, very common in Asia, Europe, United States and Australia. It is 20 to 60 cm tall, dark green, with thin roots, erect stalks, alternate leaves, yellowish blossoms and peripheral white flowers (Figure 2.2). It has a characteristic odour and bitter-aromatic taste **(Fenaroli 2005)**. It is known to contain several classes of biologically active compounds including essential oils and several phenolic compounds, primarily the flavonoids and various acetylated derivatives. The principal components of the essential oil extracted from the Chamomile flowers are the terpenoids α-bisabolol and its oxide, chamazuline **(Ganzera, Schneider and Stuppner 2006).**

Chamomile tea prepared with dried flowers has been one of the most widely used and well documented medicinal plants for centuries. As a traditional medicine, it has been used to treat wounds, ulcers, eczema, gout, skin irritations, rheumatic pain and hemorrhoids. Externally, Chamomile has been used to treat diaper rash, chicken pox, and conjunctivitis **(Srivastava and Gupta 2007)**. Moreover, Chamomile shows different pharmacological activities such as anti-inflammatory, antioxidant, anti-cancer and used for the treatment of stress and depression **(Ramadan and Emam 2012).**

Chamomile, in the form of aqueous extract, has been frequently used as a mild sedative to calm nerves and reduce anxiety and to treat hysteria, nightmares, insomnia and other sleep problems. It has been valued as a digestive relaxant and has been used to treat various gastrointestinal disturbances. Chamomile's essential oil is also used as a treatment for malaria and parasitic worm infections, colds and flu.

Chamomile is included in the "generally regarded as safe" (GRAS) list by the food and drug administration (FDA), but it must not be ingested with large amounts during pregnancy, since some of the plant's components may induce uterine contractions **(Barnes, Anderson and Phillipson 2002).**

2.2.1 Effect of Chamomile on liver and antioxidant enzymes

Medicinal plants have been the focus of many studies for their hepatoprotective and/or curative effects. **Al-Ismail and Aburjai (2004)** investigated the antioxidant activities of water and alcohol extracts of Chamomile flowers and the seeds of anise and dill. The results showed that the water extracts of Chamomile flowers and dill seeds showed higher antioxidant activity than butylated hydroxyanisole, whereas the antioxidant activities of the alcohol extracts of all three plants were lower. The water extracts showed higher antioxidant activity than the corresponding alcohol extracts. The antioxidant activity of both water and alcohol extracts decreased in the following order Chamomile flowers > dill seeds > anise seeds. The extracts also showed good free radical-scavenging activity, indicating that they act as hydrogen ions donors.

Figure 2.2: *Chamomile recutita*, (Family *Asteraceae*)

Gupta and Misra (2006) examined the hepatoprotective effect of aqueous ethanolic extract of Chamomile recutita capitula in a dose of 400 mg/ kg b.wt., by oral gavage against paracetamol-induced hepatic damage in 18 albino rats of either sex (100-150 g). The results showed that the extract of Chamomile had reversal effects on the levels of serum marker enzymes (ALT, AST and ALP) and bilirubin in addition to other parameters in paracetamol hepatotoxicity. The authors concluded that Chamomile capitula extract functions as a hepatoprotective agent and this hepatoprotective activity of Chamomile may be due normalization of impaired membrane function activity.

Gupta *et al.* (2006) studied the effect of methanolic extract of Chamomile capitula on hepatotoxic albino Wistar rats (30 male) weighing (150-200 g). Hepatotoxicity was inducing by carbon tetrachloride (CCl_4). The methanolic extract of Chamomile was orally given in doses of 150 and 300 mg/kg b.wt./day. The results showed that the methanolic extract at dose level of 300 mg/kg b.wt., showed significant antioxidant activity against CCL_4 by increasing levels of glutathione peroxidase (GPX), glutathione-s-transferase (GST), glutathione Reductase (GR), superoxide Dismutase (SOD), catalase (CAT) and glutathione (GSH) enzymes. The level of lipid peroxidation (LPO) was decreased. The authors concluded that the methanolic extract possess antioxidant activity with combined hepatoprotective action. It also has a synergistic effect to stabilize cellular membrane and antiperoxidase activity.

Yoo *et al.* (2008) selected 17 common commercial herbs and studied their relative phenolic contents, antioxidant activities and cytoprotective activities on gap–junction intercellular communication and antioxidative enzymes *in vitro* under the

same conditions. The results showed that Chamomile contained relatively high total phenolics and flavonoids. Chamomile also induced the highest antioxidant activity combined with 960 mg/100 g of vitamin C equivalent (VCE). The amount of total phenolic and total flavonoids showed a positive correlation with the antioxidant activity. Most of herbs including Chamomile enhanced cell viability and showed protective effects against oxidative stress induced by hydrogen peroxide in Chinese hamster lung fibroblast cells. Furthermore, Chamomile enhanced activity of the antioxidative enzymes such as SOD and CAT in a dose-dependent manner.

El-massry *et al.* (2009) tested some aromatic plants, including Chamomile, as ingredients to prepare a healthy drink with an acceptable flavour and taste and to evaluate their chemoprotective activity against oxidative stress induced by streptozotocin (STZ) and carbon tetrachloride (CCL_4) in rats. Infusion of Chamomile and other plants was prepared. The chemoprotective effectiveness of the blend infusion was tested by two separate biological experiments against the oxidative stress of STZ and CCL_4. Serum glucose level, ALT, AST, ALP, TBIL, LDH, TP, ALB, GR, GPX, SOD, malondialdehyde, lipid profile and plasma hemoglobin in some organs were investigated. The results showed that administration of blend infusion to intoxicated rats either with STZ or CCL_4 for four weeks significantly ameliorates most of the toxic effects and protects the pancreas and liver. The authors concluded that the blend infusion of Chamomile and other aromatic plants is safe and effective in controlling hyperglycemic effect of STZ and improve lipid metabolism. It also induces hepatoprotective activity against CCL_4 by amelioration of the associated biochemical parameters.

Ramadan and Emam (2012) examined the hypoglycemic and antioxidant effects of *Matricaria chamomilla* leave extract in Streptozotocin (STZ)-induced

diabetes in rats. The water extract of *Matricaria chamomilla* leaves at a concentration of 100 mg/kg b.wt./rat/day was orally administered to STZ-induced diabetic rats for a period of 21days. The results showed that elevated levels of blood glucose in the diabetic rats reverted back to near normal levels and serum insulin was elevated to near normal level after treatment with the Chamomile water extract. Determination of serum liver antioxidant enzymes (GST, CAT and GPX) proved the antioxidative potential of the extract, which in turn may be responsible for its hypoglycemic potential. The authors indicated that *Matricaria chamomilla* extract effectively reduced the oxidative stress induced by streptozotocin and potential reduction in blood sugar level.

Salama (2012) evaluated the protective role of *M. chamomilla* in cisplatin nephrotoxicity rat model. Thirty two rats were used in this study. Rats were injected daily with *M. chamomilla* (50 mg/kg b.wt.) intraperitoneally (IP). On day 16, animals were scarified and serum was used to determine oxidative stress index and antioxidant activities (SOD, GSH). The results showed that *M. chamomilla* significantly reduced the oxidative stress markers. The authors demonestrated that *M. chamomilla* is a promising nephroprotective compound reducing cisplatin nephrotoxicity most probably by its antioxidant activities.

Chapter III

Materials and Methods

Chapter III

Materials and Methods

The experimental work of the present study was conducted at the King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia.

3.1 Materials

3.1.1 Chamomile capitula

Matricaria chamomilla or *Matricaria recutita*, Family *Asteraceae* flowers were purchased from a local market, Jeddah, Kingdom of Saudi Arabia.

3.1.2 Herbicide

The herbicide 2, 4-Dichlorophenoxyacetic acid was purchased under the brand name (2, 4-Kill®) from the local market, Jeddah, Kingdom of Saudi Arabia.

3.1.3 Animals

Thirty six male albino rats of Wister strain weighing 230-250 grams body weight and 12 -14 weeks old were obtained from King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia.

3.1.4 Feeding of Rats

All rats were fed on commercial rat pellets obtained from Grain Silos and Flour Mills Organization, Jeddah, KSA. These pellets are consisted of 20% crude protein, 4% fat, 3.5% crude fibers, 6% ash, 1% calcium, 0.6% phosphorus, 0.5% other salts, 20 IU/g vitamin A, 2.2 IU/g vitamin D and 70 IU/g vitamin E. These constituents were thoroughly mixed and commercially manufactured in the form of pellets.

3.1.5 Kits for Biochemical Analysis

Diagnostic commercial kits for biochemical analyses were purchased from Cayman Chemicals and BioVision Incorporated, USA.

3.2 Methods

3.2.1 Preparation of Chamomile capitula Extract

Dry Chamomile flowers were weighed and grinded into a fine powder using a marble porcelain mortar and pestle. A 5% suspension (w/v) was prepared in a flask by adding boiled water. The flask was then placed on an electric shaker (200 rpm) for 4 hr., and the temperature was maintained at 37 °C. After being shaken, the flask was brought to room temperature and the mixture was filtered through a series of Whatman filter paper to obtain an aqueous infusion. The filtered aqueous extract was freeze-dried (Freeze Dryer Alpha 1-2 LD plus, Christ, Germany) and stored at -20 °C until used **(Srivastava and Gupta 2007).**

The administrated dose of the Chamomile capitula extract was 500 mg / kg body weight daily for 4 weeks according to **Kato *et al.* (2008).**

3.2.2 Determination of Plant Bioactive Constituents

Plant bioactive compounds of Chamomile capitula water extract were determined using High Performance Liquid Chromatography (HPLC, Shimadzu Corporation, Kyoto, Japan).

3.2.3 Induction of Hepatotoxicity

Experimental hepatotoxicity was induced by administration via oral gavage of an accumulative dose of the herbicide 2, 4- Dichlorophenoxyacetic acid **(**one of phenoxyacetic acid herbicides) at two dosage levels 75 and 150 mg/kg body weight daily for 4 weeks according to **Tayeb *et al.* (2010).**

3.2.4 Experimental Design and Grouping of Rats

Thirty six male rats of Wistar strain weighing 230-250 g and 12-14 weeks old were housed in plastic cages (six rats/cage) with wood shavings. Rats were kept in a temperature-controlled room at 24±1°C, 50% humidity and 12hrs /12 hrs light/dark cycle. Rats were adapted to the environment for one week prior to the start of experiment. Animals were fed on rat pellets and water was provided *ad libitum* during experimental period (4 weeks). After acclimatization period, the rats were weighted. Weighing of rats was done every week during experimental period for determining the change in body weight. Daily feed intake was calculated by subtracting the remaining feed pellets from the offered pellets every day. Body weight gain and feed efficiency ratio were then calculated. The rats were randomly distributed into six equal groups of six rats each as follows:

Group 1 (Control): Rats were fed on the basal diet and water was provided *ad libitum*, negative control group (C-ve).

Group 2 (2, 4-D$_{75}$): Rats were fed on the basal diet and received oral gavage of 75 mg/kg body weight of 2, 4-D (herbicide), to induce hepatotoxicity**,** Positive Control group1 (C+ve1).

Group 3 (2, 4-D$_{150}$): Rats were fed on the basal diet and received oral gavage of 150 mg/kg body weight of 2, 4-D (herbicide), to induce hepatotoxicity**,** Positive control group 2 (C+ve 2).

Group 4 (C$_{500}$): Rats were fed on the basal diet and received oral gavage of Chamomile capitula extract in a dose of 500 mg/kg body weight.

Group 5 (Mix$_{75}$): Rats were fed on the basal diet and received oral gavage of Chamomile capitula extract (500 mg/kg b.wt.) and 2, 4- D (75 mg/kg b.wt.) with some hours in between.

Group 6 (Mix $_{150}$): Rats were fed on the basal diet and received oral gavage of Chamomile capitula extract (500 mg/kg b.wt.) and 2, 4- D (150 mg/kg b.wt.) with some hours in between.

3.2.5 Determination of Body Weight Gain% and Feed Efficiency Ratio

Daily feed intake (FI) per group was calculated throughout the experimental period (4 weeks), body weight gain percentage and feed efficiency ratio were calculated according to the method of **Chapman, Castiilla and Campbell (1959)** using the following equations:

$$\text{Body weight gain (\%)} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

$$\text{Feed efficiency ratio (FER)} = \frac{\text{Body weight gain (g)}}{\text{Food consumed (g)}}$$

3.2.6 Anesthesia and Collection of Blood Samples

At the end of the experimental period, the rats were fasted over night. On the morning of the next day the rats were anesthetized by general volatile anesthesia using ether. Anesthesia was done by placing the rat in a large glass jar with a piece of cotton soaked with ether. After induction of mild anesthesia, the rat was rapidly pulled out and blood was collected. Blood samples were withdrawn by capillary microtubes (Micro Hematocrite Capillaries, Mucaps) from the retro-orbital plexuses of veins in inner canthus of the eye into plain tube with gel. The blood samples were centrifuged at 3000 rpm for 15 minutes. Serum samples were separated and frozen at -80 °C until used for the biochemical analyses.

After decapitation of the rats, the liver was removed by careful dissection and blotted free of adhering blood immediately after sacrificing the rats. The liver was washed with cold 0.9 % sodium chloride saline solution and dried between two filter papers then weighed. A 5% Glutaraldehyde solution was added to liver tissue for fixation pending for examination by electron microscope.

3.2.7 Serum Biochemical Analysis

Biochemical analyses were estimated using spectrophotometric techniques (Dimension Vista system, Siemens Healthcare Diagnostics Inc., USA) by different methods as explained below:

3.2.7.1 Determination of Aspartate Aminotransferase (AST)

Aspartate aminotransaminase (AST) catalyzes the transamination from L-aspartate to α-Ketoglutarate forming L-glutamate and oxaloacetate. The oxaloacetate formed is reduced to malate by malate dehydrogenase (MDH) with simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH). The change in absorbance with time due to the conversion of NADH to NAD is directly proportional to the AST activity and is measured using a dichromatic rate technique at 340 nm wave length according to **Tietz (2006).**

$$\text{L-aspartate} + \alpha\text{-Ketoglutarate} \xrightarrow[\text{pH 7.8}]{\text{AST}} \text{L-glutamate+oxalacetate}$$

$$\text{Oxalacetate} + \text{NADH} \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}$$

3. 2.7.2 Determination of Alanine Aminotransferase (ALT)

Alanine aminotransaminase (ALT) catalyzes the transamination from L- alanine to α-Ketoglutarate forming L-glutamate and pyruvate. The pyruvate formed is reduced to lactate by lactate dehydrogenase (LDH) with simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH). The change in absorbance is directly proportional to the ALT activity and is measured using a dichromatic rate technique at 340 nm wave length according to **Tietz (2006).**

$$\text{L-alanine} + \alpha\text{-Ketoglutarate} \xrightarrow[\text{pH 7.4}]{\text{ALT}} \text{L-glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH}(H^+) \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

3.2.7.3 Estimation of Serum Alkaline Phosphatase (ALP)

Alkaline phosphatase catalyzes the transphosphorylation of p-nitrophenyl phosphate (p-NPP) to p-nitrophenol (p-NP) in the presence of the transphosphorylation buffer, 2-amino-2methyle-1-propanol (AMP). The reaction is enhanced through the use of magnesium and zinc ions. The change in absorbance at 405 nm wave length due to the formation of p-NP which is directly proportional to the ALP activity, since other reactants are present in non-rate limiting quantities and is measured using a dichromatic rate technique at 405 nm according to **Tietz (2006)**.

$$\text{p-NPP} + \text{AMP} \longrightarrow \text{p-NP} + \text{AMP} + PO_4$$

3.2.7.4 Determination of Lactate Dehydrogenase (LDH)

The LDH method measures the oxidation of L-lactate to pyruvate with simultaneous reduction of NAD. The change in absorbance at 340 nm wave length due to appearance of NADH is directly proportional to the LDH activity, since other reactants are present in non- rate limiting quantities which measured by using a dichromatic rate technique at 340 nm according to **Tietz (2006).**

$$\text{L-lactate} + NAD^+ \xrightarrow[\text{pH 8.55}]{\text{LDH}} \text{Pyruvate} + \text{NADH} + H^+$$

3.2.7.5 Determination of Albumin (ALB)

In the presence of a solubilizing agent, Bromocresol Purple (BCP) binds to albumin at pH 4.9. The amount of albumin-BCP complex is directly proportional to the albumin concentration. The complex absorbs at 600 nm and is measured using a polychromatic endpoint technique at 600 nm according to **Burtis *et al.* (2006).**

$$\text{Albumin + BCP dye} \xrightarrow{\text{pH 4.9}} \text{Albumin-BCP complex}$$

(Non absorbent at 600 nm) (Absorbent at 600 nm)

3.2.7.6 Determination of Total Bilirubin (TBIL)

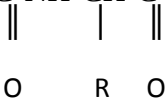
Diazotized sulfanilic acid is formed by combining sodium nitrite and sulfanilic acid at low pH. Bilirubin (unconjugated) in the sample is solubilized by dilution in a mixture of caffeine/benzoate/acetate/ Ethylene Diamine Tetraacetic Acid (EDTA). Upon addition of the diazotized sulfanilic acid, the solubilized bilirubin including conjugated bilirubin (mono and diglucoronides) and the delta form (biliprotein-bilirubin covalently bound to albumin) is converted to diazo-bilirubin, a red chromophore representing the total bilirubin which absorbs at 540 nm. A sample blank correction is used and Diazo-bilirubin is measured using a dichromatic endpoint technique at 540 nm according to **Burtis *et al.* (2006).**

$$\text{Solubilized bilirubin + Diazotized sulfanilic acid} \longrightarrow \text{red chromophore}$$

(Absorbs at 540 nm)

3.2.7.7 Determination of Total Protein (TP)

Cupric ion (Cu^{++}) reacts with the peptide linkages (-C-NH-CH-C-NH-) of protein in a basic solution.



The blue copper protein complex which formed is proportional to the total protein concentration in the sample and was measured using a dichromatic endpoint technique at 540 nm **(Henry 1974).**

$$Cu^{++} + \text{Protein} \xrightarrow{OH^-} \text{Complex}$$

(Absorbs at 540 nm)

3.2.7.8 Estimation of Serum Oxidative Stress Markers

Tests of antioxidant enzymes were performed using enzyme-linked immunosorbent assay (ELISA) technique (Microplate reader, Biotech, USA).

3.2.7.8.1 Glutathione Reductase (GR)

Glutathione (GSH) is a tripeptide widely distributed in both plants and animals. GSH serves as a co-substrate to GSH transferases in the detoxification of xenobiotics and is an essential electron donor to GSH peroxidase in the reduction of hydroperoxides. GSH is also involved in amino acid transport across membranes. Glutathione reductase is a flavoprotein that catalyzes the NADPH-dependant reduction of oxidized glutathione (GSSG) to GSH. This enzyme is essential for the GSH redox cycle which maintains adequate level of reduced cellular GSH. A high GSH/GSSG ratio is essential for protection against oxidative stress **(Foyer, Lelandais and Kunert 1994).**

$$GSSG + NADPH + H^+ \longrightarrow 2GSH + NADP^+$$

Glutathione reductase enzyme activity was determined according to the method described by **Carlberg and Mannervik (1985).**

3.2.7.8.2 Superoxide Dismutase (SOD)

Superoxide dismutases (SODs) are metalloenzymes that catalyze the dismutation of superoxide anion to molecular oxygen and hydrogen peroxide and thus form crucial part of the cellular antioxidant defence mechanisms.

$$2O_2^{\bullet -} + 2H^+ + SOD \longrightarrow H_2O_2 + O_2$$

Three types of SODs have been characterized according to their metal content: copper/zink (Cu/Zn), manganese (Mn), and iron (Fe). The SOD is widely distributed in both plants and animals. It occurs in high concentration in brain, liver, erythrocytes and kidney. The amount of SOD present in cellular and extracellular environments is crucial for the prevention of diseases linked to oxidative stress **(Sandstrom, Nilsson and Karlsson 1994).**

Superoxide dismutase (SOD) activity was assessed using a Xanthine oxidase system to generate superoxide radicals (O_2^-) as described by **Kakkar *et al*. (1995).** The rate of suppression of the reduction of Nitro tetrazolium blue (NTB) by O_2^- was monitored at 550 nm wave length.

3.2.8 Histopathological Examination

Liver tissues were examined using histopathological technique by electron microscope. The dissected liver was fixed with Glutaraldehyde in 0.2M Phosphate buffer for 5–6 h at 48^{o}C. The fixed liver was cut into approximately 1mm thickness

cubes. The cubes were post fixed in 2% Osmium tetra oxide (OsO_4) solution for 2hr at 48^{o}C and then dehydrated in an ethanol series. The pieces were embedded in tab, cut into 0.5mm thickness sections using an ultra microtome (LKB, Sweden) and mounted on Nickel grids (300 mm). The sections were double stained with uranyl acetate and lead citrate and then examined by transmission electron microscope (TEM) (Philips CM100, Netherlands) and photographed **(Hayat 1989).**

3.2.9 Statistical Analysis

Data were expressed as means ± standard deviation (SD). Statistical analysis of variance between mean values of different groups was performed using Kruskal-Wallis test followed by the Mann-Whitney test to determine the variance between all rat groups. Differences were considered significant at $P<0.05$. Statistical analysis was done using computerized SPSS program (Statistical Package for the Social Sciences, version 16).

Chapter IV

Results

Chapter IV

Results

In the current study, the results of estimation of bioactive compounds of Chamomile capitula and its effect on rats body weight, feed efficiency ratio and relative liver weight to body weight as well as biochemical constituents in the serum of hepatotoxic rats induced by 2, 4-Dichlorophenoxyacetic acid (2, 4-D) and histopathological examination of liver are depicted in Tables (4.1) to (4.6) and illustrated in Figures (4.1) to (4.23). These effects were explored when Chamomile capitula extract was orally given to hepatotoxic rats induced by 2, 4-D at two dosage levels for 4 weeks.

Table 4.1: Analytical measurements for Chamomile recutita bioactive Compounds

Chamomile recutita bioactive Compounds	Percentage
Flavonoids (apigenin 7-*O*-glucoside)	63.3%
Total phenolic compounds	23.2 %
Essential oil	1.5%
Other constituents	**12%**

4.1 Effect of Chamomile capitula extract on the initial and final body weight, body weight gain% and feed efficiency ratio in hepatotoxic rats induced by 2, 4-D.

The effect of Chamomile capitula extract on the initial and final body weight, body weight gain% and feed efficiency ratio of hepatotoxic rats induced by 2, 4-Dichlorophenoxyacetic acid (2, 4-D) is recorded in Table 4.2 and shown in Figures 4.1, 4.2 and 4.3.

Table 4.2: Effect of oral administration of Chamomile aqueous extract (500mg/ kg b.wt.) after 4 weeks on body weight, body weight gain% and feed efficiency ratio against 2,4-D-induced hepatotoxictity in rats (Mean ± SD, n=6).

Groups	Body weight (g)		Body weight gain (%)	Feed efficiency ratio (FER)
	Initial	Final		
Control (C-ve)	252.66± 11.71^{a}	345± 3.00^{a}	36.54± 0.71^{a}	5.077±0.11^{a}
2,4-D75 (C+ve1)	255.66± 4.04^{a}	329± 5.00^{a,b}	28.68± 0.34^{b,c}	2.210±0.14^{b}
2,4-D150 (C+ve2)	259± 10.14^{a}	318± 1.00^{b}	22.78± 0.73^{c}	1.111±0.13^{c,d}
C500	253.66±15.14^{a}	345.66± 5.50^{a}	36.27± 0.81^{a}	4.343±0.11^{a}
Mix75	250.66 ± 24.50^{a}	338± 16.00^{a,b}	34. 84± 0.57^{a}	3.366±0.11^{a,b}
Mix150	258.33± 14.36^{a}	334.33±9.86^{a,b}	29.42± 0.30^{a}	2.924±0.13^{a,b}

Values are presented as mean ± standard deviations.

- Control: fed on control diet, 2, 4-D_{75}: received 75 mg/kg b.wt., of 2, 4-D, 2, 4-D_{150}: received 150 mg/kg b.wt., of 2, 4-D, C_{500}: received 500 mg/kg b.wt., of Chamomile capitula extract, Mix_{75}: administrated of Chamomile capitula extract (500 mg/kg b.wt.)+ 2, 4-D (75 mg/kg b.wt.), Mix $_{150}$: administrated of Chamomile capitula extract (500 mg / kg b.wt.)+ 2, 4-D (150 mg/kg b.wt.).

- Values with different superscript letters within a column are significantly different at $p<0.05$, while those with similar or partially similar are non significant.

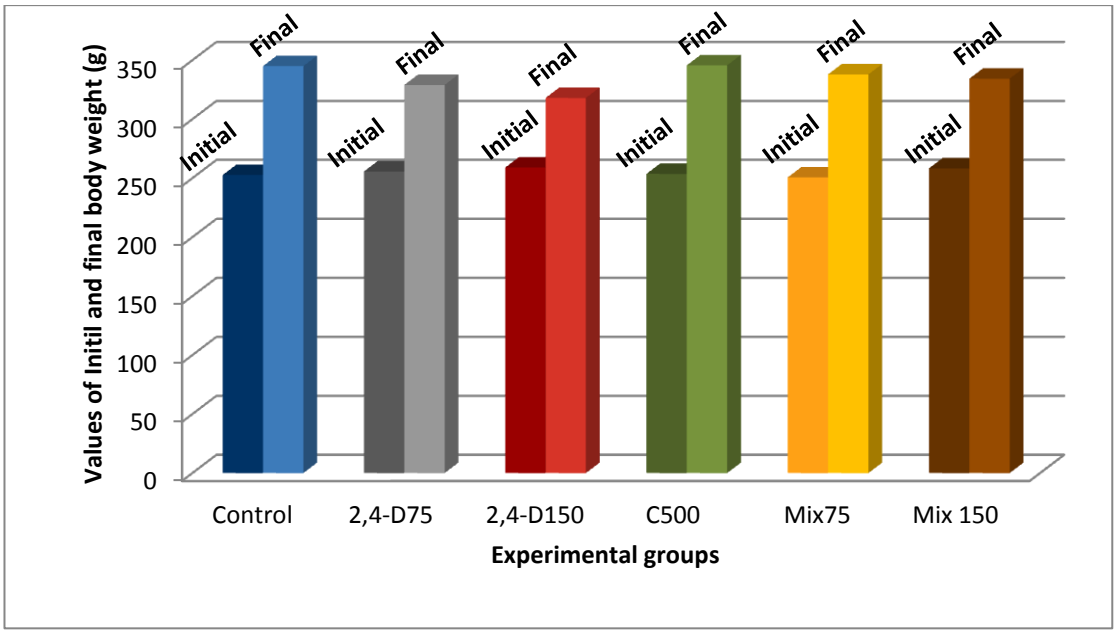


Figure 4.1: Effect of oral administration of Chamomile aqueous extract (500mg/kg b.wt., after 4 weeks) on initial and final body weight of rats in all groups against 2,4-D-induced hepatotoxicity in rats (Mean ± SD, n=6).

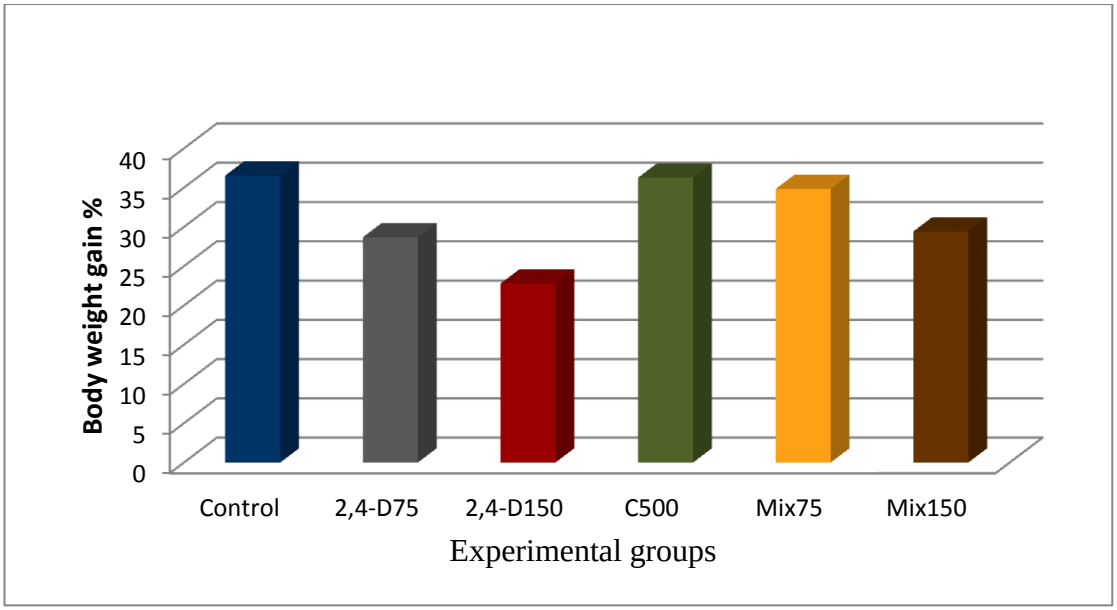


Figure 4.2: Effect of oral administration of Chamomile aqueous extract (500mg/ kg b.wt., after 4 weeks) on body weight gain percentage of rats in all groups against 2,4-D-induced hepatoxictity in rats (Mean ± SD, n=6).

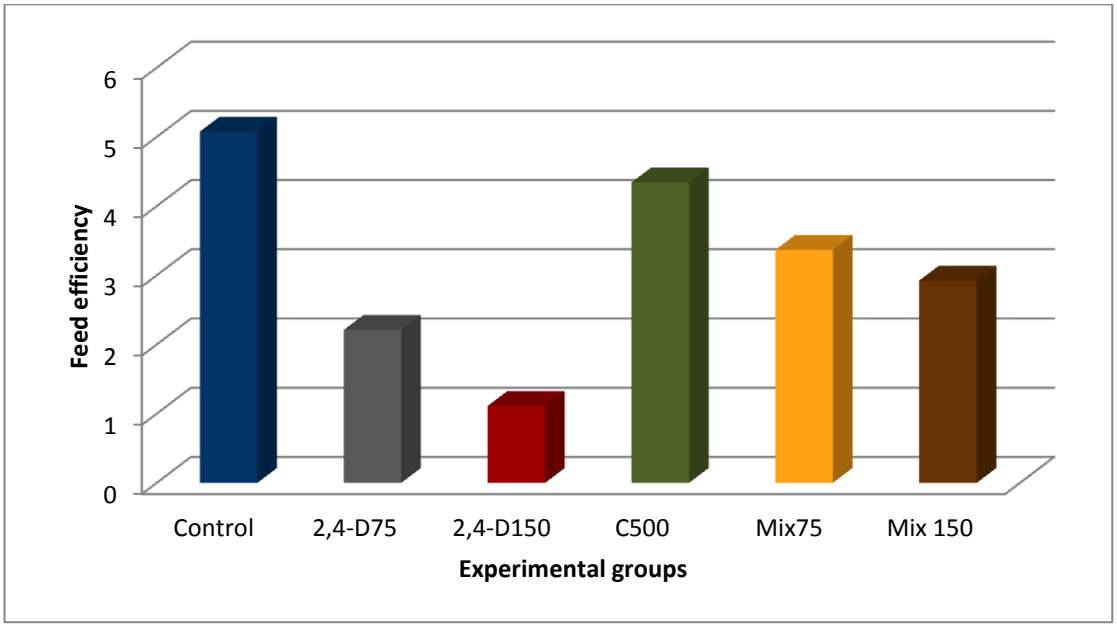


Figure 4.3: Effect of oral administration of Chamomile aqueous extract (500mg/ kg b.wt., after 4 weeks) on feed efficiency ratio against 2,4-D-induced hepatoxictity in rats (Mean ± SD, n=6).

Administration of 2, 4-D in a dose of 75 mg/kg b.wt., to rats during feeding period (28 days) showed no significant change in final body weight, while there were significant ($p<0.05$) decreases in the body weight gain% and feed efficiency ratio by 7.86 % and 56.47% respectively compared to control negative group as shown in Table 4.2 and Figs. 4.1, 4.2 and 4.3.

The results showed that oral administration of 2, 4-D in a dose of 150 mg/kg b.wt., induced a significant ($p<0.05$) decrease in final body weight, body weight gain% and feed efficiency ratio by 7.82%, 13.76 % and 78.11 % respectively compared to control negative group.

Data recorded in Table 4.2 and illustrated in Figs 4.1, 4.2 and 4.3 showed that there were no significant changes in final body weight, body weight gain% and feed efficiency ratio in male rats orally given Chamomile capitula extract in a dose of 500 mg/kg b.wt., when compared to negative control group.

Results in Table 4.2 and Figs. 4.1, 4.2 and 4.3 illustrated the effect of mix_{75} group (2, 4-Dichlorophenoxyacetic acid in a dose of 75 mg/kg b.wt., with Chamomile capitula extract in a dose of 500 mg/kg b.wt.), on initial and final body weight, body weight gain% and feed efficiency ratio. The results showed that there were no significant changes in final body weight and feed efficiency ratio when compared to 2, 4-D_{75} group, whereas a significant increase in body weight gain% by 6.16 % was shown compared to 2, 4-D_{75} group.

Oral administration of mix_{150} (2, 4-Dichlorophenoxyacetic acid in a dose of 150 mg/kg b.wt., with Chamomile capitula extract in a dose of 500 mg/kg b.wt.) showed no significant changes in initial and final body weight when compared to the group of rats orally given 2, 4-$D_{150.}$ On the other hand, there were significant ($p<0.05$)

increases in the body weight gain% and feed efficiency ratio in rats orally given mix_{150} by 6.64 % and 163.18% respectively when compared to the group of rats orally given 2, 4-D_{150}.

4.2 Effect of Chamomile capitula extract on liver absolute and relative weight in hepatotoxic rats induced by 2, 4-D.

The effect of Chamomile capitula extract on absolute and relative liver weight in hepatotoxic rats induced by 2, 4-D is recorded in Table 4.3 and shown in Fig. 4.4.

Table 4.3: Absolute and relative liver weight of 2, 4-D at two doses and Chamomile aqueous extract after 4 weeks

Groups	Absolute liver weight (g)	Relative liver weight (g/100 g body weight)
Control (C-ve)	$9.77\pm 0.12^{a,b}$	2.848 ± 0.07^{c}
2,4-D75 (C+ve1)	$10.99\pm 0.47^{a,b}$	3.265 ± 0.04^{b}
2,4-D150 (C+ve2)	11.79 ± 1.27^{a}	3.752 ± 0.13^{a}
C500	9.59 ± 0.98^{b}	2.777 ± 0.27^{c}
Mix75	$9.93\pm 0.71^{a,b}$	$2.964\pm 0.17^{b,c}$
Mix150	$10.14\pm 0.48^{a,b}$	$3.129\pm 0.02^{b,c}$

Values are presented as mean ± standard deviations.

- Control: fed on control diet, 2, 4-D_{75}: received 75 mg/kg b.wt., of 2, 4-D, 2, 4-D_{150}: received 150 mg/kg b.wt., of 2, 4-D, C_{500}: received 500 mg/kg b.wt., of Chamomile capitula extract, Mix_{75}: administrated of Chamomile capitula extract (500 mg / kg b.wt.)+ 2, 4-D (75 mg/kg b.wt.), Mix $_{150}$: administrated of Chamomile capitula extract (500 mg / kg b.wt.)+ 2, 4-D (150 mg/kg b.wt.).

- Values with different superscript letters within a column are significantly different at $p<0.05$, while those with similar or partially similar are non significant.

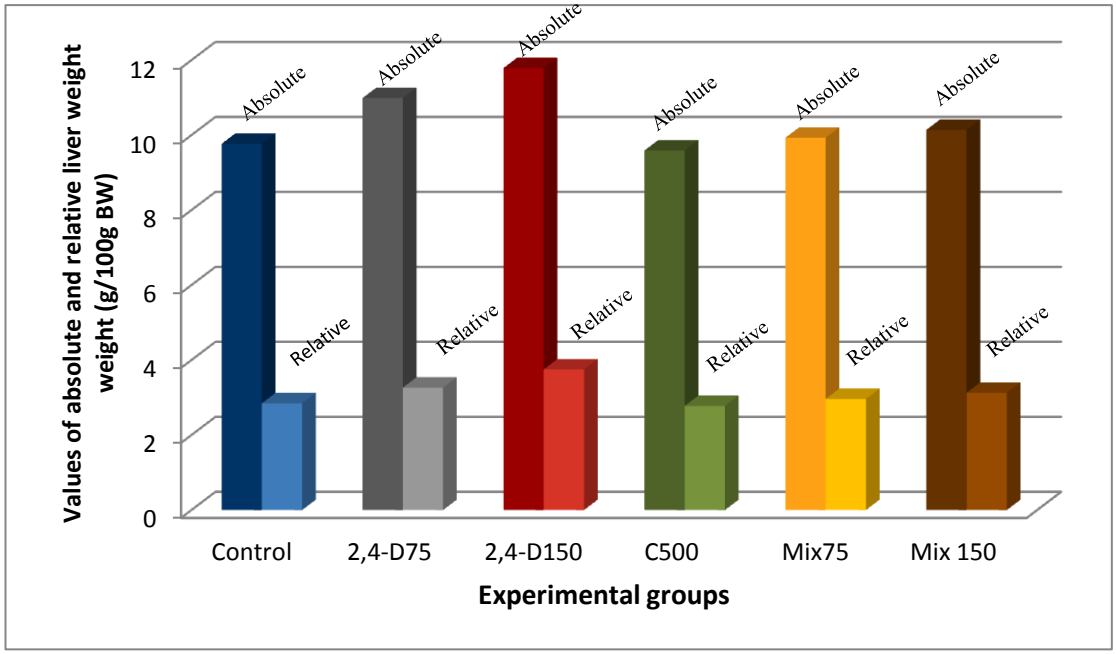


Figure 4.4: Absolute and relative liver weight of rats in all groups under investigation.

The two dosage levels 75 or 150 mg/kg b.wt., of 2, 4-D orally given to rats during experimental period (4 weeks) showed no significant change in absolute liver weight when compared to the negative control group. On the other hand, there were significant ($p<0.05$) increases in the relative liver weight by 0.4% and 0.9% respectively when compared to control negative group.

Data in Table 4.3 and Fig. 4.4 showed that there were no significant changes in absolute and relative liver weight in male rats orally given Chamomile capitula extract in a dose of 500 mg/kg b.wt., when compared to the negative control group.

Administration of mix_{75} showed no significant changes in absolute and relative liver weight when compared to rats orally given 2, 4-D_{75}.

The results showed that there was no significant change in absolute liver weight in the group of male rats orally given mix_{150} when compared to rats orally given 2, 4-D_{150}. Meanwhile, there was a significant ($p<0.05$) decrease in relative liver weight by 0.6% in rats orally given mix_{150} when compared to rats orally given 2, 4-D_{150}.

4.3 Effect of Chamomile capitula extract on serum liver enzymes AST, ALT and ALP in hepatotoxic rats induced by 2, 4-D.

The effect of Chamomile capitula extract on serum liver enzymes AST, ALT and ALP in hepatotoxic rats induced by 2, 4-D is recorded in Table 4.4 and shown in Fig.4.5.

Table 4.4: Effects of 2, 4-D at two doses and Chamomile aqueous extract after 4 weeks on serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes of rats compared to control.

Groups	AST (U/l)	ALT (U/l)	ALP (U/l)
Control (C-ve)	163.50± 7.50^{d}	56.50± 5.00^{c}	154.50± 6.94^{b}
2,4-D75 (C+ve1)	194.00± 2.00^{b}	65.00± 3.00^{b}	171.50± 1.50^{a,b}
2,4-D150 (C+ve2)	215.50± 0.50^{a}	74.33± 1.52^{a}	199.50± 2.35^{a}
C500	163.00± 6.00^{d}	56.00± 3.00^{c}	152.66± 4.93^{b}
Mix75	167.33± 8.50^{c,d}	59.00± 1.00^{c}	165.50± 2.42^{a,b}
Mix150	178.50± 5.50^{c}	65.00± 1.00^{b}	177.00± 14.73^{a,b}

Values are presented as mean ± standard deviations.

-Control: fed on control diet, 2, 4-D$_{75}$: received 75 mg/kg b.wt., of 2, 4-D, 2, 4-D$_{150}$: received 150 mg/kg b.wt., of 2, 4-D, C_{500}: received 500 mg/kg b.wt., of Chamomile capitula extract, Mix_{75}: administrated of Chamomile capitula extract (500 mg / kg b.wt.)+ 2, 4-D (75 mg/kg b.wt.), Mix $_{150}$: administrated of Chamomile capitula extract (500 mg / kg b.wt.)+ 2, 4-D (150 mg/kg b.wt.).

-Values with different superscript letters within a column are significantly different at $p<0.05$, while those with similar or partially similar are non significant.

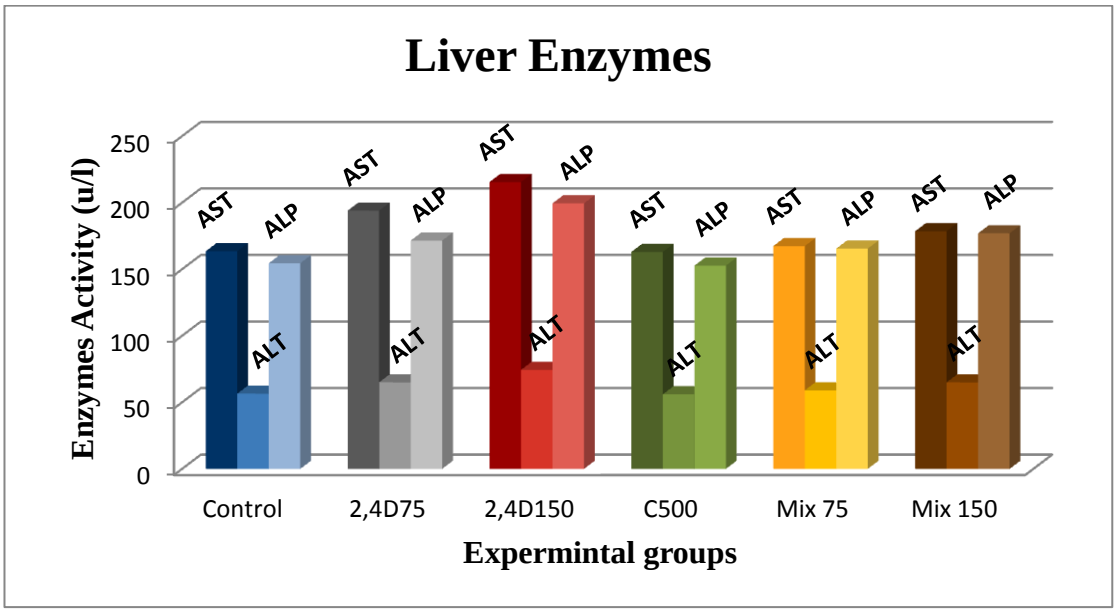


Figure 4.5: Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities in all groups under investigation.

Administration of 2, 4-D at two dosage levels 75 or 150 mg/kg b.wt., to rats during feeding period (4 weeks) significantly ($p<0.05$) increased serum levels of AST and ALT by 18.65%, 31.80% and 15.04%, 31.55% respectively when compared to the negative control group.

It is clear from Table 4.4 and Fig. 4.5 that oral administration of 2, 4-D in a dose of 75 mg/kg b.wt., to male rats for 4 weeks showed no significant change in serum ALP enzyme when compared to the negative control group. In contrast, there was a significant ($p<0.05$) increase in serum ALP enzyme by 29.12% in rats orally given 2, 4-D in a dose of 150 mg/kg b.wt., when compared to the negative control group.

Data recorded in Table 4.4 and illustrated in Fig. 4.5 revealed that there were no significant changes in serum levels of AST, ALT and ALP enzymes in male rats orally given Chamomile capitula extract in a dose of 500 mg/kg b.wt., when compared to the negative control group.

Administration of mix_{75} significantly ($p<0.05$) decreased the levels of serum AST and ALT enzymes by 13.74% and 9.23% respectively when compared to rats orally given 2, $4\text{-}D_{75}$. On the other hand, there was no significant change in serum ALP enzyme in rats orally given mix_{75} when compared to rats orally given $2,4\text{-}D_{75}$.

Concerning serum AST and ALT enzymes, data in Table 4.4 and Fig. 4.5 showed that oral administration of mix_{150} produced significant ($p<0.05$) decreases in AST and ALT enzymes by 17.16% and 12.55% respectively when compared to rats orally given 2, $4\text{-}D_{150}$. There was no significant change in serum ALP enzyme in rats orally given mix_{150} when compared to rats orally given 2, $4\text{-}D_{150}$.

4.4 Effect of Chamomile capitula extract on serum LDH, ALB, TP, and TBIL in hepatotoxic rats induced by 2, 4-D.

The effect of Chamomile capitula extract on serum LDH, ALB, TP, and TBIL in hepatotoxic rats induced by 2, 4-D is recorded in Table 4.5 and shown in Figs. 4.6, 4.7, 4.8 and 4.9.

Table 4.5: Effects of 2, 4-D at two doses and Chamomile aqueous extract after 4 weeks on serum lactate dehydrogenase (LDH), albumin (ALB), total protein (TP) and total bilirubin (TBIL) of rats compared to control.

Groups	LDH (U/l)	ALB (g/l)	TP (g/l)	TBIL (µmol/l)
Control (C-ve)	2650.66± 13.01^{c}	12.50± 0.50^{a}	74.00± 5.00^{a}	1.16± 0.02^{c}
2,4-D$_{75}$ (C+ve1)	2813.00± 30.65^{c}	10.86± 0.32^{b}	65.50± 0.50^{b}	2.33± 0.11^{a}
2,4-D$_{150}$(C+ve2)	3709.00± 47.18^{a}	10.20± 0.26^{b}	62.50± 0.50^{b}	2.50± 0.05^{a}
C$_{500}$	2668.00± 28.58^{c}	12.66± 0.57^{a}	74.00± 3.00^{a}	1.17± 0.02^{c}
Mix$_{75}$	2771.66± 38.89^{c}	11.86± 0.32^{a}	72.00± 1.00^{a}	2.00± 0.05^{b}
Mix$_{150}$	3414.33± 68.95^{b}	10.86± 0.32^{b}	68.33± 1.15^{a,b}	2.16± 0.2^{b}

Values are presented as mean ± standard deviations.

- Control: fed on control diet, 2, 4-D$_{75}$: received 75 mg/kg b.wt., of 2, 4-D, 2, 4-D$_{150}$: received 150 mg/kg b.wt., of 2, 4-D, C$_{500}$: received 500 mg/kg b.wt., of Chamomile capitula extract, Mix$_{75}$: administrated of Chamomile capitula extract (500 mg/kg b.wt.)+ 2, 4-D (75 mg/kg b.wt.), Mix $_{150}$: administrated of Chamomile capitula extract (500 mg/kg b.wt.)+ 2, 4-D (150 mg/kg b.wt.).

- Values with different superscript letters within a column are significantly different at $p<0.05$, while those with similar or partially similar are non significant.

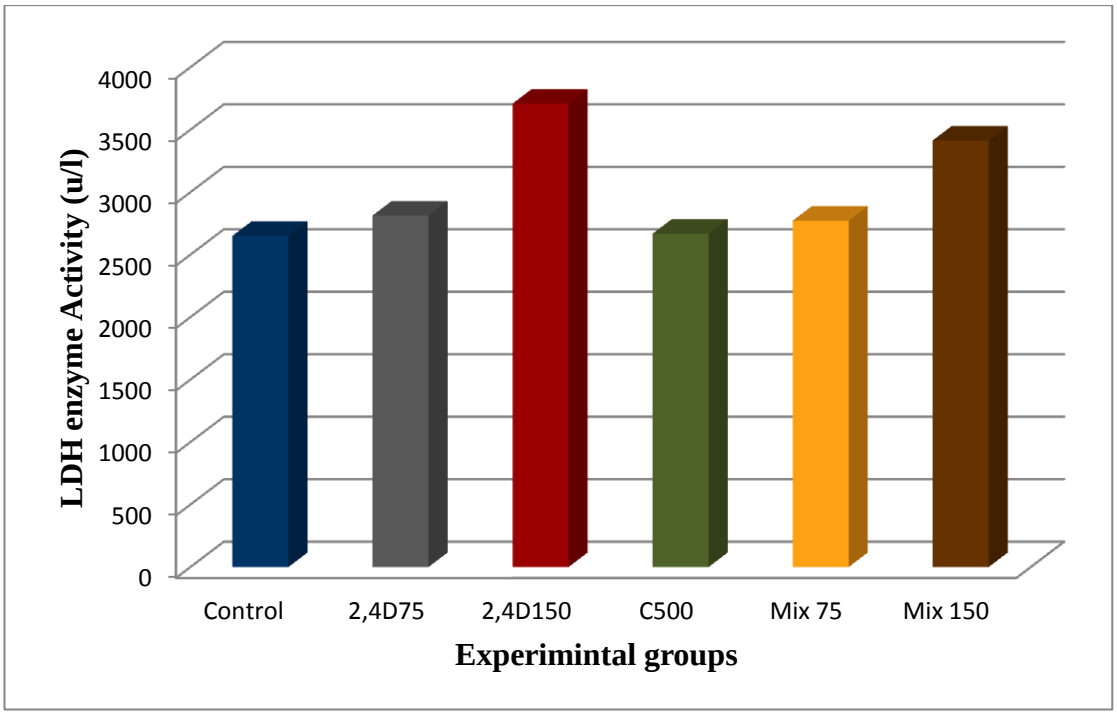


Figure 4.6: Serum lactate dehydrogenase (LDH) activity in all groups under investigation.

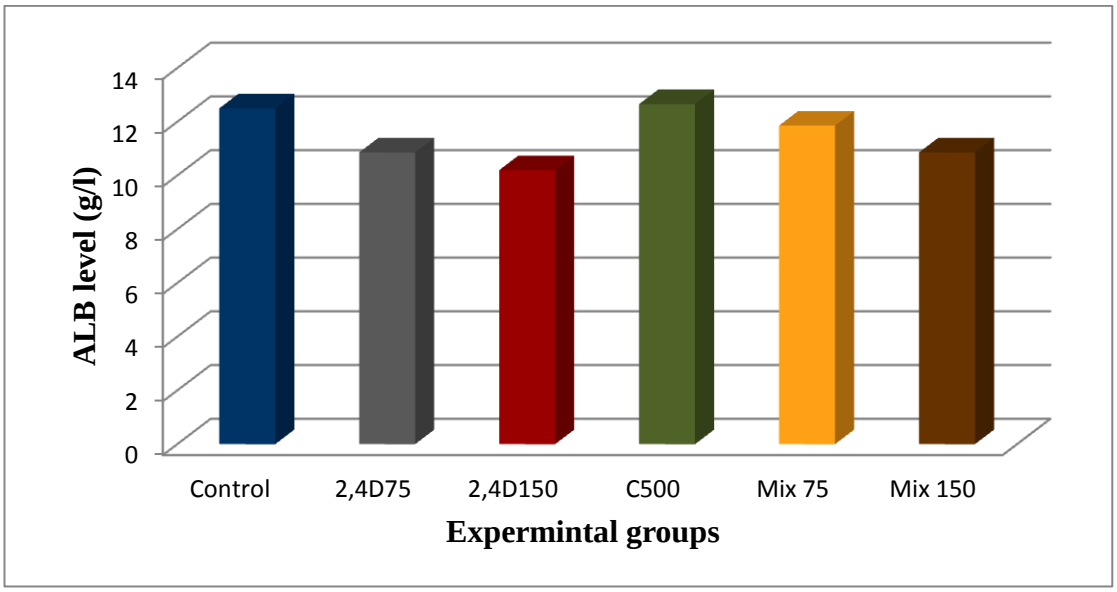


Figure 4.7: Serum albumin (ALB) level in all groups under investigation.

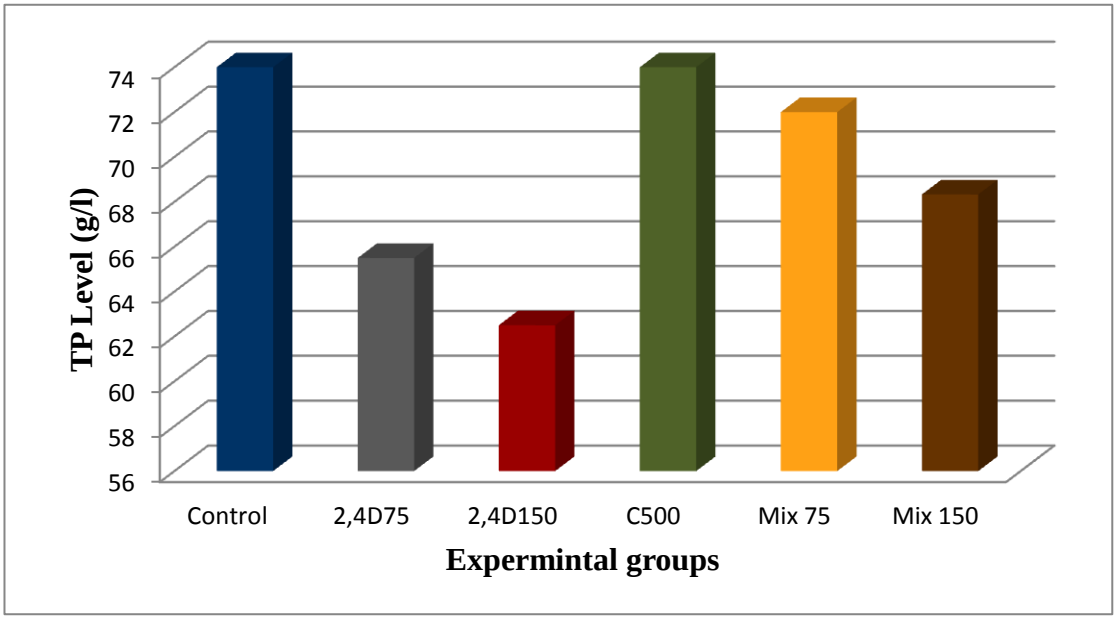


Figure 4.8: Serum total protein (TP) level in all groups under investigation.

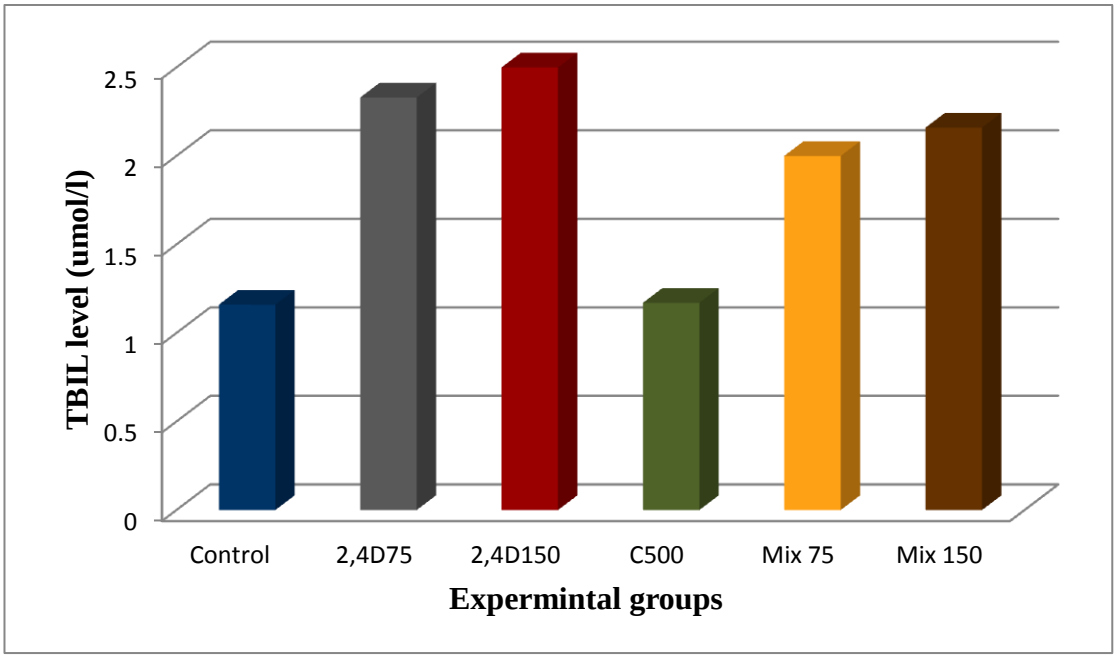


Figure 4.9: Serum total bilirubin (TBIL) level in all groups under investigation.

As seen in Table 4.5 and Figs. 4.6, 4.7, 4.8 and 4.9 the oral administration of 2, 4-D at dose of 75 mg/kg b.wt., to rats after feeding period (4 weeks) showed no significant change in serum LDH when compared to the negative control group. There were significant ($p<0.05$) decreases in serum ALB and TP by 13.12%, 11.48% respectively when compared to the negative control group. Serum TBIL showed a significant ($p<0.05$) increase by 100.86% in 2, 4-D_{75} group when compared to the control negative group.

In Table 4.5 and Figs. 4.6, 4.7, 4.8 and 4.9, the results revealed that oral administration of 2, 4-D at dose 150 mg/kg b.wt., to rats after feeding period (4 weeks) showed significant ($p<0.05$) increases in serum LDH and TBIL by 39.92% and 115.51% respectively when compared to the control negative group. Moreover, there were significant ($p<0.05$) decreases in serum levels of ALB and TP by 18.4% and 15.54% respectively when compared to the control negative group.

The results showed that there were no significant changes in serum levels of LDH, ALB, TP and TBIL in male rats orally given Chamomile capitula extract in a dose of 500 mg/kg b.wt., when compared to the control negative group.

Administration of mix_{75} showed no significant change in serum LDH compared to the group of rats orally given 2, 4-D_{75}. Conversely, there were significant ($p<0.05$) increases in serum ALB and TP by 9.21% and 9.92% respectively in rats orally given mix_{75} when compared to rats orally given 2,4-D_{75}. Serum TBIL showed a significant ($p<0.05$) decrease by 14.16% in rats orally given mix_{75} when compared to rats orally given 2, 4-D_{75}.

Serum ALB and TP data in Table 4.5 and Figs. 4.7and 4.8 showed that there was no significant change in male rats orally given mix_{150} compared to rats orally given 2, 4-

D_{150}. Meanwhile, there were significant ($p<0.05$) decreases in serum LDH and TBIL by 7.94% and 13.6% respectively in male rats orally given mix_{150} when compared to the rats orally given 2, 4-D_{150}.

4.5 Effect of Chamomile capitula extract on serum GR and SOD in hepatotoxic rats induced by 2, 4-D.

The effect of Chamomile capitula extract on serum GR and SOD in hepatotoxic rats induced by 2, 4-D is recorded in Table 4.6 and shown in Figs. 4.10 and 4.11.

Table 4.6: Effects of 2, 4-D at two doses and Chamomile aqueous extract after 4 weeks on serum antioxidant enzymes Glutathion reductase (GR) and Superoxide dismutase (SOD) of rats compared to control.

Groups	GR (U/ml)	SOD (U/ml)
Control (C-ve)	79.85 ± 1.15^{a}	94.15 ± 0.85^{a}
2,4-D75 (C+ve1)	62.46 ± 1.41^{c}	91.80 ± 0.65^{b}
2,4-D150 (C+ve2)	57.65 ± 0.25^{d}	89.83 ± 0.60^{c}
C500	81.03 ± 1.00^{a}	93.55 ± 0.39^{a}
Mix75	72.96 ± 1.10^{b}	$92.51\pm0.30^{a,b}$
Mix150	67.10 ± 0.93^{b}	91.35 ± 0.95^{b}

Values are presented as mean ± standard deviations.

- Control: fed on control diet, 2, 4-D_{75}: received 75 mg/kg b.wt., of 2, 4-D, 2, 4-D_{150}: received 150 mg/kg b.wt., of 2, 4-D, C_{500}: received 500 mg/kg b.wt., of Chamomile capitula extract, Mix_{75}: administrated of Chamomile capitula extract (500 mg/kg b.wt.)+ 2, 4-D (75 mg/kg b.wt.), Mix_{150}: administrated of Chamomile capitula extract (500 mg/kg b.wt.)+ 2, 4-D (150 mg/kg b.wt.).

- Values with different superscript letters within a column are significantly different at $p<0.05$, while those with similar or partially similar are non significant.

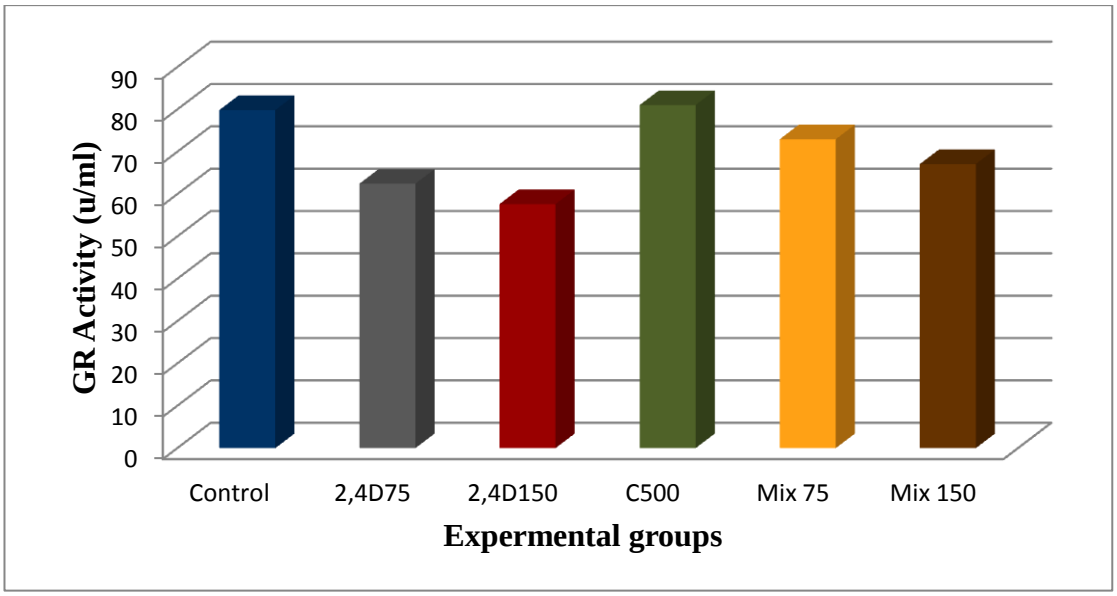


Figure 4.10: Serum glutathione reductase (GR) activity in all groups under investigation.

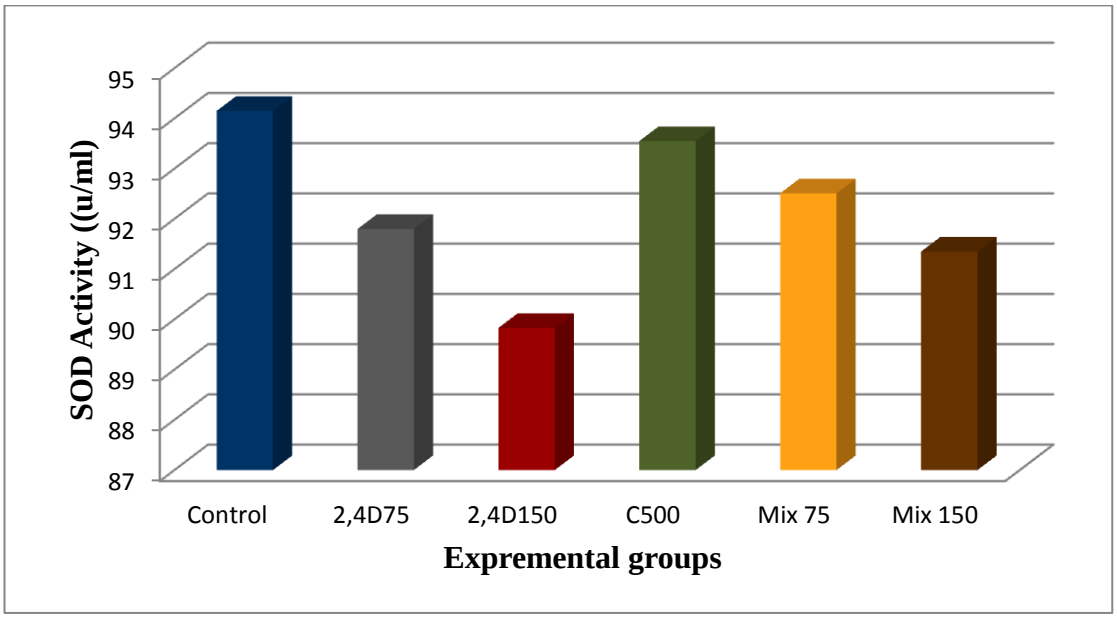


Figure 4.11: Serum superoxide dismutase (SOD) activity in all groups under investigation.

Oral administration of 2, 4-D at two dosage levels 75 or 150 mg/kg b.wt., to rats after feeding period (4 weeks) showed significant ($p<0.05$) decreases in serum GR and SOD enzymes by 21.77%, 27.80% and 2.49%, 4.58% respectively when compared to the negative control group.

Data recorded in Table 4.6 and Figs. 4.10 and 4.11 showed that there were no significant changes in serum GR and SOD enzymes in male rats orally given Chamomile capitula extract in a dose of 500 mg/kg b.wt., when compared to the negative control group.

Administration of mix_{75} induced non significant change in serum SOD enzyme when compared to rats orally given 2, 4-D_{75}. Results showed that there was a significant ($p<0.05$) increase in serum GR enzyme in male rats orally given mix_{75} by 16.81% compared to rats orally given 2, 4-D_{75}.

Concerning serum GR and SOD enzymes, data in Table 4.6 and Figs. 4.10 and 4.11 showed that there were significant ($p<0.05$) increases in the group of male rats orally given mix_{150} by 16.39% and 1.69% respectively when compared to rats orally given 2, 4-D_{150}.

4.6 Electron microscopic examination results:

The examination of ultrathin sections of liver of the control negative, positive and treated rats with Chamomile using electron microscopy that is used to determine ultrastructural organelles and showed the organelle changes which cannot be observed by light microscopy.

The electron microscopic examination of liver sections of the control negative group showed that most dark and pale hepatocytes were large polygonal with one or

two central euchromatic nuclei and fewer nuclear heterochromatic contents with apparent more electron dense nucleoli and their associated heterochromatin in deep or pale staining cytoplasm (Fig. 4.12). This was occupied by well-developed ovoid mitochondria surrounded by double membrane in which the inner one extended into mitochondrial matrix to form the cristae and rough endoplasmic reticulum (RER) as shown in (Fig. 4.13). The dark cells' cytoplasm contained numerous dense mitochondria, more rough endoplasmic reticulum and small nucleus enclosed by profilated nuclear envelop. The oval nucleus with high nucleus cytoplasmic ratio surrounded by contact nuclear envelope and had two types of chromatin: electron–dense heterochromatin around the nuclear envelope forming irregular clumps and nucleolus, the other electron-lucent euchromatin spreaded in the nucleoplasm (Fig. 4.13).

The ultrastructural lesions in hepatotoxic rats were more obvious and diverse with increase 2, 4-D dosages. The given of 2, 4-D_{75} mg/kg b.wt., induced nuclear changes in liver cells which included differences in shapes and size, irregularity and slight distention of nuclear envelope, increase in heterochromatin masses attached to inner nuclear envelop and nucleolar margination (Fig. 4.14). When increasing the dosage of 2, 4-D_{150} mg/kg b.wt., the hepatic nuclei showed variable degree of pyknosis, disaggregation and fragmentation of RER cisternae in hepatocytes appears to be a common response of the rat liver cells to treatment (Fig. 4.16). The most well known classical apoptosis is characterized by early nuclear collapse and massive condensation of chromatin with polymorph mitochondria which have dense matrices.

Concerning the mitochondria, which showed the most dramatic changes, there was considerable variations in mitochondrial population (normally abundant and closely packed or fewer); in size (increased and swollen); in shape (curved and

round) and internal structure (disorganization of cristae, matrix less electron dense, vacoulations of matrix). In all treated hepatocytes, there was an increase in lipid droplets in comparison with the control group (Figs. 4.15 and 4.17).

Electron microscopic study of Chamomile 500 mg/kg b.wt., group showed the normal control ultra structure of the liver. The hepatocytes were adjacent to each other (Fig. 4.18). The hepatocyte appeared with euchromatic nuclei containing prominent nucleoli have smooth regular outline and small amount of marginal heterochromatin. The cytoplasm contained numerous mitochondria and abundant stacked cisternae of RER as illustrated in (Fig. 4.19).

The improvement in group treated with mix_{75} was well demonstrated where the electron microscopic picture exhibited hepatocytes with intact cell membrane, many normal intact mitochondria, euchromatic nuclei with prominent nucleoli, few lipid droplets (Fig. 4.20) and normal granular endoplasmic reticulum profiles (Fig. 4.21). This improved picture of the hepatic tissue could be due to the amelioration effect of Chamomile extract and low dose of 2, 4-D at short periods.

The electron microscopic study of mix_{150} group showed partial improvement of hepatocytes in both nucleus and the mitochondria. Alterations of liver structure were noticed with decrease in number and volume of intracellular lipid droplets. Some hepatocytes with few lipid droplets while others with many large lipid droplets (Fig. 4.22). The RER appeared as parallel arrays closely adherent to (and often encircling) the mitochondria, the nuclei appeared with irregular shape (Fig. 4.23) with few necrotic cytoplasmic areas. These incomplete improvements of the hepatic tissue are suggested to be due to little amelioration effect of Chamomile extract with high dose of 2, 4-D at the short periods.

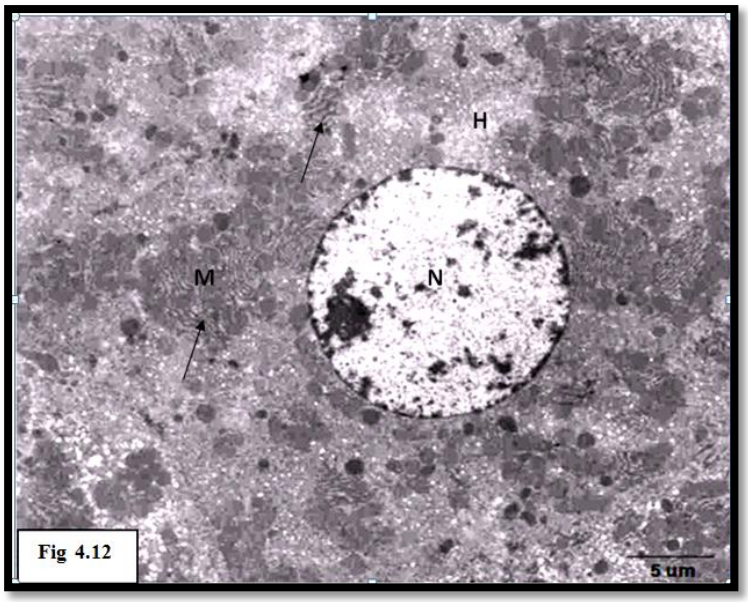


Figure 4.12: Photograph of ultrathin section of control negative liver, showing polygonal hepatocytes (H) with large central nucleus (N) has smooth regular outline and small amount of marginal heterochromatin. Numerous round or oval shaped mitochondria (M) uniformly distributed in the cytoplasm and rough endoplasmic reticulum (arrow) (Uranyl acetate and lead citrate, scale bar =5µm).

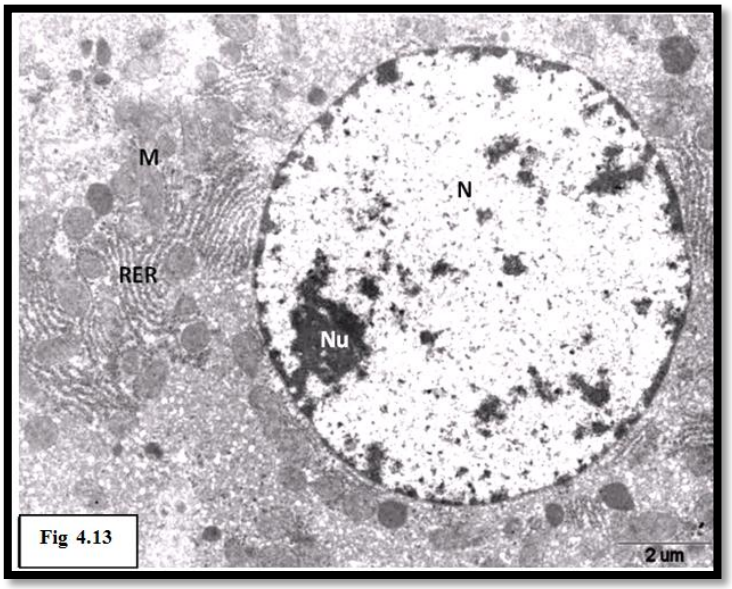


Figure 4.13: Enlarged part of the pervious photo showing: part of hepatocytes with high nucleus (N) cytoplasmic ratio and peripheral nucleolus (Nu). Cytoplasm filled with oval shaped mitochondria (M) and parallel stacks of rough endoplasmic reticulum (RER) (Uranyl acetate and lead citrate, scale bar =2 µm).

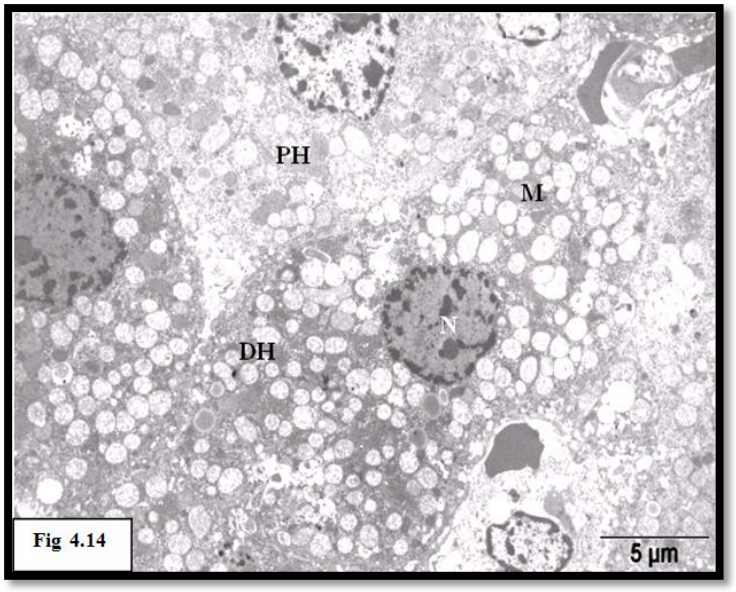


Figure 4.14: Photograph of ultrathin section of intoxicated liver (2, 4-D_{75} mg/kg b.wt.) showing: part of hepatic strand with dark (DH) and pale hepatocytes (PH) have irregular nuclei (N) and damaged mitochondria (M) (Uranyl acetate and lead citrate, scale bar =5 µm).

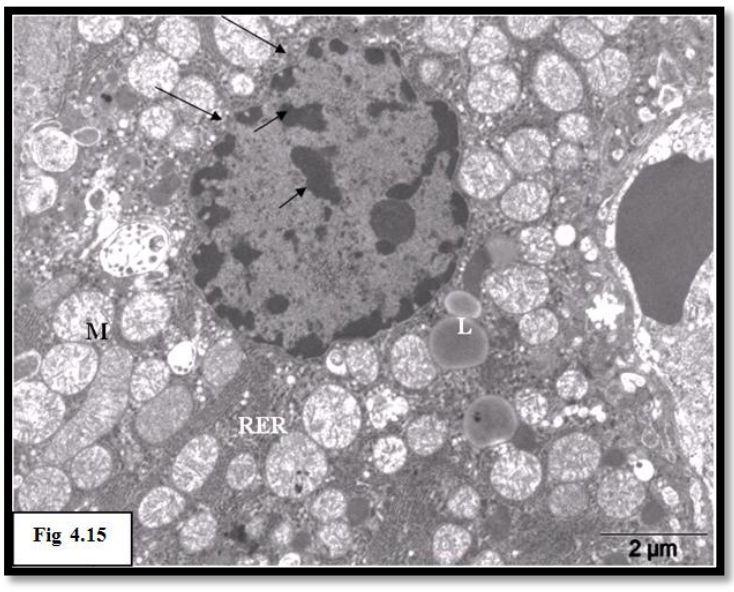


Figure 4.15: Enlarged part of the pervious photo showing: dark hepatocytes (DH) with irregular distinct nuclear envelope, heterochromatin masses (short arrows), wide nuclear pores (long arrows), lipid droplets (L), swollen mitochondria with moderate cristolysis (M) and fragmented rough endoplasmic reticulum (RER) (Uranyl acetate and lead citrate, scale bar =2 µm).

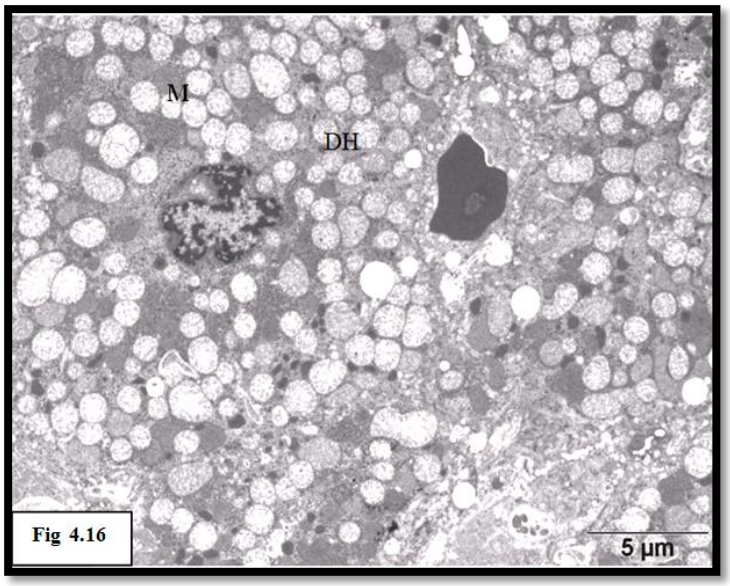


Figure 4.16: Photograph of ultrathin section of intoxicated liver (2, 4-D_{150} mg/kg b.wt.) showing: dark hepatocytes (DH) has severely lobulated electron dense nucleus (N) with low nucleus cytoplasmic ratio and damaged mitochondria (M) with low electron dense matrix (Uranyl acetate and lead citrate, scale bar =5 µm).

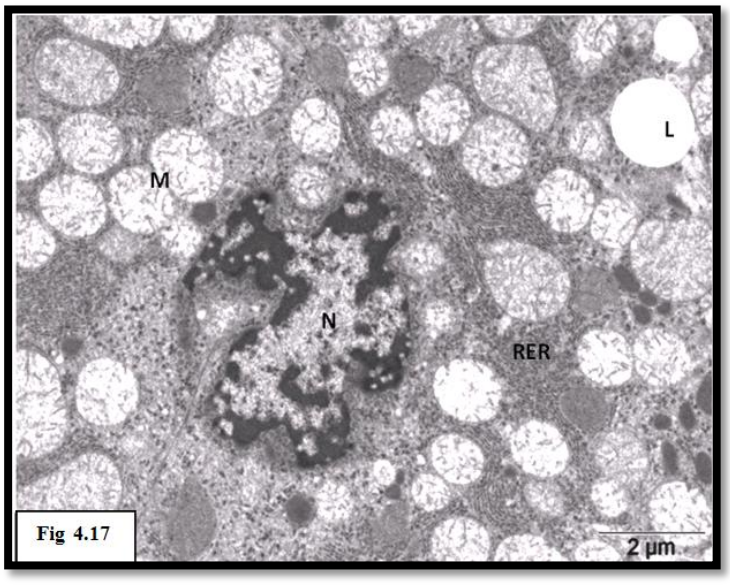


Figure 4.17: Enlarged part of the pervious photo showing: part from dark hepatocytes (DH) with apoptotic nucleus, lipid droplets (L), swollen mitochondria with advanced cristolysis (M) and fragmented rough endoplasmic reticulum (RER) (Uranyl acetate and lead citrate, scale bar =2 µm).

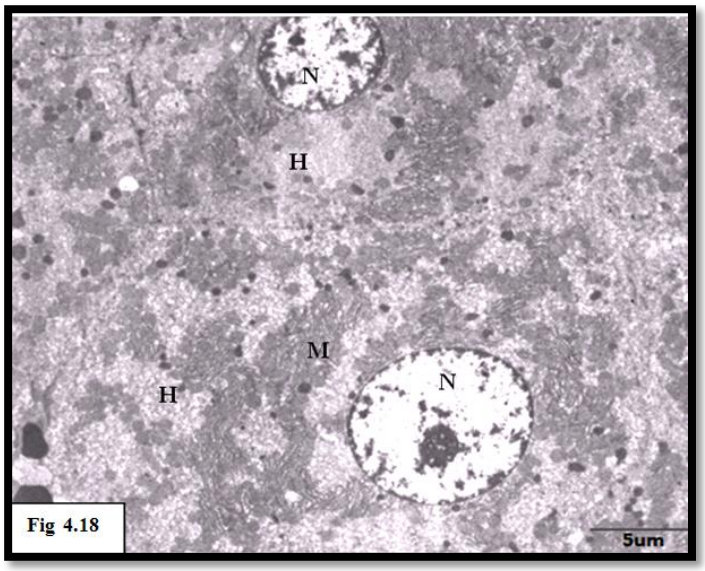


Figure 4.18: Photograph of ultrathin section of liver treated with Chamomile (C_{500} mg/kg b.wt.) showing: normal polygonal hepatocytes (H) with large central nuclei (N) in cytoplasm deep staining and normal spheroid mitochondria (M) uniformly distributed in the cytoplasm (Uranyl acetate and lead citrate, scale bar =5 µm).

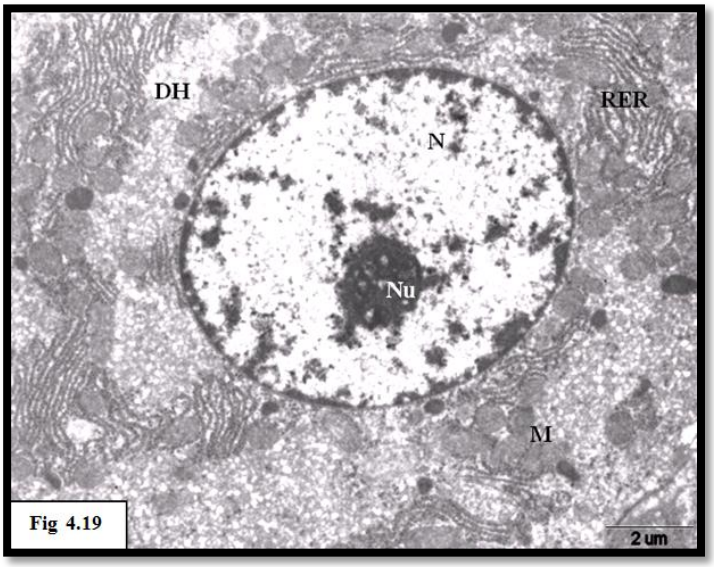


Figure 4.19: Enlarged part of the pervious photo showing: part from dark hepatocytes (DH) with nucleus (N) and clear nucleoli (Nu) have smooth regular outline and small amount of marginal heterochromatin, mitochondria (M) abundant and in stacked cisternae rough endoplasmic reticulum (RER) (Uranyl acetate and lead citrate, scale bar =2 µm).

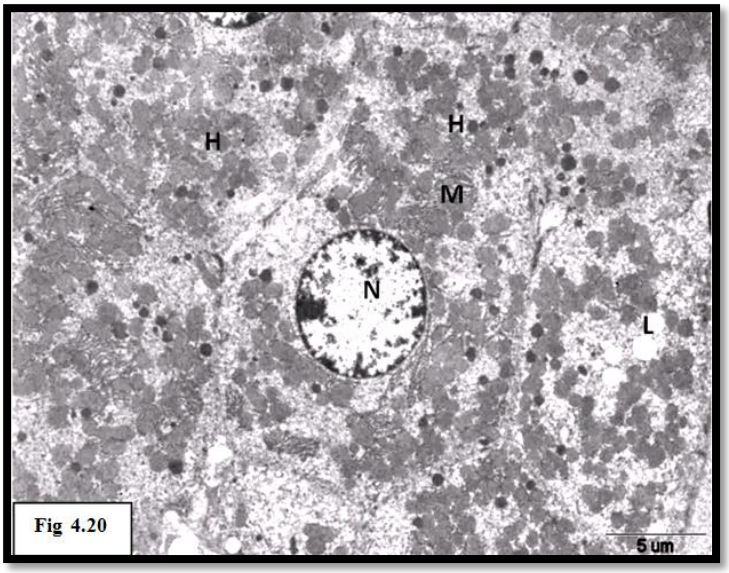


Figure 4.20: Photograph of ultrathin section of treated liver (2,4-D_{75} mg/kg b.wt.) + (Chamomile C_{500} mg/kg b.wt.) showing: adjacent hepatocytes (H) with noticeable improvement in both nucleus (N) and mitochondria (M) with few lipid droplets (L) (Uranyl acetate and lead citrate, scale bar =5 µm).

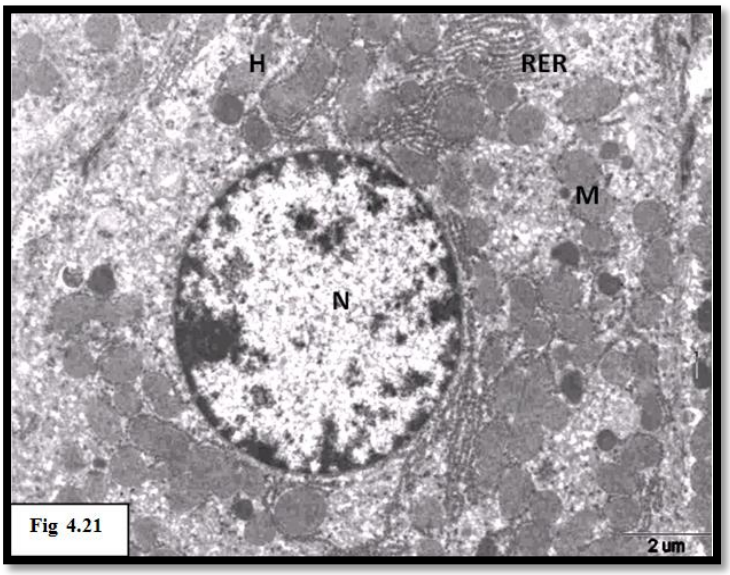


Figure 4.21: Enlarged part of the pervious photo showing: part of hepatocytes (H) has nearly normal nucleus (N) with little increased amount of heterochromatin, mitochondria (M) and uniformed rough endoplasmic reticulum (RER) (Uranyl acetate and lead citrate, scale bar =2 µm).

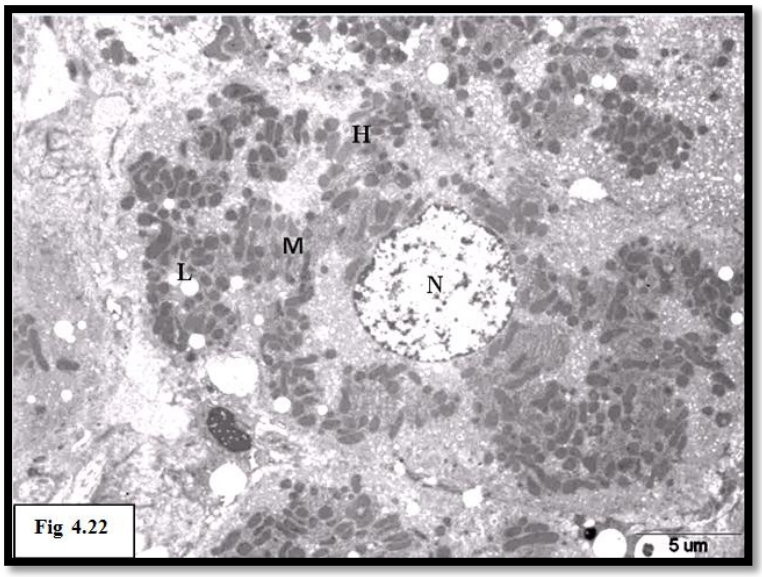


Figure 4.22: Photograph of ultrathin section of treated liver (2,4-D_{150} mg/kg b.wt.) + Chamomile C_{500} mg/kg b.wt.) showing: hepatocytes (H) with less improvement in irregular nucleus (N), perforated dense mitochondria (M) and numerous lipid droplets (L) (Uranyl acetate and lead citrate, scale bar =5 µm).

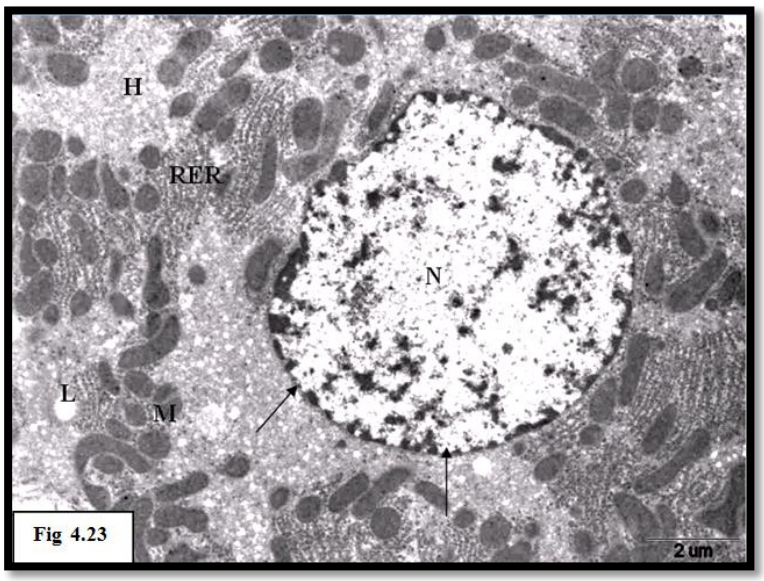


Figure 4.23: Enlarged part of the pervious photo showing: part of hepatocytes (H) characteristic by numerous dense cellular organelles specially mitochondria (M) with large number, short profiles of rough endoplasmic reticulum (RER), nucleus (N) surrounded by irregular nuclear envelop with nuclear pores (arrows) and few lipid droplet (L) (Uranyl acetate and lead citrate, scale bar =2 µm).

Chapter V

Discussion

Chapter V

Discussion

The current study was under taken to investigate the effect of Chamomile capitula aqueous extract on body weight gain percent, feed efficiency ratio and relative weight of the liver in hepatotoxic rats induced by herbicide 2, 4-D. The activities of liver enzymes (AST, ALT and ALP), antioxidant enzymes (GR and SOD) and serum levels of LDH, ALB, TP and TBIL as well as histopathological examination of the liver were also carried out.

The herbicide 2, 4-D has been extensively used in the modern agriculture despite of its short half-life in soil and aquatic environment. Toxicological studies indicated that 2, 4-D has a great potential for undesirable toxic effects **(Hassanein 2012).** The toxic effects of herbicide 2, 4-D include embryotoxicity, teratogenicity, neurotoxicity, immunotoxicity and hepatotoxicity **(Tuschl and Schwab 2003** and **Chinalia, Seleghin and Correa 2007).**

Several reports have been demonstrated that herbicide 2, 4-D can bind irreversibly to hepatic proteins in rats **(Deduffard *et al.* 1993, Sulik *et al.* 1998** and **Di paolo, Deduffard and Deduffard 2001).** A study by **Li, Grillo and Benet (2003)** revealed the presence of a novel reactive metabolite of 2, 4-D which is 2, 4-Dichlorophenoxyacetol-S-acetyl-CoA (2, 4-D-CoA). The chemically reactive

metabolites of 2, 4-D are proposed to act as mediators for induction of hepatotoxicity. However, the various toxic effects of 2, 4-D were reviewed by **Bukowska (2006).**

The present study showed that oral administration of 2, 4-D to rats at two dosage levels 75 and 150 mg/kg b.wt., for 4 weeks induced a significant ($p<0.05$) decrease in body weight gain when compared to the negative control group. This decrease in body weight of rats could be attributed to the reduction in feed intake which might be due to loss of appetite of rats due to 2, 4 –D administration. This finding agreed with the previous work of **Troudi *et al.* (2012)** which showed that there was a positive correlation between the decrease of body weight gain percent and the dose of 2, 4 –D herbicide administered to rats. Moreover, human exposure to herbicide 2, 4-D provoked reproductive damage, difficulty of respiration, loss of appetite, skin rashes, eye irritation, and chronic headache as reported by **Kogevinas (1995).** The previously reported loss of appetite in human exposed to 2, 4-D confirmed our result that rats given 2, 4-D had a decrease in the body weight gain.

Furthermore, **Tayeb *et al.* (2010)** reported that oral administration of 2, 4-D in doses of 15, 75 and 150 mg/kg b.wt., to rats resulted in a significant decrease in the body weight gain percent. This finding was attributed to the reduction in feed intake which may be due to loss of appetite of rats exposed to 2, 4-D herbicide. In addition, **Hassanein (2012)** mentioned that chronic toxicity of 2, 4-D herbicide was manifested by decreased body weight gain, altered organs weight and hematological parameters and other serum biochemical changes.

The current results revealed that oral administration of Chamomile capitula aqueous extract during experimental period (4 weeks) in a dose of 500 mg/kg b.wt.,

when given together with 2, 4-D in doses of 75 and 150 mg/kg b.wt., to rats significantly ($p<0.05$) increased the body weight gain percent as compared to rats received only 75 or 150 mg/kg b.wt., of 2, 4-D. In this respect, **Gupta and Misra (2006)** reported that Chamomile capitula extract increased the body weight in paracetamol-intoxicated rats treated with Chamomile when compared to positive control rats. This result could be explained by the high content of flavonoids (63.3%) and total phenolic compounds (23.2 %) in Chamomile which have an antioxidant effect against the oxidative stress induced by 2, 4-D, so enhancing food consumption by rats given Chamomile. Our results revealed that there was no increase in body weight gain (BWG) in rats administrated Chamomile capitula aqueous extract only**.** This may be due to short term of administration.

Liver is the most important organ of the animal body and is highly affected primarily by toxic or xenobiotics agents (**Bradberry, Proudfoot and Vale 2004** and **Gupta and Misra 2006).** Results obtained in the present study showed that oral administration of 2, 4-D at the two dosage levels 75 or 150 mg/kg b.wt., to rats for 4 weeks induced a significant ($p<0.05$) increase in the relative liver weight when compared to the negative control group. This result was consistent with previous study reported by **Tayeb *et al.* (2010)** who demonstrated that exposure of rats to 15, 75 and 150 mg/kg b.wt., of 2, 4-D increased the liver weight. The increase in liver relative weight might be due to edema inflammatory changes seen in liver tissues due to 2, 4-D administration. In this concern, some pesticides were reported to cause an increase in the relative weight of liver in experimental animals **(Undeger *et al.* 2000)**. Moreover, **Amacher, Schomaker and Burkhardart (1998)** reported that the increase in liver weight was related to the induction of enzyme cytochrome-P 450. In addition, **Bucher (1964)** reported that herbicide 2, 4-D -induced enlargement of the

liver which may be related to the increase in most of the major cellular components in response to the degenerative toxic effects of 2, 4-D herbicide.

In this study, no significant difference was observed in the relative weight of liver of rats given Chamomile capitula extract in a dose of 500 mg/kg b.wt., in combination with 2, 4-D in a dose of 75mg/kg b.wt., when compared to rats only received 2, 4-D_{75}. This finding was in consistent with that reported by **Gupta and Misra (2006).**

Results of the current study showed that oral administration of Chamomile capitula aqueous extract in a dose of 500 mg/kg b.wt., in combination with 2, 4-D in a dose of 150 mg/kg b.wt., revealed a significant ($p<0.05$) decrease in relative liver weight when compared to rats orally given 2, 4-D_{150}. Our results agreed with those obtained by **Gupta and Misra (2006)**, who found that the administration of Chamomile capitula extract decreased the relative liver weight in Chamomile-treated group compared to paracetamol control group. This recovery could be due to the high content of flavonoids (63.3%) and total phenolic compounds (23.2 %) in Chamomile. These bioactive compounds were reported to act as free radical scavengers, intercepting those radicals involved in 2, 4-D metabolism by microsomal enzymes such as cytochrome-P 450.

Our results revealed that administration of 2, 4-D at two dosage levels 75 and 150 mg/kg b.wt., to rats for 4 weeks significantly ($p<0.05$) increased serum levels AST and ALT when compared to the negative control rats. An extensive liver injury was induced by 2, 4–D due to the increase in lipid peroxidation causing membrane damage. The present results agreed with **Troudi *et al.* (2012)** who showed that there were increases in the level of transaminases (AST and ALT) of the 2, 4-D-

intoxicated groups at a dose of 126 mg/kg b.wt. This could be because of ALT and AST enzymes are the most sensitive biomarkers directly correlated with the extent of hepatic damage and toxicity. In fact, these enzymes increased and secreted into blood when hepatocellular injury occurred as mentioned by **Kalender *et al.* (2005**). However, **Kuzu *et al.* (2007)** concluded that liver necrosis and inflammatory reactions were accompanied by high levels of blood ALT and AST enzymes. In addition, **Troudi *et al.* (2012)** reported that blood aminotransferases (ALT and AST) were significantly increased in rats given 2, 4- D herbicide.

Biochemical analysis of serum ALP enzyme showed a significant ($p<0.05$) increase in serum ALP level of rats orally given 2, 4-D in a dose of 150 mg/kg b.wt., when compared to negative control rats. This result was in agreement with the study of **Kalender *et al.* (2005)** who reported that exposure of rats to diazinon insecticide (10 mg/kg b.wt.) resulted in an increase in ALP level. This increase could be due to the degenerative changes and damage in liver tissues. On the other hand, there was no significant change in serum ALP enzyme in rats given 2, 4-D at the dose of 75 mg/kg b.wt., when compared to the negative control group as has been shown by **Tayeb *et al.* (2010)**.

Coadministration of Chamomile capitula extract in a dose of 500 mg/kg b.wt., with 2, 4-D in doses of 75 and 150 mg/kg b.wt., resulted in a significant ($p<0.05$) decrease in serum ALT and AST levels when compared to rats orally given 2, 4-D in doses 75 and 150 mg/kg b.wt. Our results agreed with that of **Gupta and Misra (2006)**, who reported that the administration of Chamomile capitula extract reduced the elevated serum levels of ALT and AST induced by paracetamol. This reduction could be attributed to the protective effect of Chamomile capitula extract and the maintenance of the functional integrity of hepatic cells. Serum ALP enzyme analysis

showed no significant change at the doses of 500 mg/kg b.wt., of Chamomile mixed with 2, 4-D in doses of 75 and 150 mg/kg b.wt., when compared to rats orally given 2, 4-D at both tested doses. This result agreed with that obtained by **Gupta and Misra (2006)**, who reported that the administration of Chamomile capitula extract reduced the enhanced level of serum ALP. This reduction could be attributed to the protective effect of Chamomile capitula extract and the maintenance of the functional integrity of hepatic cells.

The antioxidant enzymes (SOD and GR) were found to limit the effects of oxidant molecules on tissues and were active in the defense against oxidative stress and cell injury as they exhibit free radical scavenging activity **(Nakbi *et al.* 2010b)**. Consequently, in our study the significant ($p<0.05$) decrease of the antioxidant enzyme activities in the liver after 2, 4-D (75 and 150 mg/kg b.wt.) intoxication as compared to the control negative group, proved the failure of antioxidant defense system to overcome the influx of reactive oxygen species generated by 2, 4-D exposure **(Tayeb *et al.* 2010)** and **Troudi *et al.* (2012)**. In addition, the decrease in SOD activity might be due to the loss of copper and zinc which are essential for the enzyme activity **(Karthikeyan, Sarala Bai and Niranjali 2007)**. Our result was in agreement with the previous study of **Tayeb *et al.* (2010**) who reported that exposure of rats to 15, 75 and 150 mg/kg b.wt., of 2, 4-D decreased the GR activity in the liver. In addition, **Troudi *et al.* (2012)** reported that exposure of rats to 126 mg/kg b.wt., of 2, 4 -D decreased the SOD activity in the liver.

Combined administration of Chamomile capitula extract in a dose of 500 mg/kg b.wt., with 2, 4-D in a dose of 150 mg/kg b.wt., produced significant ($p<0.05$) increases in serum GR and SOD activities when compared to rats orally given 2, 4-D

in a dose of 150 mg/kg b.wt. Our results agreed with those of **Gupta *et al.* (2006)**, who reported that the administration of Chamomile capitula extract increased the antioxidant enzymes activity in Chamomile treated group as compared to CCL_4 control group. This may be because Chamomile capitula extract has an antioxidant activity that affording the hepatoprotective effect against 2, 4-D toxicity via inducing cell membrane stabilization, hepatic cell regeneration and activation of antioxidant enzymes.

Co-administration of Chamomile capitula extract in a dose of 500 mg/kg b.wt., with 2, 4-D in a dose 75 mg/kg b.wt., to rats caused a significant ($p<0.05$) increase in serum GR compared to rats orally given 2, 4-D_{75}. The result of the current study agreed with **Gupta *et al.* (2006)**, who reported that the administration of Chamomile capitula extract increased the antioxidant enzymes activity in Chamomile treated group compared to CCL_4 control group. This may be due to antioxidant activity of Chamomile capitula extract that affording the hepatoprotective effect against 2, 4-D by cell membrane stabilization, hepatic cell regeneration and activation of antioxidant enzymes. On the other hand, there was no significant change in serum SOD at this dose compared to the group of rat orally given 2, 4-D_{75}.

Regarding other biomarkers of liver toxicity, the present results showed that serum levels of ALB and TP were significantly ($p<0.05$) decreased in rats after administration of 2, 4-D at two dosage levels 75 and 150 mg/kg b.wt., when compared to the negative control group. Similar results were demonstrated by **Tayeb *et al.* (2010)** who reported significant decreases in serum ALB and TP after 2, 4-D administration to rats. This is because albumin is synthesized by the liver and often transports or binds to drugs or chemicals such as 2, 4-D that may negatively influence total protein and albumin metabolism.

Combined administration of Chamomile capitula extract in a dose of 500 mg/kg b.wt., mixed with 2, 4-D in a dose 75 mg/kg b.wt., significantly ($p<0.05$) increased serum ALB and TP levels when compared to rats orally given 2, 4-D_{75}. This result was consistent with **Gupta and Misra (2006)** who reported that there was a significant increase in TP after Chamomile capitula extract treatment against paracetamol intoxication. In addition, **Kumar *et al.* (2012)** reported that the extract of Chamomile capitula induced a hepatoprotective effect and increases serum ALB levels. The hepatoprotective activity of Chamomile may be due to normalization of impaired membrane function activity. On the other hand, our results showed no significant change in ALB and TP levels after administration of Chamomile capitula extract in a dose of 500 mg/kg b.wt., combined with 2, 4-D in a dose of 150 mg/kg b.wt., when compared to rats orally given 2, 4-D_{150}. This result was in consistent with that of **Gupta and Misra (2006)** and **Kumar *et al.* (2012)**.

Lactate dehydrogenase (LDH) is intracellular enzyme that recognized as a potential marker to assess liver toxicity **(Agrahari, Pandey and Gopal 2007).** Our study showed no significant difference in serum LDH levels in rats orally given the small dose of 2, 4-D when compared with the negative control group. On the other hand, there was a significant ($p<0.05$) increase in serum LDH levels in rats orally given the large dose of 2, 4-D when compared to the negative control group .This finding may be due to the release of isozymes from destroyed liver . This result was in agreement with those obtained by **Tripathi and Shukla (1990)** and **Vander and Hunsaker (2003).** Moreover, **Tayeb *et al.* (2010)** and **Troudi *et al.* (2012)** reported that exposure of rats to 2, 4-D increased serum LDH levels and this was attributed to the functional disorder of the liver by 2, 4-D.

It was noticed that combined administration of Chamomile capitula extract in a dose of 500 mg/kg b.wt., with 2, 4-D at a dose of 75 did not show any significant changes in serum LDH when compared to rats orally given 2, 4-D at a dose of 75mg/kg b.wt., while when coadministrated with 2, 4-D at a dose150 mg/kg b.wt., it showed a significant ($p<0.05$) decrease in serum LDH when compared to rats orally given 2, 4-D at a dose150 mg/kg b.wt. The same results were obtained by **Chandrasekhar *et al.* (2012)** who reported a significant decrease in serum LDH when rats treated with Chamomile. The reduction in serum LDH could be attributed to the protective effect of Chamomile capitula extract and maintenance of the functional integrity of hepatic cells **(Gupta and Misra 2006)**.

Concerning serum TBIL analysis, the results showed that there was a significant ($p<0.05$) increase in level of TBIL in rats given herbicide 2, 4-D at doses of 75 and 150 mg/kg b.wt., when compared to the negative control group. These results are nearly similar to those mentioned by **Nakbi *et al.* (2010b)** and **Troudi *et al.* (2012)** who reported that TBIL level increased after 2, 4-D administration. It was concluded by **(Nkozi, Opoku and Terblanche 2005)** that TBIL can be used as a measure of binding, conjugation, and excretory capacity of hepatocytes. The elevated levels of total bilirubin in 2, 4-D herbicide - intoxicated rats indicated an extensive liver damage. The combined administration of Chamomile capitula aqueous extract with both doses of 2, 4-D induced a significant ($p<0.05$) decrease in TBIL serum level when compared to rats orally given 2, 4-D at both tested doses. This result agreed with that of **Gupta and Misra (2006)** who reported that treatment with Chamomile extract reduced the paracetamol-enhanced level of serum bilirubin which seems to offer the protection and to maintain the functional integrity of hepatic cells.

The ultra findings demonstrated that the cytological effects were found in all groups and their severity was dose dependent. Four weeks 2, 4-D treatment, resulted in apoptosis of some hepatocytes with shrinkage of the nuclei and condensation of the heterochromatin. Some apoptotic cells showed swelling of mitochondria and vacoulations compared to control negative group. The histopathological findings of this study were coincided with those reported by **Troudi, Mahjoubi and Zegha (2009)** and **Hussein *et al*. (2011)** who found that the liver sections of Gibberellic acid-intoxicated rats revealed that hepatocytes were swollen and appeared with severe cytoplasmic vacuolization with degeneration of their nuclei.

The herbicide 2, 4-D may be accompanied by an increase in the cytosolic Ca^{2+}, due to oxidative stress and by the breakdown of phospholipid **(Kumar, Abbas and Fausto 2005)**. These may be responsible to hepatic disturbances. A previous study disclosed that liver mitochondrial dysfunction contributed to apoptosis via the production of reactive oxygen species **(Mignotte and Vayssiere 1998)**.

The histopathological changes seen in the present study as regard to swelling of mitochondria were in accordance with the results of **Pessayre *et al.* (2001)** and **Krahenbhul (2001)** who reported that inhibition of mitochondrial function together with accumulation of reactive oxygen species led to cell death (apoptosis).

After feeding with Chamomile extract noticeable or slight ameliorations of the unfavorable effects produced in the liver by 2, 4-D intoxication, which was associated with normal intact hepatocytes nucleus and nucleolus, many normal intact mitochondria, few necrotic cytoplasmic area, euchromatic nuclei and few lipid droplets. Chamomile may probably arrest the harmful effect on liver cells through protection of cells and tissues from oxidative damage by scavenging oxygen-free

radicals and stimulate the regeneration of damaged tissues and cells as did green tea **(El Daly 2011).**

The appearance of hepatocytes treated with Chamomile was clarified by electron microscopic picture which showed partial improvement in the form of reappearance of cell organelles (mitochondria and cisternae of RER). The nuclei appeared euchromatic with prominent nucleoli. These results were coincided with **Abd El Maksoud, Abd El Maksoud and Abd El Hamid (1996)** who stated that following Gibberellic acid withdrawal, a few cells became nearly similar to those of the control group, while the majority of cells remained affected.

The increase in number of lipid droplets inside hepatocytes after 2, 4-D administration was comparable to fatty change developed in liver cells following either alcohol consumption **(McGee 1992)** or taking drug **(Hall 1994)** or treatment with styrene **(De Piceis Polver *et al.* 2003)**.

Chapter VI

Conclusion and Recommendations

Chapter VI

Conclusion and Recommendations

In conclusion the results of this study denote that oral administration of Chamomile capitula aqueous extract to 2, 4-D herbicide-intoxicated rats for 4 weeks improves body weight and feed efficiency ratio. Chamomile capitula treatment also produces hepatoprotective and antioxidant effects and alleviates degenerative changes seen in liver tissues were induced by 2, 4-D herbicide. The hepatoprotective activity of Chamomile capitula may be attributed to its antioxidant effect which maintains the functional integrity of hepatic cells. The current study recommends that intake of Chamomile capitula flowers as herbal tea may be beneficial for patients who suffer from liver disorders and oxidative stress.

The present study recommends the following:

1-Chamomile capitula aqueous extract increased albumin, total protein levels in the serum, improved liver enzymes and lactatedehydrogenase, increased serum antioxidant capacity and ameliorated the degenerative changes seen in liver tissue of hepatotoxic rats.

2- Intake of Chamomile capitula flowers as herbal tea may be beneficial for patients who suffer from liver diseases and oxidative stress.

3- Cultivation of Chamomile plant in Kigdom of Saudi Arabia and other countries should be encouraged.

4- Nutritional and health educational programs should be organized and directed to the public to be informed about health benefits of Chamomile plant.

List of

References

List of References

Abd El Maksoud, S. A., Abd El Maksoud, N. and Abd El Hamid, N. A. (1996) Chronic toxic effect of plant growth promoting hormone, gibberellic acid, on the liver cells of adult male albino rat, Assut. Med. J., vol. 20: 87-103.

Agrahari, S., Pandey, K. C. and Gopal, K. (2007) Biochemical alterations induced by monocrotophos in the blood plasma of fish channa punctatus (bloch), Pest. Biochem. Physiol., vol. 88: 268-272.

Al-Ismail, K. M. and Aburjai, T. (2004) Antioxidant activity of water and alcoholextracts of Chamomile flowers, anise seeds and dill seeds, J. Sci. Food Agric., vol. 84: 173–178.

Amacher, D., Schomaker, S. and Burkhardart, J. (1998) The relationship among microsomal enzyme induction, liver weight and histological change in rat toxicology studies, Food Chem. Toxicol., vol. 36: 831–839.

Barnes, J., Anderson, L. and Phillipson, D. (2002) Herbal medicines, London: Pharmaceutical Press.

Bongiovanni, B., De Lorenzi, P., Ferri, A., Konjuh, C., Rassetto, M., Evangelista De Duffard, A. M., Cardinali, D. P. and Duffard, R. (2007) Melatonin decreases the oxidative stress produced by 2,4-Dichlorophenoxyacetic acid in rat cerebellar granule cells, Neurotoxicity Research, vol. 11: 93-99.

Bradberry, S. M., Proudfoot, A. T. and Vale, J. A. (2004) Poisoning due to chlorophenoxy herbicides, Toxicol. Rev., vol. 23: 65-73.

Bucher, N. (1964) Effects of 2, 4-Dichlorophenoxyacetic acid on experimental animals, Proc. Soc. Exp. Biol., vol. 63: 204-205.

Bukowska, B. (2006) Toxicity of 2, 4-Dichlorophenoxyacetic acid– molecular mechanisms, Polish J. of Environ. Stud., vol. 15: 365-374.

Burtis, C. A., Ashwood, E. R., Bruns, D.E. and Tietz, N.W. (2006) Textbook of clinical chemistry and molecular biology, St. Louis: Elsevier Saunders.

Carlberg, I. and Mannervik, B. (1985) Glutathione reductase, Methods Enzymol., vol. 113: 484-490.

Celik, I., Tuluce, Y. and Isik, I. (2006) Influence of subacute treatment of some plant growth regulators on serum marker enzumes and erythrocyte and tissue

antioxidant defense and lipid peroxidation in rats, J. Biochem. Mol. Toxicol., vol. 20: 174-182.

Chandrashekhar, V. M., Nirav, M., Nidavani, R. B., Jignesh, N. and Ganapaty, S. (2012) Anti-ischemic effect of german Chamomile (matricaria recutita l.) against ischemia/reperfusion induced myocardial damage in isolated rat heart, Pharmacologia, vol. 3: 406-412.

Chapman, D., Castiilla, R. and Campbell, J. (1959) Evaluation of protein in food determination of protein and food efficiency ratio, Can. J. Biochem. and Physil., vol. 37: 676-686.

Charles, J. M., Dalgard, D. W., Cunny, H. C., Wilson, R. D. and Bus, J. S. (1996) Comparative subchronic and chronic dietary toxicity studies on 2,4–dichlorophenoxyacetic acid amine, and ester in the dog, Fundam.Appl. Toxicol., vol. 29: 78–85.

Charles, J. M., Hanley, T. R., Wilson, R. D., van Ravenzwaay, B. and Bus, J. S. (2001) Developmental toxicity studies in rats and rabbits on 2, 4–dichlorophenoxyacetic acid and its forms, Toxicol. Sci., vol. 60: 121–131.

Charles, J. M. and Leeming, N. M. (1998) Chronic dietary toxicity study on 2,4dichlorophenoxybutyric acid in the dog, Toxicol. Sci., vol. 46: 134-142.

Chinalia, F. A., Seleghin, M. H. and Correa, E. M. (2007) 2, 4-D causes, effect and control, Terrestrial and Aquatic Environmental Toxicology, vol. 1 24-33.

Conforti, F., Sosa, S., Marrelli, M., Menichini, F., Statti, G., Uzunov, D., Tubaro, A. and Menichini, F. (2009) The protective ability of mediterranean dietary plants against the oxidative damage: The role of radical oxygen species in inflammation and the polyphenol, flavonoid and sterol contents, Food Chemistry, vol. 112: 587-594.

De Piceis Polver, P., Fenoglio, C., Nano, R., Coccini, T., Bertone, V., Vaccarone, R. and Gerzeli, G. (2003) Styrene hepatotoxicity in rats treated by inhalation or intraperitoneally: A structural investigation, Histopathol., vol. 18: 49-54.

Deduffard, A. M., Deperetti, A., De cantarini, S. and Duffard, R. (1993) Effects of 2, 4-Dichlorophenoxyacetic acid butyl ester on chick liver, Arch. Environmental Contam. Toxicol., vol. 25: 204-211.

Di paolo, O., Deduffard, A. M. and Deduffard, R. (2001) Invivo and in vitro binding of 2,4-Dichlorophenoxyacetic acid to a rat liver mitochondrial protein, Chemico. Biol. Int., vol. 137: 229-241.

El Daly, A. (2011) The protective effect of green tea extract against enrofloxacin action on the rat liver; histological, histochemical and ultrastructural studies, Journal of American Science, vol. 7: 669-679.

El-massry, K., Ghorab, A., Ramadan, M. and Gad, A. (2009) Antioxidant and protective effect of aromatic plants blend infusion against oxidative stress of streptozotocine and carbon tetrachloride in rats, The egyptian journal of hospital medicine, vol. 35: 308-322.

Fenaroli, O. (2005) Fenaroli's handbook of flavour ingredients, London: CRC Press.

Foyer, C. H., Lelandais, M. and Kunert, K.J. (1994) Photooxidative stress in plants, Physiol. Plant, vol. 92: 696-717.

Ganzera, M., Schneider, P. and Stuppner, H. (2006) Inhibitory effects of the essential oil of Chamomile (matricaria recutita) and its major constituents on human cytochrome p450 enzymes, Life Sci., vol. 78: 856–861.

Garabrant, D. and Philbert, M. (2002) Review of 2, 4-Dichlorophenoxyacetic acid (2, 4-D) epidemiology and toxicology, Critical Reviews in Toxicology, vol. 32: 233–257.

Gupta, A., Chitme, H., Dass, S. and Misra, N. (2006) Antioxidant activity of Chamomile recutita capitula methanolic extracts against ccl4-induced liver injury in rats, Journal of Pharmacology and Toxicology, vol. 1: 101-107.

Gupta, A. and Misra, N. (2006) Hepatoprotective activity of aqueous ethanolic extract of Chamomile capitula in paracetamol intoxicated albino rats, American Journal of Pharmacology and Toxicology, vol. 1: 17-20

Hall, P. (1994) Alcoholic liver disease. In: Pathology of the liver, Edited by: M. Sween, P.P. Antony, P.J. Scheuer, A.D. Burt and B.C. Portmann, London: Churchill Livingstone.

Hassanein, K. (2012) Histopathological effects of 2, 4-Dichlorophenoxyacetic acid on sprague-dawley rats with special reference to its possible carcinogenicity, Vet. World, vol. 5: 24-30.

Hayat, M. A. (1989) Principles and techniques of electron microscopy, Florida: CRC Press, Inc.

Henry, R. J. (1974) Clinical chemistry principles and technics, New York: Harper and Row.

Hussein, W. F., Farahat, F. Y., Abass, M. A. and Shehata, A. S. (2011) Hepatotoxic potential of gibberellic acid (ga3) in adult male albino rats, Life Science Journal, vol. 8: 373-379.

Joshi, S., Tibrewal, P., Sharma, A. and Sharma, P. (2012) Evaluation of toxic effect of 2,4-D (2,4-Dichlorophenoxyacetic acid) on fertility and biochemical parameters of male reproductive system of albino rats, International Journal of Pharmacy and Pharmaceutical Sciences, vol. 4: 338-342.

Kakkar, R., Kalra, J., Mantha, S. V. and Prasad, K. (1995) Lipid peroxidation and activity of antioxidant enzymes in diabetic rats, Mol. Cell Biochem., vol. 151: 113-119.

Kalender, S., Ogutcu, A., Uzunhisarcikli, M., Acikgoz, F., Durak, D., Ulusoy, Y. and Kalender, Y. (2005) Diazinon-induced hepatotoxicity and protective effect of vitamin e on some biochemical indices and ultrastructural changes, Toxicology, vol. 211: 197–206.

Karthikeyan, K., Sarala Bai, B. R. and Niranjali, S. (2007) Cardioproctective effect of grape seed proanthocyanidins on isoproterenol- induced myocardial injury in rats, Int. J. Cardiol., vol. 115: 326–333.

Kato, A., Minoshima, Y., Yamamoto, J., Adachi, I., Watson, A. and Nash, R. (2008) Protective effects of dietary Chamomile tea on diabetic complications, J. Agric. Food Chem., vol. 56: 8206–8211.

Kogevinas, M. (1995) Soft tissue sarcoma and non-hodgkin's lymponmain workers exposed to phenoxy-herbicides, chlorophenols, and dioxins-2 nested case studies, Epidemiol., vol. 6: 396–402.

Krahenbhul, S. (2001) Mitochondria: Important target for drug toxicity, J. Hepatol., vol. 34: 334-336.

Kumar, J., Sourabh, R., Singh, K., Pranjali, J. and Nagori, P. (2012) Investigation of herbal extract as hepatoprotective, Res. J. Pharmaceutical Sci., vol. 1: 16-18.

Kumar, V., Abbas, A. K. and Fausto, N. (2005) Robbens and catron pathologic basis of disease, London: Elsevier Saunders.

Kuzu, N., Metin, K., Ferda Dagli, A., Akdemir, F., Orhan, C., Yalniz, M., Hanifi Ozercan, I., Sahin, K. and Halil Bahcecioglu, I. (2007) Protective role of genistein in acute liver damage induced by carbon tetrachloride, Mediators Inflamm., vol. 6: 1–6.

Li, C., Grillo, M. P. and Benet, L. Z. (2003) In vitro studies on the chemical reactivity of 2, 4-Dichlorophenoxyacetyl-s-acyl-coa thioester, Toxicol. Appl. Pharmacol., vol. 187: 101-109.

Maire, M., Rast, C., Landkocz, Y. and Vasseur, P. (2007) 2, 4-Dichlorophenoxyacetic acid: Effect on syrian hamster embrio (she) cell transformation, c-myc expression, DNA damage and apoptosis, Genetic Toxicology and Environmental Mutagenesis, vol. 631: 124-136.

Mazhar, F. M., Moawad, K. M., El-Dakdoky, M. H. and Amer, A. S. (2012) Fetotoxicity of 2, 4-Dichlorophenoxyacetic acid in rats and the protective role of vitamin E, Toxicol. Ind. Health, vol. 28: 273-280.

McGee, J. D. (1992) Alcoholic liver disease. In: Oxford textbook of pathology, Edited by: J.D. McGee, P.J. Isaacson and N.A.Wright, Oxford: Oxford University Press.

Merlin, N. J. and Parthasarathy, V. (2011) Antioxidant and hepatoprotective activity of chloroform and ethanol extracts of gmelina asiatica aerial parts, Journal of Medicinal Plants Research, vol. 5: 533-538.

Mignotte, B. and Vayssiere, J. L. (1998) Mitochondria and apoptosis, Eur. J. Biochem., vol. 252: 1-15.

Morgulis, M. S., Oliveira, G. H., Dagli, M. L. and Palermo-neto, J. (1998) Acute 2,4-Dichlorophenoxyacetic acid intoxication in broiler chicks, Poultry Science, vol. 77: 509–515.

Mountassif, D., Kabine, M., Mounchid, K., Mounaji, K., Latruffe, N. and El Kebbaj, M. S. (2007) Biochemical and histological alterations of cellular metabolism from jerboa (jaculus orientalis) by 2, 4 dichlorophenoxyacetic acid: Effects on d-3 hydroxybutyrate dehydrogenase, Pesticide Biochemistry and Physiology, vol. 10: 1-28.

Nakbi, A., Tayeb, W., Dabbou, S., Issaoui, M., Grissa, A., Attia, N. and Hammami, M. (2010a) Dietary olive oil effect on antioxidant status and fatty acid profile in the erythrocyte of 2,4-D exposed rats, Lipids in Health and Disease, vol. 9: 89-99.

Nakbi, A., Tayeb, W., Grissa, A., Issaoui, M., Dabbou, S., Chargui, I., Ellouz, M., Miled, A. and Hammami, M. (2010b) Effects of olive oil and its fractions on oxidative stress and the liver's fatty acid composition in 2,4 dichlorophenoxyacetic acid-treated rats, Nutrition & Metabolism, vol. 7: 80-89.

Nkozi, C. Z., Opoku, A. R. and Terblanche, S. E. (2005) Effect of pumpkin seed (cucurbita pepo) protein isolate on the activity levels of certain plasma enzymes in ccl4 induced liver injury in low protein fed rats, Phytother. Res., vol. 19: 341–345.

Pessayre, D., Berson, A., Fromrnty, B. and Mansouri, A. (2001) Mitochondria in steatohepatitis, Semin. Liver Dis., vol. 21: 57-69.

Ramadan, K. S. and Emam, M. A. (2012) Biochemical evaluation of antihyperglycemic and antioxidative effects of matriceria chamomilla leave extract studied in streptozotocin-induced diabitic rats, International Journal of Research in Management & Technology, vol. 2: 2249-2263.

Raseir, G., Toppari, J., Parent, A. and Bourguignor, J. (2006) Female sexual maturation and reproduction after prepubertal exposure to estrogens and endocrine disrupting chemicals: A review of rodent and human data, Mol. Cell Endocrinol., vol. 254: 187–201.

Rosso, S. B., Ca´ceres, O., Evangelista de Duffard, A. M., Duffard, R. and Quiroga, S. (2000) 2, 4-Dichlorophenoxyacetic acid disrupts thecytoskeleton and disorganizes the golgiapparatus of cultured neurons, Toxicol. Sci., vol. 56: 133-140.

Salama, R. H. (2012) Matricaria chamomilla attenuates cisplatin nephrotoxicity, Saudi J. Kidney Dis. Transpl., vol. 23: 765-772.

Sandstrom, j., Nilsson, P. and Karlsson, K. (1994) 10-Fold increase in human plasma extracellular superoxide dismutase content caused by a mutation in heparine-binding domain, J. Boil. Chem., vol. 269(29):19163-19166.

Singh, O., Khanam, Z., Misra, N. and Srivastava, M. (2011) Chamomile (matricaria chamomilla l.): An overview, Phcog. Rev., vol. 5: 82-95.

Srivastava, J. and Gupta, S. (2007) Antiproliferative and apoptotic effects of Chamomile extract in various human cancer cells, J. Agric. Food Chem., vol. 55: 9470–9478.

Sulik, M., Kisielewski, W., Szynaka, B., Kemona, A., Sulkowska, M., Sulik, A. and Baltaziak, M. (1998) Ultrastructural changes in rat hepatocytes in acute intoxication with 2, 4-Dichlorophenoxyacetic acid (2, 4-D), Rocz. Akad. Med. Bialymst., vol. 43: 314-326.

Tayeb, W., Nakbi, A., Trabelsi, M., Attia, N., Miled, A. and Hammamia, M. (2010) Hepatotoxicity induced by sub-acute exposure of rats to 2,4-Dichlorophenoxyacetic acid based herbicide "désormone lourd", Journal of Hazardous Materials, vol. 180: 225-233.

Tietz, N. W. (2006) Textbook of clinical chemistry and molecular diagnostics, Edited by: C.A. Burtis, E.R. Ashwood and D.E. Bruns, CA: Elsevier Saunders.

Timchalk, C. (2004) Comparative inter-species pharmacokinetics of phenoxy acid herbicides and related organic acids: Evidence that the dog is not a relevant species for evaluation human health risk, Crit. Rev. Toxicol., vol. 200: 1–19.

Tripathi, G. and Shukla, S. P. (1990) Malate and lactate dehydrogenases of a freshwater catfish: Impact of endosulfan, Biomed. Environ. Sci., vol. 3: 52-64.

Troudi, A., Amara, I., Samet, A. and Zeghal, N. (2012) Oxidative stress induced by 2, 4-phenoxyacetic acid in liver of female rats and their progeny:Biochemical and histopathological studies, Environmental Toxicology, vol. 27: 137-145.

Troudi, A., Mahjoubi, S. A. and Zegha, l. N. (2009) Hepatotoxicity induced by gibberellic acid in adult rats and their progeny, Exp. Toxicol. Pathol., vol. 18: 127-133.

Tuschl, H. and Schwab, C. (2003) Cytotoxic effect of the herbicide 2, 4-Dichlorophenoxyacetic acid in hepg2 cells, Food and Chemical Toxicilogy, vol. 41: 385-393.

Undeger, U., Institoris, L., Siroki, O., Nehez, M. and Desi, I. (2000) Simultaneous geno- and immunotoxicological investigations for early detection of organophosphate toxicity in rats, Ecotoxicol. Environ. Safe., vol. 45: 43–48.

U.S. Environmental Protection Agency (2002). Toxicity and Exposure Assessment for Children's Health, Washington: U.S. Environmental Protection Agency.

Uyanikgil, Y., Ates, U., Baka, M., Bicer, S., Oztas, E. and Ergen, G. (2009) Immunohistochemical and histopathological evaluation of 2,4-Dichlorophenoxyacetic acid-induced changes in rat kidney cortex, Bull. Environ. Contam. Toxicol., vol. 82: 749–755.

Vander, D. L. and Hunsaker, L. A. (2003) Methylglyoxal metabolism and diabetic complications: Roles of aldose reductase, glyoxalase-i, betaine aldehyde dehydrogenase and 2-oxoaldehyde dehydrogenase, Chem. Biol. Interact., vol. 143: 341–351.

Wang, Y., Tang, H., Nicholson, J., Hylands, P., Sampson, J. and Holmes, E. (2005) Metabonic strategy for the detection of the metabolic effects of Chamomile(matricaria recutita l.) ingestion, J. Agricultural and Food Chem., vol. 53: 191-196.

Yilmaz, H. R. and Yuksel, E. (2005) Effect of 2, 4-Dichlorophenoxyacetic acid on the activities of some metabolic enzymes for generating pyridine nucleotide pool of cells from mouse liver, Toxicol. Ind. Health, vol. 21: 231-237.

Yoo, K., Lee, C., Lee, H., Moon, B. and Lee, C. (2008) Relative antioxidant and cytoprotective activities of common herbs, Food Chemistry, vol. 106: 929–936.

Arabic

Summary

التأثير الوقائي الكبدي لمستخلص الكاموميل على الفئران المصابة بالتسمم الكبدي بـــ٢و٤ داي كلورو فينوكسي استيك اسيد

دلال عبدالعزيز البارودي

الملخص

استهدفت هذه الرسالة دراسة تأثير الإعطاء الفموى لمستخلص أزهار نبات البابونج لمدة ٢٨ يوم على الفئران المصابة بتسمم الكبد المحدث بـــــ٢و٤ داي كلوروفينوكسى حمض الأستيك. وشملت الدراسة تأثير ذلك على كلاً من وزن الجسم، معدل التحويل الغذائى، مستوى إنزيمات الكبد والبروتين الكلى والألبيومين والبيليروبين وانزيم لاكتات ديهيدروجينيز، ومستوى الانزيمات المضادة للأكسدة فى مصل الدم وكذلك الفحص الهستوباثولوجى لأنسجة الكبد.

وقد استخدم فى هذه الدراسة عدد ٣٦ فأر ذكر بالغ ، تم توزيعهم على ست مجموعات وكانت المجموعة الأولى ضابطة سالبة وتم تغذيتها على غذاء قياسي جاهز تم شراؤه من مستودعات حبوب ومطاحن جدة بالمملكة العربية السعودية. وتم إعطاء فئران المجموعتين الثانية والثالثة مبيد الحشائش ٢و٤ داي كلوروفينوكسى حمض الاستيك عن طريق الفم بجرعتين هما ٧٥ و١٥٠ مجم/ كجم من وزن الجسم على التوالى ، لإحداث تسمم كبدى بها . وتم إعطاء فئران المجموعات الرابعة والخامسة والسادسة مستخلص أزهار نبات البابونج بجرعة ٥٠٠ مجم/كجم من وزن الجسم منفرداً ومتحداً مع المبيد ٢و٤ داي كلوروفينوكسى حمض الاستيك بالجرعتين المنخفضة والمرتفعة على التوالى لمدة ٢٨ يوم ، وتم تسجيل أوزان الفئران قبل بدء التجربة وفى نهايتها لمعرفة معدل الزيادة فى وزن الجسم ، وكذلك حساب كمية الغذاء المستهلك يومياً وحساب معدل التحويل الغذائى . وفى نهاية فترة التجربة

تم أخذ عينات من الدم لإجراء التحليلات البيوكيميائية ، وتم حساب الوزن النسبى للكبد ، وكذلك تم أخذ الكبد لإجراء الفحص الهستوباثولوجى بإستخدام المجهر الإلكتروني.

وكانت من أهم النتائج المستخلصة من الدراسة أن كلاً من الجرعة المرتفعة والمنخفضة من المبيد قد أدت إلى تأثيرات سمية متفاوتة تظهر في نقصان ملحوظ في أوزان الفئران وانخفاض المتناول الغذائي وتغيرات ملحوظة في نسيج الكبد وزيادة وزنه. كما لوحظت زيادة في مستويات انزيمات الكبد مع انخفاض في مستويات الألبيومين والبروتين الكلي بالإضافة إلى الإنخفاض في نشاط بعض الإنزيمات المضادة للأكسدة وذلك مقارنة بالمجموعة الضابطة. وقد ظهرت هذه التأثيرات السمية في المجموعة التي تم اعطاؤها المبيد بالجرعة المرتفعة أكثر منها في الجرعة المنخفضة وكذلك أظهرت النتائج أن المجموعات المتسممة بالجرعتين المستخدمتين من المبيد والمعالجة بالمستخلص المائي للبابونج بجرعة ٥٠٠ مجم/كجم من وزن الجسم قد أظهرت تحسن ملحوظ من أعراض التسمم بالمبيد مقارنة بالمجموعات الضابطة لكل من جرعتي المبيد وقد كان التحسن ملحوظ في المجموعة التي تم اعطاؤها المبيد بالجرعة المنخفظة أكثر منه في الجرعة المرتفعة. ولم يلاحظ أي تأثير على المجموعة التي تم اعطاؤها مستخلص البابونج بمفرده.

ويتضح من نتائج هذه الدراسة أن مستخلص أزهار نبات البابونج له تأثير وقائي للكبد ومضاد للأكسدة فى الفئران المصابة بالتسمم الكبدى المحدث بـــــ٢و٤ داي كلوروفينوكسى حمض الاستيك. توصى هذه الدراسة بأن تناول أزهار نبات البابونج كمشروب عشبي قد يكون مفيدا للمرضى الذين يعانون من أمرض الكبد وكذلك فى حالات الإجهاد التأكسدى.

التأثير الوقائي الكبدي لمستخلص الكاموميل على الفئران المصابة بالتسمم الكبدي بـــ٢و٤ داي كلورو فينوكسي استيك اسيد

دلال عبدالعزيز البارودي

بحث مقدم لنيل درجة الماجستير في الإقتصاد المنزلي
(غذاء وتغذية)

تحت إشراف

د .ريهام أبو المحاسن عرفات
د. ثناء عبد الفتاح الخولي

كلية الإقتصاد المنزلي
جامعة الملك عبدالعزيز

جدة- المملكة العربية السعودية

رجب ١٤٣٤هـ - مايو ٢٠١٣م