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Effect of Tubers *Cyperus rotundus* Phenolic Extract and Taurine Compound on Some Biochemical Parameters in Induced Myocardial Infarction in Female Rats

A thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
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List of Abbreviations

abs	Absorbance
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
AIP	Atherogenic index of plasma
ALT	Alanine aminotransferase
Alb	Albumin
RO [•]	Alkoxy radical
α	Alpha
AST	Aspartate aminotransferase
ATI	Atherogenic index
β	Beta
B.W	Body weight
CVD	Cardiovascular disease
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
CAT	Catalase
Cp	Ceruloplasmin
C-18	Column-18
Conc	Concentration
CHD	Coronary heart disease
CK	Creatine kinase
CPK	Creatine phosphokinase
cAMP	Cyclic adenosine monophosphate
<i>C.rotundus</i>	<i>Cyperus rotundus</i>
CM	Cylomicrons
Cys	Cysteine
CSD	Cysteine sulphinat decarboxylase
Da	Dalton

dL	Deciliter
°C	Degree celsius
DNA	Deoxy ribose nucleic acid
DPX	Disteren plasticizer xylene
D.W	Distill water
DTNB	5,5-dithio bis(2-nitro benzoic acid)
ECG	Electrocardiograph
FA	Fatty acid
γ	Gamma
Glu	Glutamic acid
GSH	Glutathione
GSSG	Glutathione disulfide
GOT	Glutamic oxaloacetic transaminase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GST	Glutathione - S - transferase
g	Gram
HDL-C	High density lipoprotein cholesterol
HFCD	High fat cafeteria diet
HPLC	High performance liquid chromatography
H	Hour
HSA	Human serum albumin
•OH	Hydroxyl radical
HOCl	Hypochlorous acid
IP	Intraperitoneally
IDL	Intermediate density lipoprotein
IHD	Ischemic heart disease
ISO	Isoproterenol hydrochloride
KD	Kilodalton
Kg	Kilogram
LDH	Lactate dehydrogenase
LV	Left ventricular
LVH	Left ventricular hypertrophy

LDL-C	Low density lipoprotein cholesterol
LOO •	Lipid peroxy radical
LOOH	Lipid hydro peroxides
L	Liter
MDA	Malondialdehyde
mRNA	Messenger ribose nucleic acid
µg	Microgram
µL	Microliter
µmol	Micromole
mL	Milliliter
mg	Milligram
mm	Millimeter
min	Minute
M	Molarity
MW	Molecular weight
MI	Myocardial infarction
ng	Nanogram
nm	Nanometer
NADPH	Nicotinamide adenine dinucleotide phosphate
NSTEMI	Non ST elevation myocardial infarction
OD	Optical density
ODC	Ornithine decarboxylase
PPD	Para phenylene diamine
RO ₂ •	Peroxy radical
PAL	Phenylalanine ammonia-lyase
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
SD	Standard deviation
STEMI	ST elevation myocardial infarction
O ₂ • ⁻	Superoxide anion
SOD	Superoxide dismutase
TBA	Thio barbituric acid
TCA	Tri chloroacetic acid

TG	Triglycerides
TnC	Troponin C
TnI	Troponin I
TnT	Troponin T
UV	Ultra-Violate
VLDL	Very low density lipoprotein cholesterol
WHO	World Health Organization

Summary

In the present investigation, an attempt has been made to examine the effect of phenolic extract of tubers of *Cyperus rotundus* and taurine compound against isoproterenol – induced myocardial infarction in female rats.

The work involved the following studies :

Analytical study: It involved extraction and identification of phenolic content of tubers of *Cyperus rotundus*. The UV –Vis. spectra and high performance liquid chromatography of phenolic extract are proved the presence of (Tannic acid, Rutin, p- Coumaric acid, Campherol, Catechin, Gallic acid) in the extract .

Biochemical study: Myocardial infarction was induced by injected of isoproterenol hydrochloride (85 mg/kg) to rats at an interval of 24 h for two days. We used (60) of the female rats (*Rattus norvegicus*) which were divided into 10 groups, each group contain of 6 rats. Group(1) received distill water as control group, group(2): ISO group; animals were injected I.P. with (85 mg/kg) of isoproterenol hydrochloride (ISO), twice an interval of 24 h, i.e., on 22th and 23th day), the three and four groups were administrated orally with (15 and 30mg/kg) phenolic extract of tubers of *Cyperus rotundus* respectively once daily for 21 days, the fifth and sixth groups were injected (100 and 200 mg/kg) of taurine respectively once daily for 21 day, the seventh and eighth groups were pretreated with phenolic extract of tubers of *Cyperus rotundus* orally (15 and 30mg/kg) respectively, once daily for 21day, then injected I.P. with

(85mg/kg) ISO, twice an interval of 24 h , i. e ., on 22th and 23th day, the ninth and tenth groups were injected with taurine (100 and 200 mg/kg) respectively, once daily for 21 day, then injected I.P. with (85mg/kg) of ISO, twice an interval of 24 h, i. e., on 22th and 23th day.

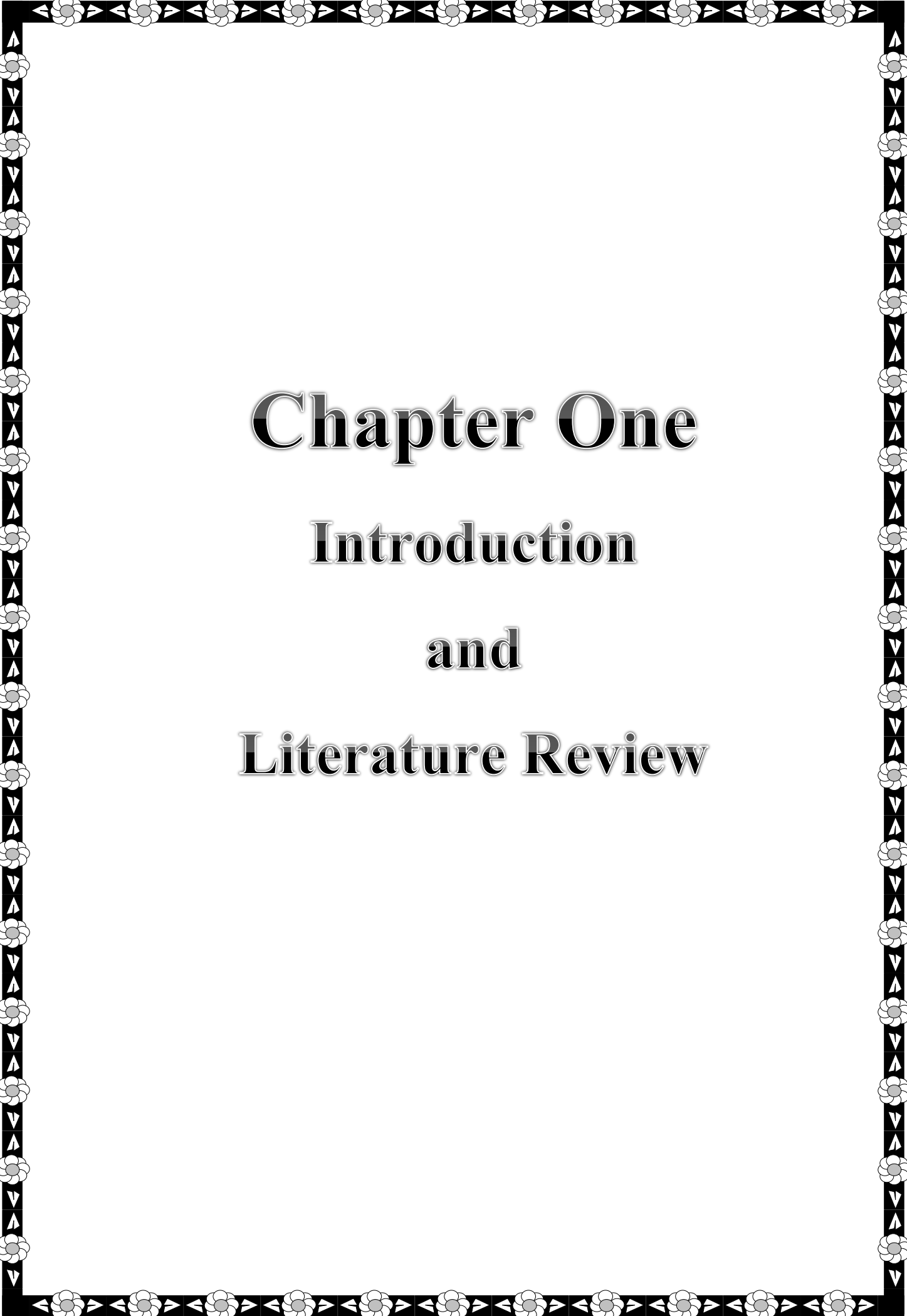
The results showed a significant increase ($p \leq 0.05$) in the concentration of serum (Troponin- I, LDH, CPK, ALT, AST, MDA, Cp, uric acid, TC, TG, VLDL, LDL and atherogenic index) in group (2) in comparison with group (1), treatment with phenolic extract of tubers of *Cyperus rotundus* at dose (15, 30mg /kg) for 21 days, caused a significant decrease ($p \leq 0.05$) in the concentration of serum (Troponin-I, LDH, CPK, ALT, AST, MDA, Cp, uric acid, TC, TG, VLDL, LDL and atherogenic index) in groups (7 and 8). Treatment with taurine at dose (100,200 mg /kg) for 21 days, caused a significant decrease ($p \leq 0.05$) in the concentration of serum (Troponin-I, LDH, CPK, ALT, AST, MDA, Cp, uric acid, TC, TG, VLDL, LDL and atherogenic index) in groups (9 and 10) in comparison with group (1). Results indicated a significant increase ($p \leq 0.05$) in the concentration of tissue MDA in group (2) in comparison with group (1), treatment with phenolic extract of tubers of *Cyperus rotundus* at dose (15, 30mg /kg) for 21 days, caused a significant decrease ($p \leq 0.05$) in the concentration of tissue MDA in groups (7 and 8) in comparison with group (2). There was a significant decrease tissue MDA ($p \leq 0.05$) in groups (9 and 10) in comparison with group (2). There was a significant decrease ($p \leq 0.05$) in the concentration of serum Alb and GSH in group (2) in comparison with group (1), treatment with phenolic extract of tubers of *Cyperus rotundus* at dose (15, 30mg /kg) for 21 days, caused a significant increase ($p \leq 0.05$) in the concentration of serum Alb and GSH in groups (7 and 8) in comparison with group (2).

Also, there was a significant increase ($p \leq 0.05$) in the concentration of serum Alb and GSH in groups (9 and 10) in comparison with group (2).

Results indicated a significant decrease ($p \leq 0.05$) in the concentration of tissue GSH in group (2) in comparison with group (1), treatment with phenolic extract of tubers of *Cyperus rotundus* at dose (15, 30mg /kg) for 21 days, caused a significant decrease ($p \leq 0.05$) in the concentration of tissue GSH in groups (7 and 8) in comparison with group (2). GSH decreased significantly ($p \leq 0.05$) in groups (9 and 10) in comparison with group (2).

Histopathological Study: Histopathological studies showed that animals' treatment with ISO caused heart damage including severe necrosis, which was also accompanied by congestion and accumulation of inflammatory cells. In contrast, cardiomyocytes necrosis and congestion appeared less marked in phenolic extract and taurine treated rat. Degenerative changes in various degrees were observed in microscopic examination of heart in animal group treated with ISO to induced MI and heart damage. In this group, showed a large ventricle area of degenerated, vacuolated myocardial cell associated with infiltration of mononuclear cell and myocardial necrosis which were reduced by the treatment of phenolic extract of tubers of *Cyperus rotundus* and treatment with taurine. The results that consumption of phenolic extract of tubers of *Cyperus rotundus* and taurine could reduce the risk of cardiovascular diseases.

The final conclusion is that the phenolic extract of tubers of *Cyperus rotundus* and taurine have a cardioprotective effect against ISO-induced MI in rats.



Chapter One

Introduction

and

Literature Review

1-Introduction and Literature Review

1-1- Introduction :

Plants are the oldest friends of mankind. They not only provided food and shelter but also served the humanity to cure different ailments, according to the world health organization (WHO, 2001), about three-quarters of the world population relies upon traditional remedies (mainly herbs) for the health care of its people (Calixto, 2005). Historians from all around the world have produced evidence to show that apparently all primitive peoples used herbs and often in a sophisticated way.

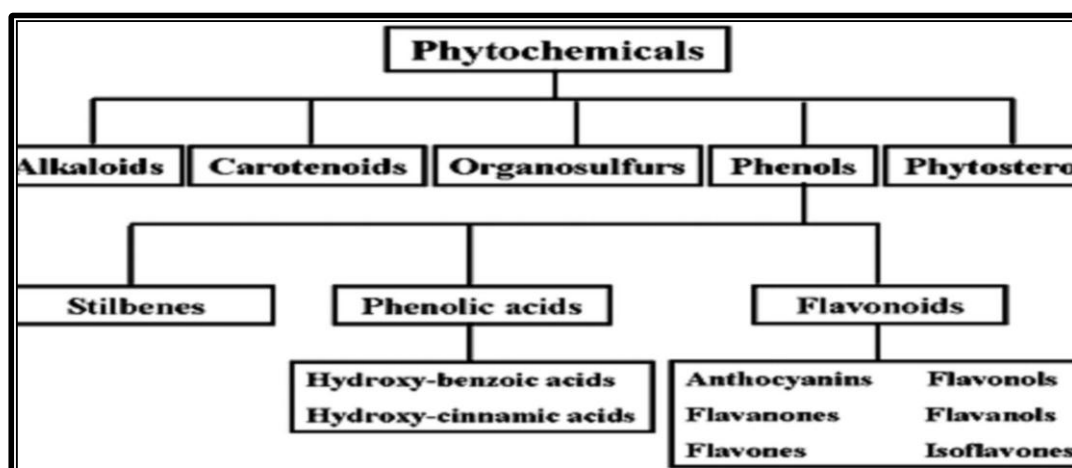
The world health organization (WHO, 2001) defined the medicinal plant as any herbal preparation produced by subjecting plant material to extraction, fractionation, purification, concentration or other physical or biological process which may be produced for immediate consumption or as a basis for herbal product. The term "herbal drug" determines the part/parts of a plant used for preparing medicine (for example: leaves, flowers, seeds, roots, barks, stem....etc). Studies on the use of plant extracts for controlling disease have shown the importance of natural chemicals (phytochemicals) as possible sources of non-phytotoxic and easily biodegradable, alternative fungicides and antibiotics (Okigbo *et al.*, 2009).

Several plant extracts with potential therapeutic properties for the treatment of hypertension and complications such as coronary heart disease, angina, arrhythmias and congestive heart failure have been identified (Somova *et al.*, 2003; Ghasemzadeh and Ghasemzadeh, 2011). Plant parts used included leaves, flowers, stems, roots and seeds (Said *et al.*, 2008; Said *et al.*, 2008).

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Fruits and vegetables contain ascorbic acid, carotenoids, tocopherols and phenolic compounds showed antioxidant properties (Prior, 2003; Rohrmann *et al.*, 2007).

The (phyto) of the word phytochemicals is derived from the Greek word phyto, which means plant. Therefore, phytochemicals are plant chemicals (figure 1-1). Which are produced by the plants in self-defence to protect them from pest, microbes and environmental stress factors. In past, plants and herbs have been reported to contain different phytochemical compounds with a wide range of activities, which may help in the development of new drug (Menichini *et al.*, 2009; Lei *et al.*, 2011).



Figure(1-1) : Classification of phytochemicals (Menichini *et al.*, 2009).

In fact, many phytochemicals, such as polyphenols are rapidly degraded and metabolized in the human body. Further more, genetic variation in pathways affect absorption, metabolism and distribution of these natural substances (Manach *et al.*, 2009). More than 10,000 phytochemicals have been described (Maria *et al.*, 2010). Phytochemicals are plants-derived chemical compounds, which are non-essential nutrients, some of which show health promoting properties (Dharmesh *et al.*, 2010), namely primary constituents which includes amino acids, sugar, protein and chlorophyll etc and secondary constituents

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consists of alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins and phenolic compounds (Alinmoladun *et al.*, 2007). Majority of phytochemicals have been known to have valuable treatment activities like insecticidal, antifungal, antibacterial (Edeogo *et al.*, 2005) and antioxidant activities (Krishnaiah *et al.*, 2007).

1-2- Plant Secondary Metabolites :

Secondary metabolites are more limited in the plant kingdom and mostly accumulated by plant cells in smaller quantities than primary metabolites and synthesized in specialized cells at particular development stages making their extraction and purification difficult (Roze *et al.*, 2011).

These secondary metabolite or products exert in general a profound physiological effect on the mammalian system and, thus are known as active principles of plant (Pussa *et al.*, 2006).

The classifications of these compounds are different, some scientist classified it according to the chemical structure of these compounds and others classified it according to the source of metabolism process. The most common compounds are :- (Hartmann, 1996).

- **Phenolic Compounds.**
- **Alkaloids.**
- **Volatile oil**
- **Terpenoid Compounds.**
- **Resins**
- **Saponins**

1-2-1-Phenolic Compounds:

Phenolic compounds possess an aromatic ring bearing one or more hydroxyle substituents (Scalbert *et al.*, 2005). Phenolic compounds are found in almost every plant-derived food, fruits and vegetables contain several thousand structurally diverse phytochemicals, of which a large fraction are polyphenols

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(Balasundrama *et al.*, 2006). The beneficial effects of polyphenols are mainly attributed to their antioxidant properties (Miller and Ruiz-Larrea, 2002; Young-Joon, 2003; Pan and Ho, 2008). Due to their antioxidant activity,

Polyphenol compounds and fruit extracts have been reported to have positive effects on cancer, cardiovascular disease, immune disorders, microbial infections, neurodegenerative diseases and viral infections (Le-Marchand *et al.*, 2000; Xu *et al.*, 2000). Polyphenols occupies a unique place in science and the only class of biologically active natural products that result of their presence in the food and beverages derived from plants and inserted into the formulas of well-marketed cosmetics (Banas and Vikerman, 2003; Arakawa *et al.*, 2004) parapharmaceutical products (Banas and Vikerman, 2003). Polyphenols exhibit multiple pharmacological properties such as anti- microbial, anti-allergenic and anti- inflammatory activities (Del Rio *et al.*, 2013). The bioavailability and biological properties of dietary polyphenols vary to a great extent and depend on their chemical structure (Manach *et al.*, 2005; Loke *et al.*, 2008). They include simple compounds, such as phenolic acids and also more complex molecules such as condensed tannins (figure1-2) (Cheynier, 2005).

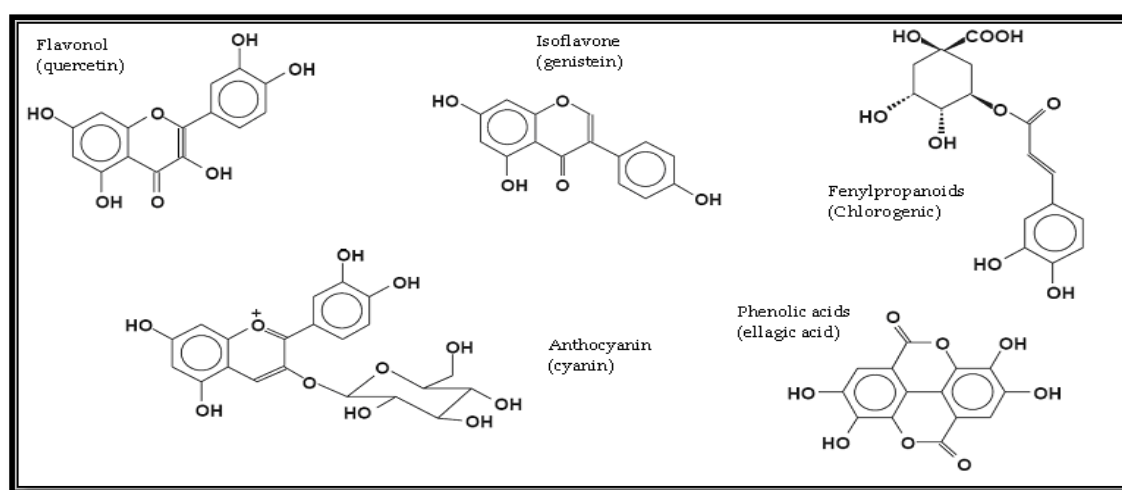


Figure (1-2): Families and examples of polyphenols (Cheynier, 2005).

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The biosynthetic pathways of phenolic compounds in plants are quite well known (Kondratyuk and Pezzuto, 2004). Phenolic compounds are generally synthesised via the shikimate pathway, which starts with the formation of phenylalanine and tyrosine. In (figure 1-3), phenylalanine is deaminated to form cinnamic acid in a reaction catalysed by phenylalanine ammonia-lyase (PAL), the key enzyme in phenolic biosynthesis (Tomás-Barberán and Espín, 2001; Chen *et al.*, 2009). Hydroxycinnamoyl and *p*-coumaroyl coenzyme A esters are the common structural precursors of various phenolic classes. The final products of the flavonoid branch are anthocyanins, starting with the formation of chalcones and with the rest of flavonoids being formed from intermediates of anthocyanin biosynthesis (Schijlen *et al.*, 2004).

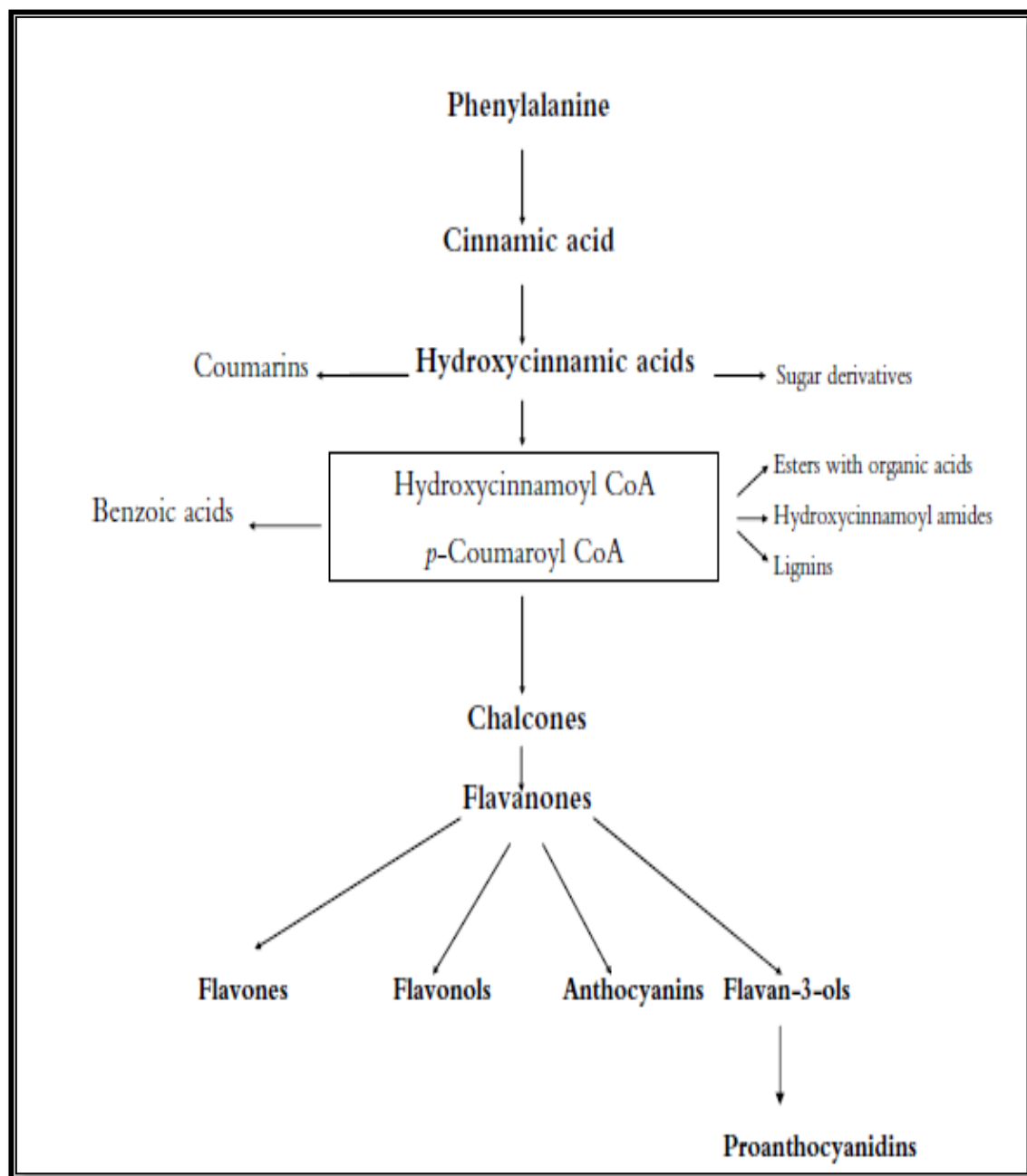


Figure (1-3) : Phenolic compounds biosynthesis (Macheix *et al.*, 1990).

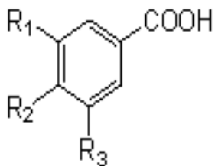
1-2-1-1- Phenolic acids :

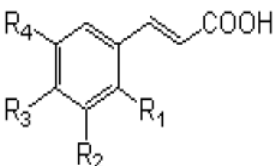
Phenolic acids are one group of aromatic secondary plant metabolites broadly distributed throughout the plant kingdom (Yordi *et al.*, 2012). Interest in phenolic acids stems from their importance as protective factors against cancer and heart diseases and this is due to their properties as antioxidant

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(Chandrasekara and Shahidi, 2010). The antioxidant activity of phenolic acid depends on the numbers and positions of the hydroxyl groups in the molecule (Musonda and Chipman, 1998). As a result, the antioxidant activity of phenolic acids increases the higher the degree of hydroxylation (Naczki and Shahidi, 2006). They are derivatives of either hydroxycinnamic acid or hydroxybenzoic acid and can be divided into two groups (Saulnier *et al.*, 2007): those with hydroxybenzoic acid have a general structure C1-C6 (Macheix *et al.*, 1990) derivatives including p-hydroxybenzoic, protocatechuic, vanillic, syringic, gallic acids and hydroxycinnamic acid derivatives including p-coumaric, caffeic, ferulic and sinapic acids (Adom and Liu, 2002).

The different phenolic acids (figure 1-4) differ in the number and position of the hydroxyl and methoxyl groups attached to the aromatic ring (Macheix *et al.*, 1990; Robbins, 2003)





Benzoic acids

Acids	R1	R2	R3
Gallic	OH	OH	OH
Vanillic	H	OH	OCH3
Syringic	OCH3	OH	OCH3

Cinnamic acids

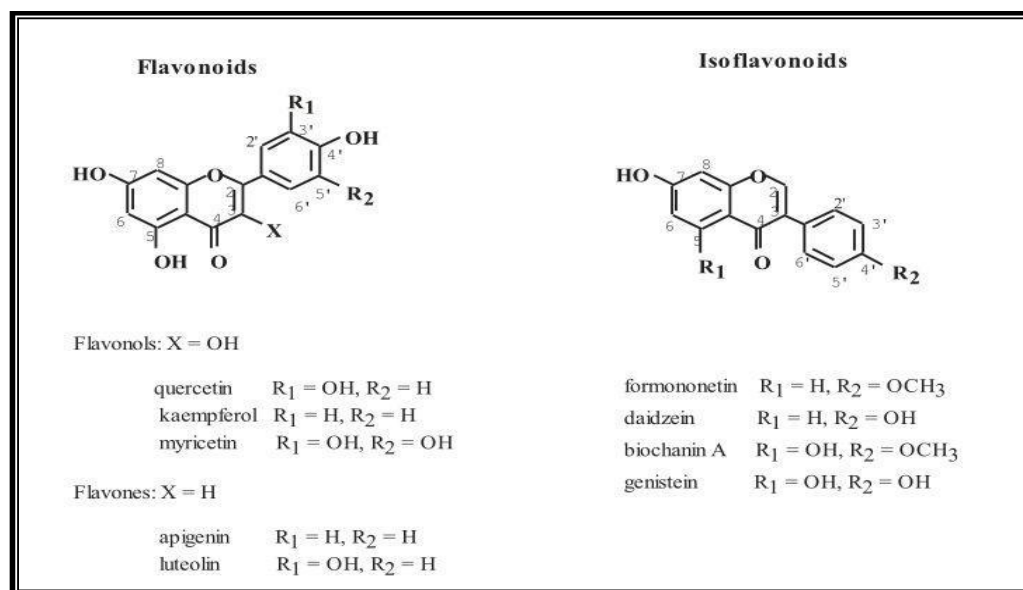
Acid	R1	R2	R3	R4
Ferulic	H	H	OH	OCH3
p-coumaric	H	H	OH	H
Caffeic	H	H	OH	OH
Sinapic	H	OCH3	OH	OCH3

Figure (1-4): Basic structure of benzoic and cinnamic acid derivatives
(Macheix *et al.*, 1990; Robbins, 2003).

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1-2-1-2- Flavonoids:

Flavonoids are low molecular weight secondary metabolites that are produced by plants, and generally are described as non-essential for plant survival, unlike primary metabolites. Secondary products are biologically active in many ways, and over 10,000 structural variants of flavonoids have been reported (Williams and Grayer, 2004; Ferrer *et al.*, 2008). Flavonols, flavones and isoflavones constitute three major subclasses of flavonoids. Flavonols and flavones have similar C-ring structure with a double bond at the 2-3 position. Flavones, as opposed to flavonols, lack a hydroxyl group at the 3-position (Miller, 1996). Major flavonols are quercetin (3,5,7,3',4'-pentahydroxyflavone), kaempferol (3,5,7,4'-tetrahydroxyflavone) and myricetin (3,5,7,3',4',5'-hexahydroxyflavone). The most abundant flavones in plants are luteolin (5,7,3',4'-tetrahydroxyflavone) and apigenin (5,7,4'-trihydroxyflavone). B-ring of isoflavonoid is connected to 3-C (Silva *et al.*, 2002). Most frequently occurred isoflavonoids are isoflavones with a double bond between 2- and 3-C. Major isoflavones are genistein (5,7,4'-trihydroxyisoflavone), daidzein (7,4'-dihydroxyisoflavone), formononetin (7-hydroxy-4'-methoxyisoflavone) and biochanin A (5,7-dihydroxy-4'-methoxyisoflavone) all these structures are illustrated in (figure 1-5). (Andrea *et al.*, 2003).



Figure(1-5): Chemical structure of flavonoids and isoflavonoids
(Andrea *et al.*, 2003).

1-3- Botanical Classification of *Cyperus Rotundus* :

Cyperus rotundus is botanically classified as the following : (Townsend and Guest, 1985).

Kingdom : Plantae Plants

Subkingdom : Tracheobionta Vascular plants

Superdivision: Spermatophyta Seed plants

Division: Magnoliophyta Flowering plants

Class: Liliopsida Monocotyledons

Subclass: Commelinidae

Order: Poales (Cyperales)

Family: Cyperaceae

Genus: *Cyperus*

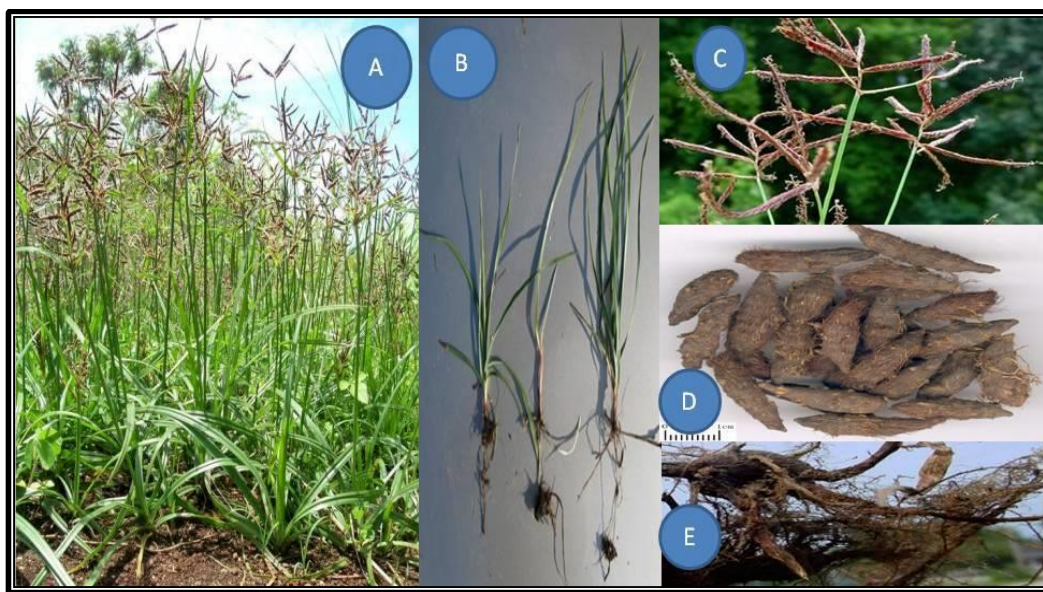
Species : *rotundus*

Cyperus genus belongs to the Sedge family (Cyperaceae) of large families in the plant kingdom and its economic importance as well as the importance of medical (Townsend and Guest, 1985).

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Cyperus rotundus is a highly variable perennial sedge. Flowering stems are erect, up to 60 cm tall, 3-sided, smooth with swollen bases (basal bulbs). The leaves which have a distinct midrib are linear usually shorter than the flowering stem, up to 7 mm wide and emerge from a sheath around the shoot base. The inflorescence is a terminal, open umbel subtended by several leafy bract, 2-6 cm long, support 3-8 reddish-brown to purplish-brown, spikelets are up to 3.5 cm long and 2 mm wide (Allan *et al.*, 1978).

Rhizomes are wiry, dark and persistent, connecting a network of daughter shoots and tubers. The tubers are dark brown to black, irregularly shaped and 1-2 cm long when fully grown. Each tuber has an apical bud and several lateral buds (Meena *et al.*, 2010). (Picture 1-1).



Picture (1-1): Phenology of *Cyperus rotundus* L: **A.** Natural cultivated in agricultural land; **B.** Uprooted plant; **C.** *cyperus rotundus* flower spikes; **D.** *cyperus rotundus* seeds and **E.** *cyperus rotundus* roots.

Scientists have reported that rhizomes and tubers of *cyperus rotundus* possess antidiarrheal, antioxidant, anti-inflammatory, antipyretic, antifungal, antidiabetic, antimalarial, antilipidemic, antibacterial, antiviral, anti-tumoral, cardioprotective, and wound-healing properties (Kilani *et al.*, 2008a; Sundaram *et al.*, 2008; Neffatti *et al.*, 2008; Shivakumar *et al.*, 2009; Dang *et al.*, 2011).

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The leaves and seeds of *Cyperus rotundus* are rich sources of volatile oils with efficient bactericidal and fungicidal compounds that improve the digestive system, stimulate appetite, and relieve irritation (Nima *et al.*, 2008). Tannins, alkaloids, essential oils (α -longipinane, β -selinene, cyperene and caryophyllene oxide) and flavonoids (anthocyanidins, catechins, flavans, flavones, flavanonols and isoflavane) are the active ingredients responsible for the biological and therapeutic properties of *C. rotundus*, and they are used in several medicines (El-Kaream, 2012; Ghannadi *et al.*, 2012).

1-3-1- Chemical Constituent :

Phytochemical surveys revealed that the plant contained flavonoids, tannins, glycosides, monoterpenes, sesquiterpenes, sitosterol, alkaloids, saponins, terpenoids, essential oils, starch, carbohydrates, protein and amino acids (Chandratre *et al.*, 2011; Sivapalan and Jeyadevan, 2012).

Cyperus rotundus contained many secondary metabolites such as sesquiterpenes (with diverse skeletons such as patchoulane, rotundane, eudesmane, guaiane, cadinane and caryophyllene types), quinones, flavonoids (visnagin, khellin, ammiol, isorhamnetin, and tricetin), saponins, alkaloids, phenolic acids (salicylic acid, protocatechuic acid, caffeic acid and p coumaric acid), coumarins and steroids (steroidal glycoside, sitosteryl-(6'-hentriacontanoyl)- β -D-galactopyranoside) (Huang, 1999).

Cyperus rotundus L rhizomes is characterized by perfumery smell attributable to volatile oils that make up the (0.5-0.9%) and contains 27 compound monoterpene, sesquiterpene. Sesquiterpene epoxides, and sesquiterpene ketones. Studies have shown the existence of compounds which belonging to a group sesquiterpenoids for example copadiene ($C_{15}H_{22}$) (Duka, 1992).

Four chemotypes (H-, K-, M- O), of essential oils rhizome from different parts of world have been reported (Kalani *et al.*, 2008a) The H-type from Japan

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was found to contain α -cyperone (36.6%), β -selinene (18.5%), cyperol (7.4%) and caryophyllene (6.2%) (Kilani *et al.*, 2008a).

The M-type from China, Hong Kong, Japan, Taiwan and Vietnam had α -cyperone (30.7%), cyperotundone (19.4%), β -selinene (17.8%), cyperene (7.2%) and cyperol (5.6%) (Kalani *et al.*, 2008b).

The O-type from Japan, Taiwan, Thailand, Hawaii and the Philippines was characterized by cyperene (30.8%), cyperotundone (13.1%) and β -elemene (5.2%). In addition, the Hawaiian O-type had cyperotundone (25.0%) and cyperene (20.7%) as the major compounds. Finally, the K-type, also from Hawaii, was dominated by cyperene (28.7%), cyperotundone (8.8%), patchoulenyl acetate (8.0%) and sugeonyl acetate (6.9%) (Komai and Tang, 1989).

1-3-2- Biological Activities:

Cyperus rotundus has a vibrant multi-events because it contains antioxidant compounds and anti-carcinogenesis, and they are able to protect the liver and other organs from the impact of chemicals that exposed them. This plant has a great benefit to human beings because of the characteristics that it owns. These characteristics are follows : pharmacological activities of *Cyperus rotundus* linn (Singh *et al.*, 2012). (figure 1-6).

1-3-2-1- Antioxidant Activity:

A combination of spices (*Piper nigrum*, *Piper longum* and *Zingiber officinale*), herbs (*Cyperus rotundus* Linn. and *Plumbago zeylanica*) and salts make up Amrita Bindu. The study was focused on evaluating the antioxidant property of individual ingredients in Amrita Bindu against the free radical 2,2'-azino-bis-(3-ethylbenzothiazoline -6- sulphonic acid (ABTS) .The analysis revealed the antioxidant potential of the ingredients in the following order:

Piper nigrum > *Piper longum* > *Cyperus rotundus* > *Plumbago zeylanica* > *Zingiber officinale*. These results reveal that Amrita Bindu, a salt-spice-herbal mixture containing *C. rotundus* Linn. exerts a promising

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antioxidant potential against free radical induced oxidative damage (Natarajan and Paulsen, 2000).

1-3-2-2-Anti Inflammatory Activity:

The alcoholic extract (70%) possessed anti inflammatory activity against carrageenan induced oedema and also found it effective against formaldehyde induced arthritis in albino rats (Sundaram *et al.*, 2008). Its anti-inflammatory relative effect was higher than that of hydrocortisone (75.9% versus 47.3% in carrageenan-induced oedema model; 55.1% versus 35.6% in formaldehyde induced arthritis model (Singh *et al.*, 1970, Singh, and Gilca, 2010).

In another study the petroleum ether extract of the rhizomes showed anti-inflammatory activity against carrageenan induced oedema in albino rats (Seo *et al.*, 2001). The triterpenoid obtained by chromatographic separation from petroleum ether extract revealed a high potent anti-inflammatory activity. *C.rotundus* has also reported as protective in inflammatory bowel disease (AL-Hilli, 2009).

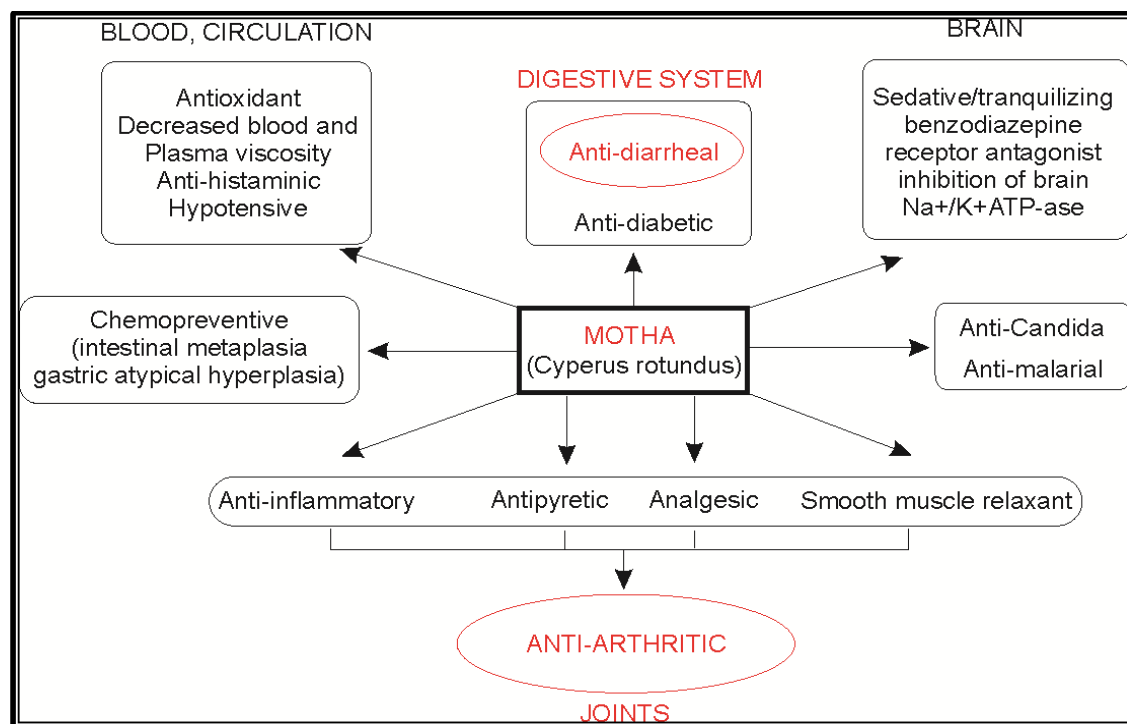


Figure (1 -6) :Pharmacological activities of *Cyperus rotundus* linn

(Singh *et al.*, 2012).

1-3-2-3- Antilipidemic and Cardioprotective Activity:

Atherosclerosis resulting from hyperlipidemia, leads to blockage of coronary arteries, interrupted blood circulation, cardiac ischemia, and myocardial infarction (Manimegalai and Venkatalakshmi, 2012). Atherosclerosis is caused by low-density lipoproteins in the oxidized form, which may result in a heart attack (Mansour *et al.*, 2009). Jahan *et al.* (2012) first conducted a study on isoproterenol (ISO), a synthetic catecholamine, to identify the hypolipidemic potential of *C. rotundus*. They stated that a 200 mg/kg b.w. extract of *C. rotundus* can potentially decrease the level of ISO-induced cytosolic enzymes and maintain the cardio-membrane by restoring the level of antioxidant enzymes in heart tissues. At high concentrations, ISO can disturb cardiac muscles, leading to oxidative stress-induced cardiotoxicity (Upaganlawar *et al.*, 2011). In turn, cytosolic enzymes are released, leading to cell necrosis, contractile failure, and finally myocardial infarction.

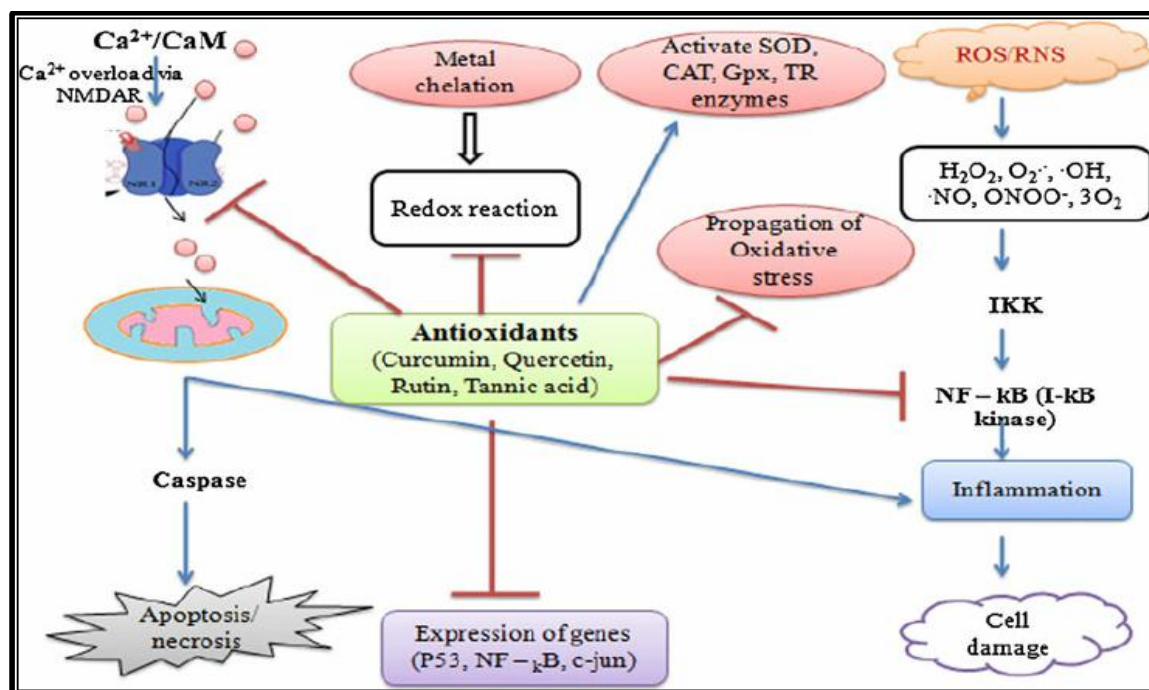
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The organ weight (liver, kidney, and spleen), serum triglyceride level, and the total cholesterol level in obese rats can be significantly reduced by orally administering 300 mg/kg of aqueous tuber extract of *C. rotundus* daily for 40 days along with a high-fat cafeteria diet (HFCD). A new herbal supplement containing the tuber extract of *C. rotundus* was suggested for controlling obesity (Athesh *et al.*, 2014).

1-4 -The Antioxidant Activity of Phenolic Compounds :

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Aswatha *et al.*, 2008). The antioxidative effect is mainly due to phenolic components, such as flavonoids and phenolic acids, (Prior, 2003). Many of these phytochemicals possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of cancer in several human populations (Aswatha *et al.*, 2008).

Polyphenols clearly improve the status of different oxidative stress biomarkers (Manach *et al.*, 2005). Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction (Sies, 1992). Polyphenols together with other phenolic structures of plant origin have been reported as scavengers of reactive oxygen species (ROS) and are seen as promising therapeutic drugs for free radical pathologies (Khasawneh *et al.*, 2011; Boligon *et al.*, 2012). Some studies suggests that the biological actions of these compounds are related to their antioxidant activity (Koleva *et al.*, 2002; Pourmorad *et al.*, 2006). (Figure1-7)



Figure(1-7): Mechanisms of antioxidants defenses against free-radical – induced damage in humans (Aseervatham *et al.*, 2013).

1-5- Phenolic Compounds and Cardiovascular Disease:

A number of studies has demonstrated that consumption of polyphenols limits the incidence of coronary heart diseases (Pandy and Rizvi, 2009). Plant-derived foods and beverages are rich in phenolic compounds which show protection properties against cancer, cardiovascular disease and aging (Piotr *et al.*, 2012).

Polyphenols are potent inhibitors of LDL oxidation and this type of oxidation is considered to be a key mechanism in development of atherosclerosis (Aviram *et al.*, 2000). Both human and animal in vivo studies have shown that the level at which LDL oxidizes, decrease with increasing phenolic concentration (Weinbrenner *et al.*, 2004; Marrugat *et al.*, 2004; Covas *et al.*, 2006). Other mechanisms by which polyphenols may be protective against cardiovascular diseases are antioxidant, anti-platelet, anti-inflammatory effects as well as increasing HDL, and improving endothelial function (Garcia-Lafuente *et al.*, 2009).

1-6- Myocardial Infarction :

Myocardial infarction (MI) is a clinical problem defined as acute necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand (Kesarwani and Azmi, 2014). MI is a condition of heart muscles damage and refers to tissue death when one or more coronary arteries which supply oxygen-rich blood to the heart muscle become suddenly blocked (Bishop *et al.*, 2005). Acute myocardial infarction is a disease which affects the patient in an extremely stressful way. It is described as a threat that leads to a life crisis in one's whole life (Eriksson *et al.*, 2010). Usually this is because one of the coronary arteries that supplies blood to the heart develops a blockage due to an unstable buildup of white blood cells, cholesterol and fat.

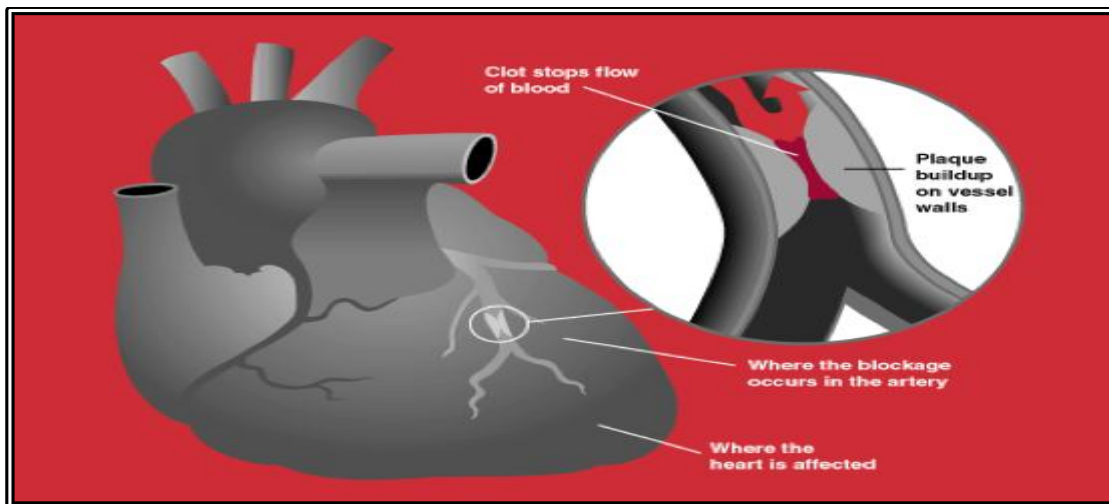


Figure (1-8): Myocardial infarction (Andrade-Castellanos and Colunga-Lozano, 2014).

A person having an MI usually has chest pain that is felt behind the sternum and sometimes travels to the left arm or the left side of the neck. Moreover, the person may have dyspnea , sweating, nausea, vomiting, abnormal heart beats and anxiety. Women experience fewer of these signs than men, but frequently have shortness of breath, weakness, a feeling of indigestion, and fatigue (Kosuge *et al.*, 2006). In many cases, in some estimates as high as 64%, the person

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does not have chest pain or other symptoms (Valensi *et al.*, 2011). These are called silent myocardial infarctions. Important risk factors are earlier cardiovascular disease, old age, smoking, high blood levels of certain lipids (LDL and TG) and small levels of (HDL) cholesterol, diabetes, high blood pressure, lack of physical activity, obesity (Graham *et al.*, 2007; Devlin and Henry, 2008). MI can be defined from a number of different perspectives related to clinical, (ECG), biochemical and pathologic characteristics. One of the proposed mechanisms of infarction refer to the free radicals that produced by multiple mechanisms and play an important role in cell membrane damage including mitochondrial membrane damage, thereby produce the necrosis which lead to infarction (Opie, 2004). The process of infarction progresses over several hours, and therefore most patients are presented, when it is still possible to salvage myocardium and to improve the outcome (Haslett *et al.*, 2002; Snell, 2004).

1-6-1- Causes of Myocardial Infarction:

The propagation and severity of the disease among individuals and groups are linked to a number of factors, some are constitutional and therefore immutable, but others are obtained and are potentially capable of control.

1-6-1-1- lifestyle :

Smoking harms nearly every organ of the body, it causes many diseases and reduces the health of smokers. In general, smoking causes coronary heart disease, the leading cause of death (Smith *et al.*, 2006). This causes of about 36% and obesity the cause of 20% of coronary artery disease (Kivimäki *et al.*, 2012). Lack of exercise has been linked to 7-12% of cases (Kivimäki *et al.*, 2012; Lee *et al.*, 2012). Job stress appears to play a minor role, accounting for about 3% of cases (Kivimäki *et al.*, 2012). Psychosocial factors including, low socioeconomic status, social isolation and negative emotions increase the risk

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of and are associated with worse outcomes after MI (Kumar *et al.*, 2003). There is little evidence that dietary saturated fat or polyunsaturated fat intake affects risk (Chowdhur *et al.*, 2014).

1-6-1-2- Disease :

Diabetes mellitus (Buse *et al.*, 2007). Hypertension (Smith *et al.*, 2006). dyslipidemia/hypercholesterolemia (abnormal levels of lipoproteins in the blood), particularly high low-density lipoprotein, low high-density lipoprotein and high triglycerides (Smith *et al.*, 2006) and Obesity (Kumar *et al.*, 2003).

1-6-1-3- Others:

At any given age, men are more at risk than women, particularly before menopause (Leander *et al.*, 2001). But in general women live longer than men, ischemic heart disease causes slightly more total deaths in women (Graham *et al.*, 2007). Family history of IHD or MI, particularly if one has a first-degree relative who suffered a premature MI (defined as occurring at or younger than age 55 years men or 65 women (Graham *et al.*, 2007). Women who use combined oral contraceptive pills have a diffidently increased risk of MI, particularly in the presence of other risk factors, such as smoking (Khader *et al.*, 2003). An increased incidence of a heart attack is related with time of day especially in the morning hours, more specifically around 9 am (Muller *et al.*, 1985; Beamer *et al.*, 1987; Cannon *et al.*, 1997).

1-6-2- Signs and Symptoms :

The beginning of symptoms in myocardial infraction is usually gradual, over some minutes, and infrequently instantaneous (Jennings, 2013). Chest pain is the most common sign of acute MI and is often described as a sensation of tightness, pressure, or squeezing. Chest pain due to ischemia of the heart muscle is termed angina pectoris. Pain radiates most often to the left arm, but

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may also radiate to the lower jaw, neck, right arm, back, and upper abdomen (Berger *et al.*, 1990; Van de Werf *et al.*, 2008). Dyspnea happens when the damage to the heart limits the output of the left ventricle, causing left ventricular failure and consequent pulmonary edema.

Other signs include diaphoresis (Mallinson, 2010). Weakness, light-headedness, nausea, vomiting, and palpitations. These symptoms are likely induced by a massive surge of catecholamines from the sympathetic nervous system (Little *et al.*, 1986). Which happens in response to pain and the hemodynamic abnormalities that result from cardiac dysfunction. Loss of consciousness (due to inadequate blood flow to the brain and cardiogenic shock) and sudden death (frequently due to the development of ventricular fibrillation) can occur in MI (Van de Werf *et al.*, 2008). The most common symptoms of MI in women include dyspnea, weakness, and sleep disturbances. In women, chest pain may be less predictive of coronary ischemia than in men (McSweeney *et al.*, 2003). At least one-fourth of all MI are silent, without chest pain or other symptoms (Kannel, 1986; Valensi *et al.*, 2011). These cases can be discovered later on electrocardiograms, using blood enzyme tests or at autopsy without a prior history of related complaints. Estimates of the prevalence of silent MI vary between 22 and 64% (A silent course is more common in the elderly (Valensi *et al.*, 2011). In patients with diabetes mellitus (Davis *et al.*, 2004) and after heart transplantation, probably because the donor heart is not fully innervated by the nervous system of the recipient (Rubin *et al.*, 2001).

1-7-The Model of Myocardial Infarction Induction Using Isoproterenol:

1-7-1- Chemistry:

Isoproterenol hydrochlorid is an L- β -(3,4-dihydroxyphenyl)- α -isopropyl amino ethanol hydrochloride with a molecular formula of $C_{11}H_{17}NO_3.HCl$. Its molecular weight is 247.7. The hydrochloride salt of isoproterenol is a white to off-white crystalline powder of melting point 170-171 °C. It is soluble in water and ethanol. figure (1-8).

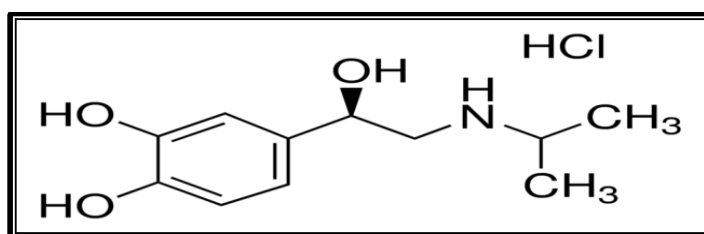


Figure (1-9): Structure of isoproterenol

(Upaganlawar *et al.*, 2011).

1-7-2-Mechanism of Action and Biological Effects of Isoproterenol:

Isoproterenol (ISO), a synthetic catecholamine and β -adrenergic agonist, has been documented to produce myocardial infarction in large doses. The pathophysiological changes associated with myocardial infarction induced by isoproterenol mimics to a greater extent with those occurring in humans (Upaganlawar *et al.*, 2011). Isoproterenol is a β -adrenergic receptor agonist that increases cytosolic cAMP. In the case of β -adrenergic agonist action, the circulating hormones or drug is the first “messenger”, interacting with β -adrenergic receptor on the external surface of the target cells (Rendon and Lopez, 2001). The drug hormone receptor complex activates the enzyme adenyl cyclase on the internal surface of the plasma membrane of the target cells. This

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accelerates the intracellular formation of cyclic adenosine monophosphate (cyclic AMP), the second “messenger” which then stimulates or inhibits various metabolic or physiological processes (Hu *et al.*, 2006). It has been shown that isoproterenol induces myocardial cell injury similar to that reported for myocardial infarction, myocardial ischemia, cardiac stress and cardiac myopathy (Sudhakumari *et al.*, 2012). Several studies have investigated the molecular and cellular mechanism of isoproterenol-induced cell injury of the myocardium (Curti *et al.*, 1990; Capozza *et al.*, 1992; Chagoya de Sanchez *et al.*, 1997; Rendon and Lopez, 2001). Among these, the investigation by Chagoya de Sanchez *et al.* (1997) establishes a long-term, integrated model of isoproterenol-induced myocardial cell damage encompassing structural, biochemical and physiological aspects.

The enhanced adenylate cyclase activity as result of isoproterenol induction increases cAMP formation, which in turn would have lead to the higher lipid accumulation in the myocardium (Subhash *et al.*, 1978). Isoproterenol is reported to increase lipolysis (Mohan and Bloom, 1999) and this may play a role in isoproterenol-induced myocardial necrosis.

Hypertriglyceridemia and high levels of ester cholesterol in serum and heart tissue are the major factors responsible for the altered cardiovascular functions during isoproterenol- induced myocardial infraction (Freedman *et al.*, 1998). Accelerated degradation of membrane phospholipids by phospholipase and lysophospholipase has also been proposed to be related to membrane dysfunction and irreversible ischemic injury (Farber and Young, 1981). The administration of isoproterenol increases the activities of myocardial cholesterol ester synthetase and triglyceride lipase with simultaneous decline in the activities of cholesterol ester hydrolase and lipoprotein lipase. Accumulation of ester cholesterol occurs when the rate of esterification by cholesterol ester synthetase exceeds the rate of hydrolysis, which in turn results in myocardial membrane damage. Peroxidation of endogenous lipid is a major factor in the

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cytotoxic action of isoproterenol (Namikawa *et al.*, 1992). A growing body of evidence is emerging which suggests that reactive oxygen-derived free radicals play a crucial role in the pathogenesis of isoproterenol-induced myocardial infarction (Nirmala and Puvanakrishnan, 1994).

1-7-3-Structural and Functional Changes Induced by Isoproterenol in Heart :

Myocardial infarction induced by isoproterenol has been reported to show many metabolic and morphologic aberrations in the heart of experimental animals. It induces myocardial necrosis by a multiple step mechanism (Upaganlawar *et al.*, 2010). Isoproterenol has been reported to cause oxidative stress in the myocardium, which results infarct like necrosis of heart muscle (Upaganlawar *et al.*, 2012).

Administration of isoproterenol induces a significant elevation of the ST-segment elevation with an enhancement in ventricular wall motion (Yamamoto and Katori, 1995). It also elevates mean heart rate with an imbalance in autonomic regulation of cardiac automaticity, which accounts for the 27% incidence of arrhythmias in rats (Rote and Connor, 1992). A progressive enlargement of the LV cavity that is out of proportion to mass, similar to that observed in discrete myocardial infarction also commonly occurs in isoproterenol-induced myocardial necrosis (Teerlink *et al.*, 1994).

Injection of isoproterenol stimulates the development of left ventricular hypertrophy(LVH) with an increase in relative left ventricle weight, LV protein content and LV beta-myosin heavy chain levels (Ocaranza *et al.*, 2002).

1-7-4-Metabolic Changes During Isoproterenol–Induced Myocardial Infarction :

The isoproterenol-induced alterations in experimental animals includes increase in heart weight, marked electrocardiographic changes, increase in the level of serum marker enzymes and lipid peroxides and decrease in the levels of

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antioxidant (Manikandan *et al.*, 2002). Isoproterenol administration produces a marked increase in CPK, LDH, phospholipase and significant decrease in cardiac glycogen, creatine phosphate and phospholipid level (Raza *et al.*, 2012).

Injection of isoproterenol administration causes a dose dependant increase of lysosomal enzyme activity in vitro and in vivo (Prince *et al.*, 2011). The increase in the serum lysosomal hydrolase activities in isoproterenol treated rats is mainly due to the decreased stability of the membranes, which is usually reflected by the lowered activity of cathepsins D in mitochondrial and microsomal fractions (Xu *et al.*, 2011). During isoproterenol-induced myocardial infarction the cytoplasm of cardiac myocytes becomes more acidic due to lactate accumulation. The infiltration of inflammatory cells at the infract regions and altered lysosomal fragility are responsible for the increased activity of these enzymes (Ravichandran *et al.*, 1991).

A considerable body of clinical and experimental evidence now exists suggesting the involvement of free radical mediated oxidative process in the pathogenesis of isoproterenol-induced myocardial infarction (Nirmala and Puvanakrishnan, 1996). Alterations in tissue defense systems including chemical scavengers or antioxidant molecules and the enzymes catalase, superoxide dismutase, and glutathione peroxidase have been reported in isoproterenol-induced myocardial infarction (Sathish *et al.*, 2003; Sasikumar and Devi, 2000). The administration of isoproterenol produces necrotic lesions in the myocardium and increases lipid peroxidation in the cardiac tissue, which plays a significant part in the pathogenesis of myocardial infarction (Ziaee *et al.*, 2015). A significant depletion of cardiac glutathione (GSH) has been reported in isoproterenol-induced myocardial infarction in rats (Hagar, 2002). Depletion of GSH is known to result in enhanced lipid peroxidation and excessive lipid peroxidation can cause increased GSH consumption and increase the susceptibility of the myocardial cells to reactive oxygen metabolites (Patel *et al.*, 2010).

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Ornithine decarboxylase (ODC) is an initial rate-limiting enzyme in the synthesis polyamines (putrescine, spermidine and spermine). They play a role in cell growth and differentiation. Isoproterenol induces an increase in ODC activity and putrescine and spermidine level in the heart. These polyamines are one of the intracellular factors that contribute to cardiac injury (Tipnis *et al.*, 2000).

1-8 -Taurine :

1-8-1- Properties and Synthesis of Taurine :

Taurine (2-aminoethanesulfonic acid), seafood is the most rich source of it, is a conditionally essential amino acid which is not incorporated into proteins, but rather is found free in most mammalian tissues, such as heart, retina, liver, muscle, platelets (Huxtable, 1992). It was considered as a nonessential nutrient in human beings until 1975 when it was found that formula-fed, pre-term infants were not able to sustain normal plasma or urinary taurine levels (Raiha *et al.*, 1975). Furthermore, *in vivo* study has demonstrated that low levels of taurine were associated with various pathological lesions, including cardiomyopathy, retinal degeneration, and growth retardation (Sturman, 1993). It is well known that taurine has many biological and physiological properties, such as antioxidation, anti-inflammation, osmoregulation, membrane stabilization, modulation of cellular calcium levels. (Schaffer *et al.*, 2010). For example, taurine can react with hypochlorous acid, a strong oxidant produced by neutrophils, to form taurocholate chloramine, which not only plays a direct antioxidant role, but also plays an anti-inflammatory role by reducing inflammatory media (Schaffer *et al.*, 2009). For cholesterol metabolism, taurine is considered only because of its conjugation with bile acids. The chemical structure of taurine, shown in (figure, 1-9 A), reveals that it lacks the carboxyl group typical of other amino acids, but does contain a sulfonate group. The major route for the biosynthesis of taurine, shown in (figure, 1-9 B) is from methionine and cysteine via cysteinesulfinic acid decarboxylase (CSD), and

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typically requires oxidation of hypotaurine to taurine as the final step (Stipanuk, 1986).

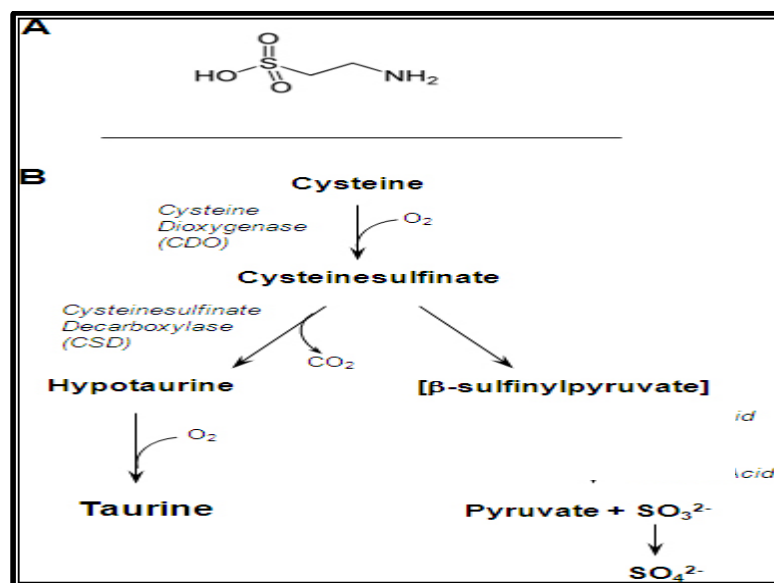


Figure (1-10): Structure and formation of taurine. **A:** The chemical formula of taurine is C₂H₇NO₃S MW=125.15. **B:** This oversimplified diagram shows the main steps in the conversion of L-cysteine to taurine. (Stipanuk, 1986).

1-8-2 -Physiological Properties:

1-8-2-1- Antidiabetic Effect of Taurine :

In both forms of diabetes, insulin dependent (Type 1) and non-insulin dependent (Type 2), taurine exerts a multitude of beneficial actions. Supplementation of taurine has been reported to prevent the triggering of platelet aggregation in Type 1 diabetes, a major risk factor of cardiovascular diseases (Franconi *et al.*, 2004). In models of diabetic mice, the researchers found that taurine supplementation yields specific beneficial effects on levels of malondialdehyde (MDA), a marker of lipid peroxidation resulting from free radical damage. Dietary supplementation of taurine is able to correct abnormal elevations of MDA and thus to prevent depletion of glutathione in diabetics

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(Lim *et al.*, 1998). Diabetes is notable for its impact on eyesight and to produce a generalized decline in the content of free amino acids in the retina and retinal pigment epithelium of the eye. Taurine supplementation has been reported to reduce the deleterious action of diabetes on amino acid transport systems, which results in alteration of the cellular amino acid balance. It has also been found as an effective method for correcting the antioxidative imbalances that produce diabetic cataracts (Obrosova and Sterens, 1999). Taurine supplementation has also been reported to prevent diabetic nephropathy, which is the leading cause of end-stage renal disease in over half of Type 1 diabetics. Report by Trachtman *et al.* (1995) indicated that taurine supplementation is able to exert beneficial changes in the kidneys of diabetic rats, specifically reducing proteinuria and albuminuria by nearly half. This effect is attributed to the ability of taurine to decrease lipid peroxidation and reduce accumulation of advanced glycosylation end products within the kidney.

1-8-2-2- Antilipidemic Effect of Taurine :

According to animal study, taurine may reduce blood lipid levels (Niittynen *et al.*, 1999). The cholesterol-lowering effect of taurine can be explained by its effects on bile acid metabolism. It has been suggested that taurine stimulates bile acid synthesis, which is closely related to the enhancement of cholesterol 7 α -hydroxylase activity, thus reducing serum lipid values (Murakami *et al.*, 1999).

Taurine has been reported to attenuate the elevation in total and LDL cholesterol levels in people consuming high fat/ high cholesterol diet (Zhang *et al.*, 2004). Report by Murakami *et al.* (1999) indicate that the administration of taurine lowers serum LDL and VLDL by 44% and elevates HDL by 25% in experimental mice. High cholesterol diets produce hypercholesterolemia (elevated cholesterol), atherosclerosis and accumulation of lipids on coronary artery. Report by Park and Lee, (1998) that taurine supplementation able to exert hypocholesterolemic and hypotriglyceridemic effects in rats fed a high

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cholesterol diet. The antilipidemic and cholesterol-lowering action of taurine may lie in its ability to promote the degradation of potentially detrimental cholesterol to relatively harmless bile acids.

1-8-2-3- Antioxidant Effect of Taurine :

Physicians regularly recommend the use of antioxidant vitamins (A, beta-carotene, C and E) and the mineral selenium as counter-measures to free radicals. Recently, the value of taurine as a potent antioxidant has been discovered. If present in sufficient concentration, taurine protects against oxidative cellular damage (Schaffer *et al.*, 2009). The antioxidant properties of taurine are also seen in its ability to inhibit neutrophil burst and subsequent oxidative stress, which results in reperfusion injury to heart tissue (Raschke *et al.*, 1995). Taurine prevents the inactivation of superoxide dismutase by H_2O_2 (Pecci *et al.*, 2000). It also acts as an antioxidant by preventing changes in the levels of non-enzymatic free radical scavengers (Tadros *et al.*, 2005).

1-8-2-4- Other Effects of Taurine on Cardiovascular Diseases:

In heart failure patients, the left ventricular function has been improved after a dietary supplement of taurine (2-4 g/day) alone (Ito *et al.*, 2014), and combined with diuretic agents (Azuma *et al.*, 1983). The mechanism by which taurine improves cardiac performance might result from the fact that taurine plays a role in the regulation of intracellular calcium homeostasis through the modulation of cation fluxes (Azuma *et al.*, 1992). Abdel-Reheim (2016) reported that taurine significantly protected against the toxic effects of ISO *via* alleviating the altered cardiac biomarker enzymes which may be mediated by attenuate the oxidation mechanism system. In addition, hyperlipidemia and its cardiovascular complications were markedly ameliorated through increasing HDL level and enhancement of LDL turnover.

1-9- Cardiac Markers of MI :

1-9-1-Troponins:

Myofibrillar proteins are the building blocks of the contractile apparatus. These proteins comprise a thick filament, containing only myosin, and a thin filament containing actine, tropomyosin and troponin. Smooth muscle does not contain troponin. Consequently, the troponin complex is located only in the thin filament of the myocyte. It control muscle contraction by regulating the calcium- dependent interaction between myosin and actin. It is a complex of 3 polypeptide chains. These isoforms are designated troponin C, troponin I, and troponin T (Guyton and Hall, 2006). The composition of the thin filament is shown in figure (1-10).

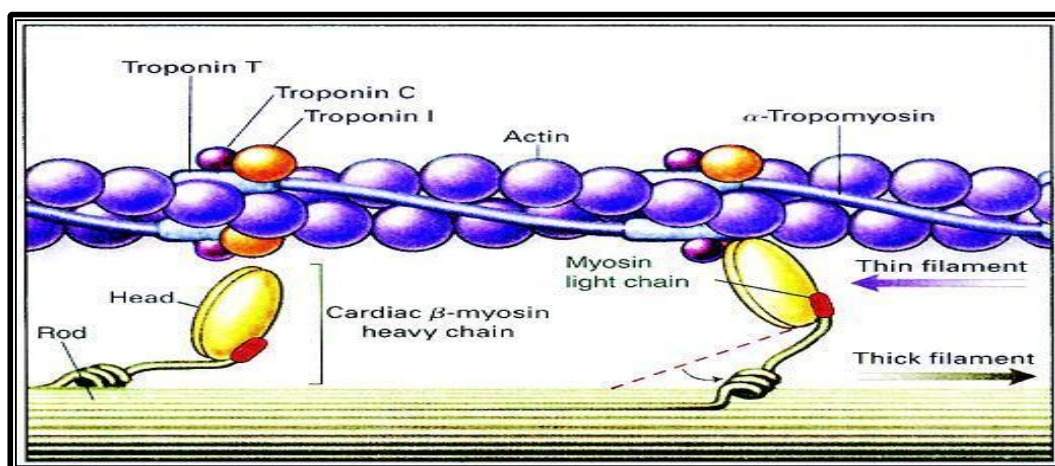


Figure (1-11): The composition of the thin filament
(Bach *et al.*, 2004).

The three troponin isoforms consist of subunits with different structures and functions:

Troponin C (TnC): is not heart specific. It is a Ca^{+2} -binding protein containing four metal-binding sites. Two sites located in the C-terminal globular domain of TnC, bind both Ca^{+2} and Mg^{+2} , whereas two other sites located in the N-terminal domain specifically bind Ca^{+2} (Guyton and Hall, 2006; Panteghini *et al.*, 2004).

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Troponin I (TnI), inhibitory subunit: -inhibits actomyosin ATPase activity, and this inhibition is reversed by the addition of Ca^{+2} -saturated TnC (Panteghini *et al.*, 2004).

TroponinT (TnT):- tropomyosin-binding subunit.

TnI and TnC interact tightly with each other and the strength of their interaction depends on the saturation of Ca^{2+} -binding site of TnC (Allan *et al.*, 2006).

These cardiac troponins (cTns) appear in the blood as early as 3-4 hours of the acute episode and remain elevated for 4-14 days (Kumar and Cannon, 2009).

1-9-2- Lactate Dehydrogenase (LDH):

The enzyme LDH (EC 1.1.1.27) catalyses the reversible transfer of 2 electrons and one hydrogen ion from lactate to NAD resulting in pyruvate and NADH. LDH is a tetramer (MW 135.000 D) composed of M- (muscle; MW 34.000) and of H- (heart; MW 34.000 D) subunits. The subunits are encoded by different genes. Because of the tetramer structure, LDH consists of 5 isoenzymes LDH- 1 to 5. LDH- 1 contains four H subunit and the predominant from the heart, but also occurs in erythrocytes, brain, pancreas, kidney and stomach. After AMI, serum LDH activities start to rise 12 to 18 hours after the onset of symptoms, peak at 48- 72 hours, and return to normal by 6- 10 day (Nicolas *et al.*, 2007).

1-9-3- Creatine Phosphokinase(CPK):

Creatine kinase (CK) (EC 2.7.3.2) is an enzyme located in the cytosol and mitochondria of the cell. It is involved in cellular energy metabolism by catalyzing the reversible transfer of a high energy phosphate group from adenosine triphosphate (ATP), resulting in ADP and creatine phosphate. CK consists of 2 subunits M and B, each with a molecular weight of 41 KDa. So three composition of CK might occur: the iso-enzymes CK-BB, CK-MB, and CK-MM. CK-MM is most abundant in skeletal muscle (97-99%) and myocardial muscle (about 80%). CK-MB is part of myocardial muscle (nearly 20%) and of skeletal muscle (1- 3%). CK-BB is most abundant in brain

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and intestines (Chattington *et al.*, 1994; McLean *et al.*, 2008). Serum CK activity increases following MI beginning within 6 hours and peaking on an average at 24 hours and returning to normal within 2-3 days. (Chattington *et al.*, 1994).

1-9-4-Alanine Amino Transferase (ALT) Enzyme :

ALT catalyse transamination reaction, showed in (figure,1-11), is found in kidney, heart, muscle and increase concentration in liver compared with other tissues of the body. ALT is purely cytoplasmic catalysing the transamination reaction. Any type of liver cell injury can reasonably increases ALT levels. Marked elevations of ALT levels greater than more of normal value that affect mainly hepatocytes such as viral hepatitis and toxin-induced liver damage (Clifton and Jr., 2003). Viral hepatitis like A, B, C, D and E may be responsible for a marked increase in aminotransferase levels. Continuation of elevated ALT for more than six months after an occurrence of acute hepatitis is used in the diagnosis of chronic hepatitis. Measure ALT and AST of intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury (Meybodi *et al.*, 2008).

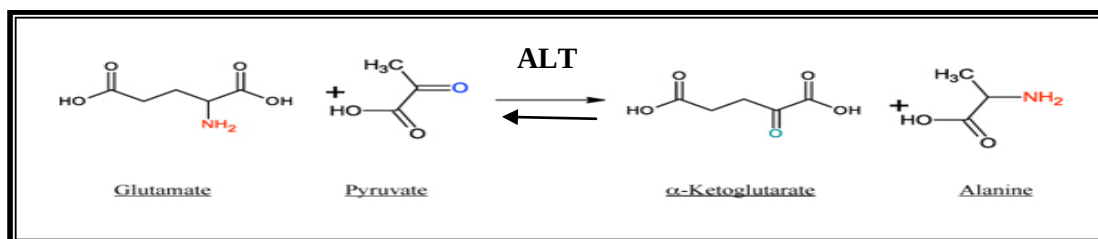


Figure (1-12): Reaction ALT enzyme(Clifton and Jr., 2003).

1-9-5- Aspartate Amino Transferase (AST) Enzyme :

AST catalyse transamination reaction, showed in (figure,1-12). AST exist in two different isoenzyme forms which genetically differ, the mitochondrial and cytoplasmic form. AST is found in highest concentration in heart compared to other tissues of the body such as liver, skeletal muscle and kidney. Elevated mitochondrial AST seen in extensive tissue necrosis during

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myocardial infarction as well as in chronic liver diseases like liver tissue degeneration and necrosis. About 80% of AST activity of the liver is contributed by the mitochondrial isoenzyme, while most of the circulating AST activity in normal people is derived from the cytosolic isoenzyme. However the ratio of mitochondrial AST to total AST activity has diagnostic importance in identifying the liver cell necrotic type condition and alcoholic hepatitis. AST elevations often prevail in patients with cirrhosis and even in liver diseases that typically have an increased ALT (Thapa and Walla, 2007).

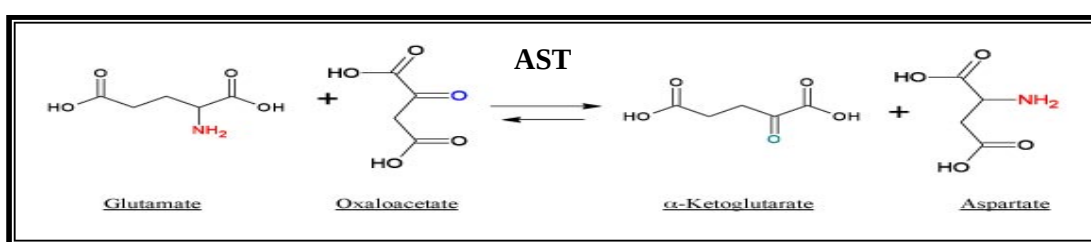


Figure (1-13) : Reaction AST enzyme (Thapa and Walla, 2007).

1-10- Free Radicals and Reactive Oxygen Species:

Free radicals are unstable compounds with an unpaired electron in outer shell (Valko *et al.*, 2007). the biomolecular damage caused by attack of reactive species upon the constituents of living organisms”. Oxidative damage to cellular components impairs physiological functions. Reactive species can be reactive oxygen species (ROS) or reactive nitrogen species (RNS) (Ashok *et al.*, 2005).

Free radicals are normally generated in substantial amounts as a by-product of various internal metabolic processes in aerobic organisms such as phagocytosis, neutrophils defense, autooxidation of catecholamine and carboxylation or hydroxylation reactions. All of these processes happen in various ways at different times and sites (Hass, 2006).

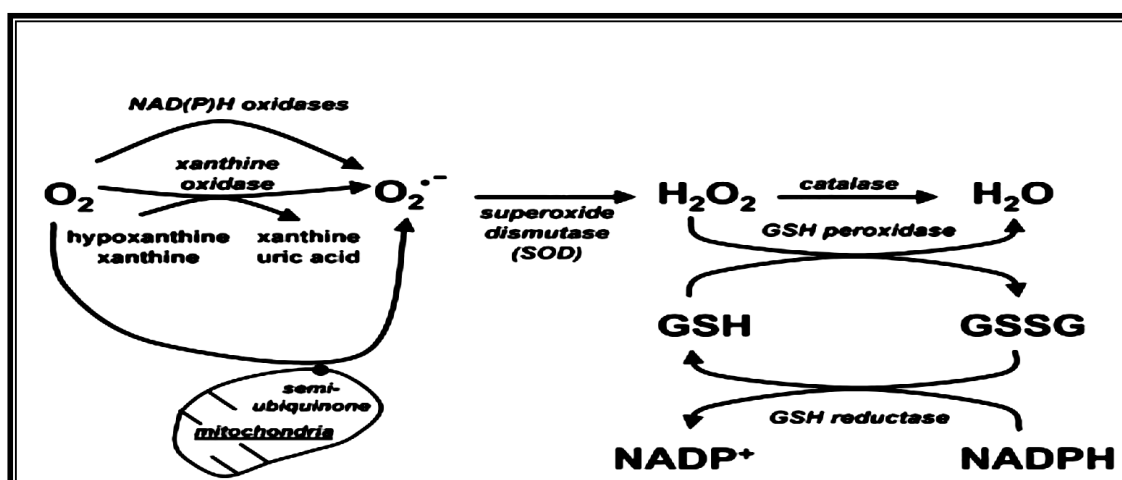
Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are describes free radicals and other non-radical reactive derivatives (Pacher *et al.*, 2007). The reactivity of radicals is generally stronger than non-radical formed from molecules by the homolytic cleavage of a chemical bond and via redox

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reactions, once formed these highly reactive radicals can start a chain reaction (Fialkow *et al.*, 2007). ROS and RNS includes radicals such as superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$) and nitric oxide radical; and non radicals like hydrogen peroxide (H_2O_2) and peroxynitrate ($ONOO^-$) (Wattanapitayakul and Bauer, 2001).

At high concentrations, ROS can be important mediators of damage to cell structures, nucleic acids, lipids and proteins (Dalle-Donne *et al.*, 2005). Free radicals and reactive oxygen species (ROS) are known to be generated in ischemia and oxidative stress (Zbeger *et al.*, 2004). Superoxide ($O_2^{\bullet -}$) radical is responsible for lipid peroxidation and also has the capability to decrease the activity of other antioxidant defense system enzyme such as glutathione peroxidase (GPx), it causes damage to the ribonucleotide which is required for DNA synthesis (DeCoursey and Ligeti, 2005). The protonated form of $O_2^{\bullet -}$ is hydroperoxyl ($HO_2^{\bullet}$), which is more reactive and able to cross the membrane and causes damage to tissue. hydroxyl ($OH^{\bullet}$) radical is most reactive chemical species.

Hydrogen peroxide (H_2O_2) is not a radical but it produces toxicity to cell by causing DNA damage (Lobo *et al.*, 2010). Metal induced generation of ROS attack DNA and other cellular components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation (Waris and Ahsan, 2006). In general, the production of these species may occur enzymatically, or non-enzymatically, as accidental by products or major products of reactions (Cannio *et al.*, 2000). The superoxide anion which plays a role, as a precursor of other reactive species is produced from one-electron reduction of oxygen, this process is mediated by enzymes such as NADPH oxidase and xanthine oxidase, or non-enzymatic by redox-reactive compounds such as the semi-ubiquinone compound of the mitochondrial electron transport chain (Droge, 2002), as illustrated in figure (1-13).



Figure(1-14): Pathways of ROS production and clearance (Droge, 2002).

1-11- Lipid Peroxidation :

Oxidative stress represents an imbalance between the production of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Some oxidative species can even act as messengers through a phenomenon called redox signaling (Benzie, 2003). In humans, oxidative stress is involved in many diseases examples include atherosclerosis, Parkinson's disease, heart failure, myocardial infarction, Alzheimer's disease (de Diego-Otero *et al.*,2009).

Lipid peroxidation (figure 1-14) refers to the oxidative degradation of lipids. It is the process whereby free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene $-CH_2-$ groups that possess especially reactive hydrogens(Ayala *et al.*, 2014).

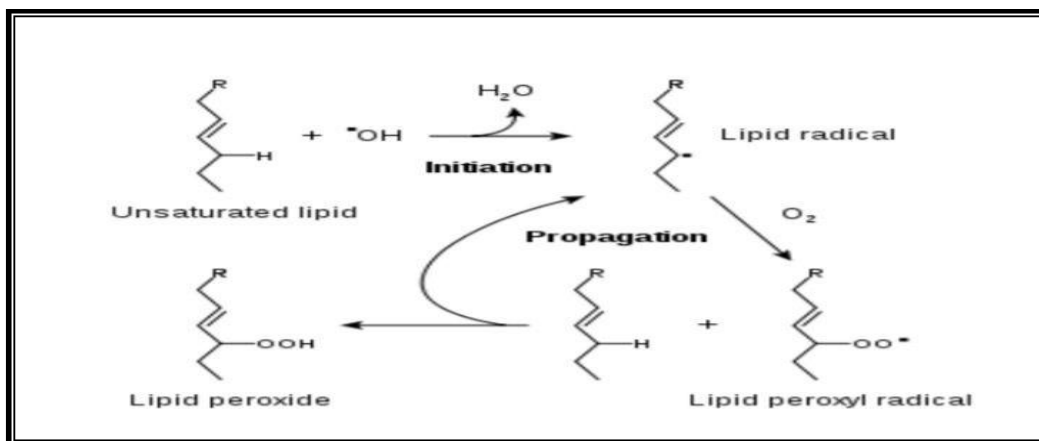


Figure (1-15) : Lipid peroxidation process (Ayala *et al.*, 2014).

Polyunsaturated fatty acids in cholesterol esters, phospholipids and triglycerides are subject to free radical -initiated oxidation and can participate in chain reactions that amplify damage to biomolecules. A key feature of lipid peroxidation is the breakdown of these polyunsaturated fatty acids to yield a broad array of smaller fragments, three to nine carbons in length, including aldehydes (Kaur *et al.*, 2008).

The important reactive aldehydes originated from lipid peroxidation is including malondialdehyde (MDA) and glyoxal. MDA is in many instances the most abundant individual aldehyde resulting from lipid peroxidation and has been shown to disturb aminophospholipid organization in the membrane bilayer of erythrocytes (Thandavarayan *et al.*, 2011). Malondialdehyde is able to impair several physiological mechanisms of the human body through its ability to react with molecules such as DNA and proteins. It is therefore useful to consider this molecule as something more than a lipid peroxidation product (Del Rio *et al.*, 2005). MDA is used as a biomarker to measure the level of oxidative stress in an organism (Del Rio *et al.*, 2005).

1-12- Antioxidants :

Antioxidants are any substances that delay or inhibit oxidative damage to a target molecule. At a time one antioxidant molecule can react with single free radicals and are capable to neutralize free radicals by donating

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one of their own electrons (Willcox *et al.*, 2004).

Antioxidants prevent cell and tissue damage as they act as scavenger. Cells produce defense against excessive free radicals by their preventative mechanisms, repair mechanisms, physical defenses and antioxidant defenses (Benz, 2008). The body has developed several antioxidant defense systems classified into two groups such as enzymatic and non enzymatic. The enzymatic defense system includes different endogenous enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and non enzymatic defense system included vitamin C, Ceruloplasmin, albumin, uric acid and reduced glutathione (GSH), as well as, lipid – soluble molecules such as the carotenoids and vitamin E (Ghone *et al.*, 2008). as illustrated in figure (1-15).

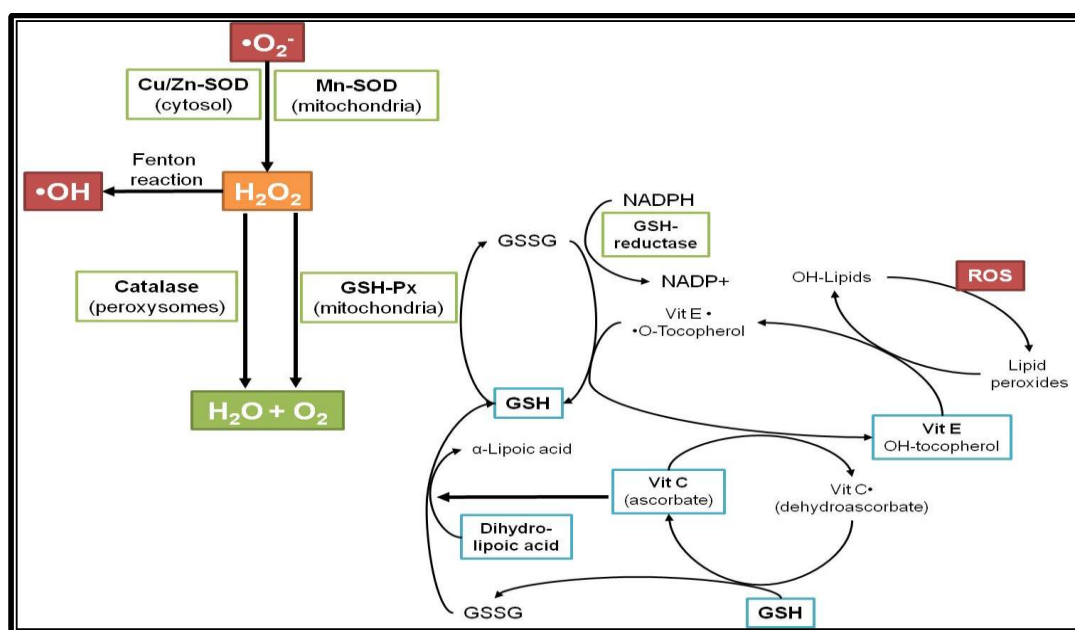


Figure (1-16): Antioxidants defenses in the organism (Jevas, 2016).

1-12-1- Ceruloplasmin (Cp) :

Ceruloplasmin (Cp) is one of the main proteins taking part in copper metabolism by distributing it in the human body. It is responsible for carrying more than 95% of copper in blood serum (Adamczyk-sowa *et al.*, 2016). Cp is

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an α -glycoprotein composed of a single chain polypeptide of 1046 amino acids and has a molecular weight of approximately 151 KDa (Healy and Tipton, 2007). Cp is synthesized mainly in the liver as a single chain polypeptide, and after the incorporation of six atoms of copper in the biosynthetic pathway, it is secreted into the plasma as an α_2 -glycoprotein (Gutteridge, 2005).

A principal function of Ceruloplasmin is the transport and delivery of copper to tissues, copper utilisation in the biosynthesis of cytochrome c oxidase and also ceruloplasmin can transfer copper to metal-free superoxide dismutase.

Ceruloplasmin possesses ferroxidase activity in that it oxidises the more toxic ferrous ions to the less toxic ferric form. The oxidation of Fe^{2+} to Fe^{3+} by Cp is thought to reduce oxidative stress by inhibiting the fenton reaction which (in simple chemistry) uses Fe^{2+} to generate ROS (Shukla *et al.*, 2006).

In normal healthy human adults the circulating levels of ceruloplasmin are approximately 300 mg/ml of plasma. This increases about 2–3 times during the acute phase of inflammation (Healy and Tipton, 2007). High serum Cp levels were found in patients with multiple cardiovascular disorders including arteriosclerosis, unstable angina and peripheral arterial disease. Prospective studies also showed that serum Cp may be an independent risk factor for cardiovascular disease. The association between Cp and cardiovascular events is well explained by describing a role for Cp in LDL oxidation by vascular cells (free radical-mediated, transition metal dependent mechanism) (Ahmed *et al.*, 2012).

1-12-2- Albumin (Alb):

Albumin is the most abundant protein of blood plasma. It is a single-chain, polypeptide with a molecular weight of (65-70) KDa containing 585 amino acids (Yang *et al.*, 2014). Serum albumin represents 60 % of total plasma proteins and its half-life is ~ 20 days in normal conditions (Taverna *et al.*, 2013). Albumin has many important physiological functions. Among them,

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regulates colloidal osmotic pressure, and transports numerous endogenous compounds such as fatty acids (FA), hormones, bile acids, amino acids, metals and toxic metabolites. Additionally, there is a wide variety of drugs that are delivered to their targeting organs/tissues by binding with HAS. Therefore, HAS not only protects the bound drugs against oxidation and influences the *in vivo* drug distribution, but also alters the pharmacokinetic and pharmacodynamic properties of drugs (Yang *et al.*, 2014).

Several studies have suggested that albumin as a powerful oxidants scavenger in human plasma can inhibit hydroxyl radicals and peroxy radicals (Lim *et al.*, 2007). Serum albumin is a negative acute phase protein synthesized in liver. Lower levels of serum albumin within the “normal” range are associated with increased risk of all-cause and cardiovascular mortality, as well as with coronary heart disease (CHD) and stroke incidence (Ahmed *et al.*, 2012).

1-12-3 -Uric acid :

Uric acid is the major product of the catabolism of the purine nucleotides, adenine and guanine, the bulk of purines ultimately excreted as uric acid in the urine arise from degradation of endogenous nucleic acids, it is approximately 75% of uric acid is lost in the urine (Berg *et al.*, 2002). Uric acid conceivably acts as a powerful antioxidant in plasma (Nyyssonen *et al.*, 1997). Uric acid efficiently inhibits various kinds of oxidative damage to membranes, including damage by lipid peroxidation (Ames *et al.*, 1993). The antioxidative role of the uric acid, is its ability to scavenge hydroxyl radical, peroxy radicals and reducing the effect of lipid peroxidation induced by various model systems (Muraoka and Miura, 2003).

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Uric acid has emerged as a simple and independent marker of morbidity and mortality in a variety of cardiovascular disease states, and it has been clearly associated with oxidative stress and inflammation in several pathological conditions (Letsas Konstantinos *et al.*, 2010).

Serum uric acid is a risk factor for coronary artery disease, as well as for hypertension, metabolic syndrome, and kidney disease. (Sun *et al.*, 2015), also Uric acid is associated with an increased risk for myocardial infarction and stroke (Euser *et al.*, 2009).

1-12-4 – Glutathione (GSH):

Glutathione (γ -glutamyl cysteinyl glycine) is a tri-peptide of the amino acids cysteine, glycine, and glutamic acid. Glutathione is a non-enzymatic antioxidant compound found in living cells. It takes up and gives off hydrogen.

A deficiency of glutathione can cause hemolysis (destruction of red blood cells, leading to anemia) and oxidative stress. Glutathione is essential in intermediary metabolism as a donor of sulfhydryl groups which are essential for the detoxification of acetaminophen. Glutathione is the major endogenous non-enzymatic antioxidant produced by the cell. Glutathione participates directly in the neutralization of free radicals, reactive oxygen compounds, and maintains exogenous antioxidants such as vitamins C and E in their reduced (active) forms. In addition, through direct conjugation, glutathione plays an important role in the detoxification of many xenobiotics (foreign compounds) both organic and inorganic (Zhao, 2001; Voet and Voet, 2004).

Glutathione exists in reduced form (GSH) and oxidized form (GSSG). In the reduced state, the thiol group of cysteine is able to donate a reducing equivalent ($H^+ + e^-$) to other unstable molecules, such as reactive oxygen species. In donating an electron, glutathione itself becomes reactive, but readily reacts with another reactive glutathione to form glutathione disulfide (GSSG). Such a

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reaction is possible due to the relatively high concentration of glutathione in cells (up to 5 mM in the liver). GSH can be regenerated from GSSG by the enzyme glutathione reductase. In healthy cells and tissue, more than 90% of the total glutathione pool is in the reduced form (GSH) and less than 10% exists in the disulfide form (GSSG). An increased GSSG/GSH ratio is considered as indicative of oxidative stress (Garrett and Grisham, 2005).

1-13 -Lipids and Lipoproteins:

1-13-1- Lipids:

The prevalence of lipid abnormalities in humans has shown a steady rise in various countries of the world over the years (Okosun and Matthew, 2015). Lipids are a diverse and ubiquitous group of compounds which have many key biological functions, such as acting as structural components of cell membranes, serving as energy storage sources and participating in signaling pathways. The term “lipid” has been loosely defined as any of a group of organic compounds that are insoluble in water but soluble in organic solvents. These chemical features are present in a broad range of molecules such as fatty acids, phospholipids, sterols, sphingolipids, terpenes and others (Fahy *et al.*, 2011). Plasma lipids consist of triacylglycerol (triglycerides) 16%, phospholipids 30%, cholesterol 14%, cholesterol esters 36% and a much smaller fraction of unesterified long-chain fatty acids or free fatty acids 4% (Murray *et al.*, 2003).

1-13-1-1- Total Cholesterol (TC):

It is a lipid molecule present in the diet, mainly synthesized in the small intestine and the liver, it is mainly esterified with fatty acids present in the plasma cholesterol may be utilized in the liver to form cell membrane component or bile acids, or may be excreted in the bile. (Gaw *et al.*, 1999).

A study conducted by Patil *et al.* (2007) showed that serum total cholesterol was positively and significantly associated with cardiovascular disease (CVD). Cholesterol plays a major role in human heart health. High cholesterol in serum

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is a leading risk factor for human cardiovascular disease such as coronary heart disease and stroke . Excess cholesterol in the bloodstream can form plaque (a thick, hard deposit) in artery walls.

The cholesterol or plaque build-up causes arteries to become thicker, harder and less flexible, slowing down and sometimes blocking blood flow to the heart. When blood flow is restricted, angina (chest pain) can result. A heart attack will result when blood flow to the heart is severely impaired and a clot stops blood flow completely (Hongbao, 2004).

1-13-1-2- Triglyceride (TG):

Triglycerides are fatty acid esters of glycerol, each containing three fatty acids. They are derived from three primary sources: the diet, storage depots in adipocytes and biosynthesis particularly in the liver (Menotti *et al.*, 1994). High levels of triglycerides is linked to heart disease. Triglyceride is the most common type of fat in the body. Many people who have heart disease have high triglyceride levels. A high triglyceride level combined with low HDL cholesterol or high LDL cholesterol seems to speed up atherosclerosis, which is the buildup of fatty deposits in artery walls that increase the risk for heart attack and stroke (Hongba, 2004) .

1-13-1- 3- Lipoproteins :

Lipoproteins are macromolecular complexes carrying lipids and proteins in plasma. Lipoprotein particles vary in composition, size, density and function (Ginsberg, 2002), Although different types of lipoprotein particles circulate in the plasma, The lipoproteins present in plasma are: chylomicrons (CM), very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), Intermediate – density lipoprotein (IDL) and high-density lipoproteins (HDL) (Jonas A, 2002).

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1-13-1-3-1- High Density Lipoprotein (HDL):

HDL are often called good cholesterol. They remove excess cholesterol from tissues and vessel walls and carry it to the liver where it is removed from blood and discarded. The amount of serum HDL is inversely correlated with the incidence of cardiovascular diseases, the higher level the lower risk of atherosclerosis and vice versa. HDL carries cholesterol in the blood from other parts of the body back to the liver for excretion. So HDL helps keep cholesterol from building up in the walls of the arteries (Stein and Stein, 1999; Das, 2003; Bandeali and John, 2012).

1-13-1-3-2- Low Density Lipoprotein (LDL):

LDL are often called bad cholesterol, they are formed primarily by the break down of VLDL. They contain little triglycerides and huge amount of cholesterol (60-70% of total cholesterol). Although the particles are much smaller than chylomicron and VLDL, LDL particles can vary in size and chemical structure. These variations represent subclasses within the LDL class. Serum from a human being with great quantity of LDL will be clear. LDL carries cholesterol in the blood and deposits it in the body tissues and in the walls of blood vessels, a condition known as atherosclerosis (Murray *et al.*,2000). LDL carries most of the cholesterol in the blood, and the cholesterol from LDL is the main source of damaging buildup and blockage in the arteries. Thus, the more LDL we have in our blood, the greater our risk of heart disease (Segrest *et al.*,2001).

1-13-1-3-3- Very Low Density Lipoprotein (VLDL):

VLDL is made in the liver by the combination of cholesterol, triglycerides and apoprotein. VLDL transports endogenous triglycerides, phospholipids, cholesterol and cholesteryl esters. It functions as the body's internal transport mechanism for lipids. VLDL levels have been correlated with accelerated rates of atherosclerosis, and are elevated in a number of diseases and metabolic states. These lipoprotein particles are smaller than chylomicrons and contain less

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triglyceride but contain significant amount of cholesterol (10-15% of cholesterol). As the VLDL circulates in the blood, triglycerides are deposited and the particles get smaller, eventually becoming low density lipoproteins (LDL). Serum with large mass of VLDL will be cloudy (McEneny *et al.*, 2000; Niu and Evans, 2011).

1-14 – Atherogenic Index :

Atherogenic lipoprotein profile of plasma is an important risk factor for coronary heart disease (CHD). It is characterized by high ratio of low-density lipoprotein cholesterol (LDL-C) to high-density lipoprotein cholesterol (HDL-C) (Campos *et al.*, 1992; Stampfer *et al.*, 1996).

The atherogenic index of plasma (AIP) also defined as the base 10 logarithm of the ratio of the concentration of triglyceride (TG) to high density lipoprotein cholesterol (HDL-C), where each concentration is expressed in mmol/L (Asztalos *et al.*, 2005; Kanthe *et al.*, 2012). This quantity has been shown to reflect the distribution of particle sizes in lipoprotein subclasses and correlates significantly with the presence of risk factors for atherosclerosis such as gender, age, dyslipidemia and diabetes as well as positive findings on coronary angiography (Dobiasova *et al.*, 2011).

1-15- Aim of the Study:

In the present study, an attempt has been made to assess the preventive effects of phenolic extract of tubers of *Cyperus rotundus* and taurine against isoproterenol-induced myocardial infarction in rats, an experimental animal model for myocardial infarction of human beings, as following:-

The main objectives of the work are:

- 1- Extraction of phenolic compounds from tubers of *Cyperus rotundus*, Identification of phenolic compounds containing in the extract and determination of phenolic compounds containing in the extract.

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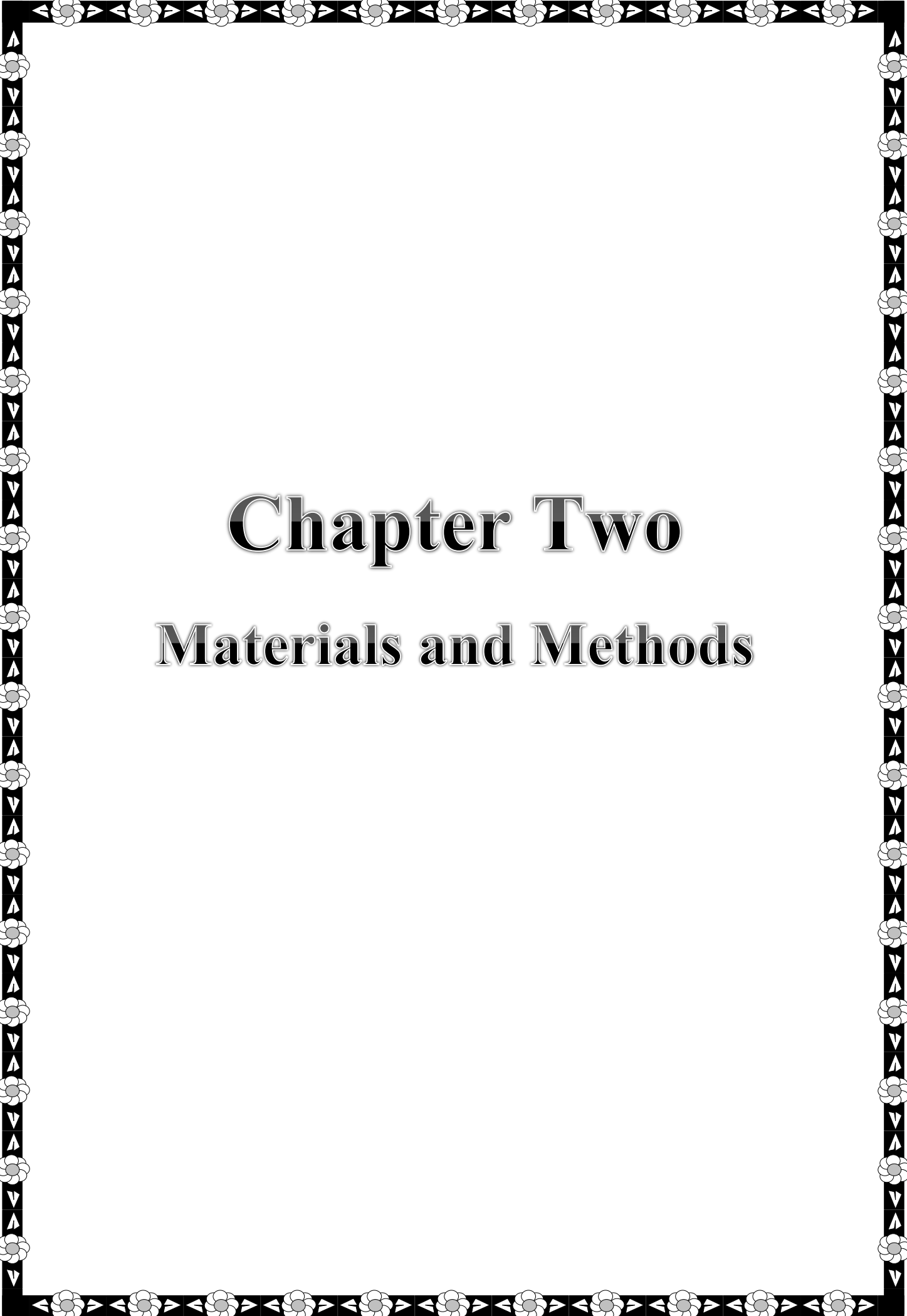
- 2- Investigation of the cardio protective effects of phenolic extract of tubers of *Cyperus rotundus* and taurine in experimentally induced myocardial infarction by assaying the levels of serum cardiac enzymes and troponin I.
- 3- Investigation of the antioxidant activity and the antilipidemic of phenolic extract of tubers of *Cyperus rotundus* and taurine in experimentally induced myocardial infarction.
- 4- Assay of malondialdehyde (MDA) and glutathione (GSH) using the heart tissue homogenate.
- 5- Study of the histopathological pattern to confirm the protective action of phenolic extract of tubers of *Cyperus rotundus* and taurine against isoproterenol-induced myocardial infarction in rats.

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Chapter Two

Materials and Methods

2- Materials and Methods

2-1-Materials

2-1-1- Chemicals :

The study used specific chemicals which are shown with their suppliers in Table (2-1).

Table (2-1): Chemicals and their suppliers.

No	Material	Supplied Company
1	Acetic acid	BDH(England)
2	Acetic acid anhydrate	BDH(England)
3	Alanine transaminase (ALT) Kit	Randox (UK)
4	Albumin kit	Biolabo (France)
5	Ammonia	BDH(England)
6	Aspartate transaminase (AST) Kit	Randox (UK)
7	Chloroform	BDH(England)
8	Cholesterol kit	Biolabo (France)
9	Copper sulfate	BDH(England)
10	Creatine Phosphokinase (CPK) Kit	Boilabo (France)
11	Di ethyl Ether	BDH(England)
12	Eosin stain	BDH(England)
13	Ethanol	BDH(England)
14	Ferric chloride	BDH(England)
15	Formalin	BDH(England)
16	Glacial acetic acid	BDH(England)
17	Heamatoxylin stain	Fluka Chemical AG Switzerland
18	n- Hexane	BDH(England)
19	Hydrochloride acid	BDH(England)

20	Iodide	BDH(England)
21	Isoproterenol hydrochloride	Cayman(British)
22	Lactate dehydrogenase (LDH)	Biolabo (France)
23	Lead acetate	BDH(England)
24	Mercury Chloride	Riedel dehaenage(Germany)
25	Methanol	BDH(England)
26	n-Propanol	BDH(England)
27	Paraffin wax	Merck (Germany)
28	Para-phenylene diamine (PPD)	BDH(England)
29	Potassium citrate	BDH(England)
30	Potassium hydroxide	BDH(England)
31	Potassium iodide	BDH(England)
32	Reduced Glutathione	CDH(India)
33	Sodium acetate trihydrate	BDH(England)
34	Sodium azide	BDH(England)
35	Sodium carbonate anhydrate	BDH(England)
36	Sodium hydroxide	BDH(England)
37	Sodium citrate	BDH(England)
38	Sodium chloride	BDH(England)
39	Sodium phosphate acidic NaH_2PO_4	Fluka Garnatie (Switzerland)
40	Sodium phosphate basic Na_2HPO_4	Fluka Garnatie (Switzerland)
41	Sulfuric acid	BDH(England)
42	Taurine	BDH(England)
43	Thio barbituric acid (TBA)	BDH(England)
44	Trichloro acetic acid (TCA)	BDH(England)
45	Tris- HCl	BDH(England)
46	Troponin Kit	My Biosource (USA)

47	Xylene	FlukaGarnatie (Switzerland)
48	α -naphthol	BDH(England)

2-1-2- Equipments and Apparatus :

Table (2 –2): explained the equipments and apparatus which were used in this study.

Table (2–2): The Equipments and their manufactures.

No	Equipments	Supplied company
1	Animals balance _ Stanton 461	Germany
2	Centrifuge	Universal 16A, (Germany)
3	Digital camera	Nikon (Japan)
4	Elisa	Bio Tek , P.N 7331000 ,USA
5	High-Performance Liquid Chromatography (HPLC)	Shimadzu 10AVP.(Japan)
6	Incubator	Memmert , (Germany)
7	Light microscope	Olympic (Japan)
8	Mixer	(China)
9	Oven	Gallen Kamp, (England)
10	Rotary evaporator	Buchi (switzland)
11	Rotary microtome	Reichert- Jwng 2030
12	Sensitive balance	Denver(Germany)
13	UV/VIS spectrophotometer	T 60, PG Instruments Ltd, (Germany)
14	Vacuum pump	PG Instruments Ltd, Germany
15	Water bath	Thomas Sc. Ap.(England)

2-1-3- Plant Collection :

Samples of tubers of *Cyperus rotundus* were collected from local market of Nasiriyia city, Thi-Qar, Iraq. It cleaned after that broke and grinded it by using electric grinder.

2-2- Methods:**2-2-1- Extraction of Phenolic Compounds:**

(500 g) of plant powdered material was defatted by washing five times with n-hexane (1L) at (60°C), then it was mixed with (800mL) of acetic acid (2% v/v), the mixture was placed in conical flask volume (2000mL) and put in water bath (60°C) for 8 hrs, then the extraction process done by reflex condenser. The mixture was heated at 50°C (water bath) for 15 min and left to cool. The suspension was filtered by Buchner funnel by Whitman No.1 filter paper and by the use of vacuum pump. The precipitate was canceled and the filtrate volume was measured.

n-propanol was added in to filtrate with the same volume of filtrate. Then (NaCl) was added until it become solution super saturated. Then, it was evaporator by using rotary evaporator until drying (Gayon, 1972).

2-2-2- Qualitative Tests of Some Active Compounds in Plant**Extract:****2-2-2-1- Detection of Phenolic Compounds in Plant Extract:**

Filter paper (Whitman No.1) was wetted in (2mL) from extract and some drops from ferric chloride(1%) was added to it after that it was exposed to ammonia vapor, appearance bluish- green color indicate presence of phenolic compounds (Gayon, 1972).

2-2-2-2- Detection of Flavonoid Compounds in Plant Extract:

(1mL) of alcohol potassium hydroxide reagent (Ethanolic KOH (5N)) was added to (1mL) of extract, appearance of yellow precipitate indicates presence of flavonoids (Harbone, 1984) .

2-2-2-3- Detection of Tannin Compounds in Plant Extract:**1- Lead acetate hydrate (1%) reagent:**

(1mL) of Lead acetate hydrate was added to (1mL) of extracts, appearance of white or light brown precipitate indicated presence of tannins.

2- Ferric chloride (1%) reagent:

Some drops of these reagent was added to (1mL) of extracts, appearance bluish green color indicated presence of tannins (Harbone, 1984).

2-2-2- 4- Detection of Alkaloid Compounds in Plant Extract:

Wagner reagent: It was prepared by (2g) of iodine and (6g) of potassium iodide were dissolved in (100mL) of distilled water.

Some drops of this reagent was added to (1mL) of extracts, appearance reddish brown precipitate indicated presence of alkaloids (Harbone, 1984).

2-2-2-5-Detection of Carbohydrates Compounds in Plant Extract Using Molisch Reagent:

(0.5mL) of α -naphthol (dissolve 1 gm α -naphthol in 25 mL of ethanol) was added to (1mL) of extracts in test tube with shaking thereafter concentration sulphuric acid was added slowly with slope, appearance violet ring indicated presence of carbohydrates (Hawk *et al* ., 1954) .

2-2-2-6- Detection of Saponin Compounds in Plant Extract :

(1mL) of extracts was added to (1mL) of HgCl_2 (5%), appearance white precipitate indicated presence of Saponins (Haddad, 1965).

2-2-2-7- Detection of Glycoside Compounds in Plant Extract**Using Benedict Reagent:**

Benedict reagent : it was prepared by dissolving (173 g) of potassium citrate and (100 g) of sodium carbonate anhydrate was solved in (800mL) of distilled water heated at (100°C). This solution was cooled and filtered. Copper sulfate(it was prepared by dissolving (17.30 g) of copper sulfate in (100mL) distilled water and completed the volume to (1L).Then the two prepared solutions were mixed with (1:1) volume ratio.

1- Before glycoside dissociation

(1mL) of Benedict reagent was added in test tube containing (1mL) of extract and heated in boiling water bath for (15 min), the appearance of red or brown precipitate indicated the presence of reduced saccharides.

2- After glycoside dissociation

Several drops of HCl were added to (5mL) of extracts and heated in water bath at (40°C) for (25 min) to cleavage the glycoside bonds, the solution was adjusted by added sodium hydroxide (2M) , thereafter equal volume from Benedict reagent was added to extract and this solution was heated for (10 min), appearance of red or brown precipitate more than the precipitate that appearance in first test indicates presence of glycosides (Al-khazrajy, 1991).

2-2-2-8-Detection of Triterpinoid Compounds in Plant Extract:

Adding (1mL) of concentration sulfuric acid to (1mL) of extracts that was dissolved in chloroform, the appearance purple red color indicated the presence of triterpinoids (Harbone, 1984).

2-2-2-9- Detection of Triterpine and Sterol Compounds in Plant Extract Using Liebermann – Burchard Reagent:

(10) drops of acetic anhydrate was added to (2mL) of solution of extracts in test tube thereafter two drops of $\text{Con.H}_2\text{SO}_4$ and this solution was mixed carefully to become color of solution dark blue and it was converted to green color that indicated presence of triterpines and sterols (Harbone, 1984).

2-2-3- Investigation of Active Compounds in Plant Extract by UV-VIS. (Spectrophotometer):

The absorption spectra of plant constituents can be measured in very dilute solution against a blank solvent by using an automatic recording spectrophotometer. The solvent used for UV spectroscopy was water since most classes of compound showed some solubility in it. The method was performed by using water extraction. The sample solution absorbance was recorded by measuring the range scan from 190 nm to 800 nm on a double beam UV-VIS spectrophotometer (Ikbal, 2004).

2-2- 4 - Investigation of Phenolic Extract by HPLC Technique:

The extract were analyzed on HPLC (High- Performance Liquid Chromatography) column, C18-ODS (25 cm, 4.6 mm, 5 µm), using mobile phase of MeOH: Acetic acid: D.W.(10:2:88) mL (A) and MeOH: Acetic acid: D.W. (90: 3: 7)mL (B) at flow rate of 1.0 mL/min. Analysis was performed using the following gradient elution (0-4min) 40%, (5-8 min) 50%, (9-10min) 60% (A)., detection UV at 254 nm (Bharathi *et al.*, 2010).

The separation occurred on liquid chromatography Shimadzu 10 AV-LC equipped with binary delivery pump model LC-10A Shimadzu, the eluted peaks were monitored by UV-Vis 10 A-SPD spectrophotometer.

Calculation:

$$\text{Sample Conc. (ppm)} = \frac{\text{Conc.of Standard} \times \text{Area of Sample}}{\text{Area of Standard}} \times \frac{\text{dilution factor}}{\text{Weight o f Sample}}$$

2-2-5- Animals and Housing:

Sixty adult female rats (*Rattus norvegicus*) weighing (190-200 g) of 9-10 weeks old were used in the present study. Animals were housed in the animal house of the Biology Department, College of Science, Thi-Qar University, Iraq. Experiments were achieved between January -2017 / February-2017. Animals were housed in iron boxes bedded with wooden chips. During the experimental period six animals were kept in each box and they were housed under standard laboratory conditions (12h light: 12h dark photoperiod (LD) at 22 ± 2 C° and relative humidity 45-55% (Coskun *et al.*, 2004). Animals were fed on standard rat pellet and tap water *ad libitum*. The standard pellet contains wheat 66.6%, soya 25.6%, and sun flower oil 4.4%, lime stone 1.5%, salt 0.63%, methionine 0.158%, choline chloride 0.062% and trace elements 0.05% (Krinke, 2002).

2-2- 6- Induction of Myocardial Infarction:

Myocardial Infarction was induced in female rats by intraperitoneally injection (IP) of Isoproterenol hydrochloride ISO (85mg/kg bw). ISO solution was given once a day for two days (22th and 23th day) after the experimental period. ISO was first weighed individually in eppendorf tubes for each animal according to the weight and then solubilized in 0.5 mL of distill water, just prior to the injection (Hassan *et al.*, 2015).

2-2-7- Administration of Laboratory Animals:

Laboratory animals were divided into ten groups (6 rats in each group), designed as follows:

Group-1: a control group; treated orally with distill water for 21 days .

Group-2: ISO (Isoproterenol hydrochloride) group; were injected I.P. with (85mg/kg, body weight) of ISO, twice an interval of 24 h , i.e ., on 22th and 23th day).

Group-3: treated orally with 15 mg /kg of phenolic extract of tubers of *C. rotundus* once daily for 21 days .

Group-4: treated orally with 30 mg /Kg of phenolic extract of tubers of *C. rotundus* once daily for 21 days .

Group-5: injected I.P. with (100mg/kg) of taurine for 21 days.

Group-6: injected I.P with (200mg/kg) of taurine for 21 days.

Group-7: pretreated orally with (15mg/kg) of phenolic extract of tubers of *C. rotundus*. once daily for 21 days, then injected I.P. with (85mg/kg) of ISO, twice an interval of 24 h, i.e., on 22th and 23th day).

Group-8: pretreated orally with (30mg/kg) of phenolic extract of tubers of *C. rotundus*. once daily for 21 days, then injected I.P. with (85mg/kg) of ISO, twice an interval of 24 h, i.e., on 22th and 23th day).

Group-9: injected with (100mg/kg). of taurine once daily for 21 days, then injected I.P. with (85mg/kg) of ISO, twice an interval of 24 h, i.e., on 22th and 23th day).

Group-10: injected with (200mg/kg) of taurine once daily for 21 days, then injected I.P. with (85mg/kg) of ISO, twice an interval of 24 h , i.e ., on 22th and 23th day).

2-2- 8- Blood Samples:

8 mL of blood were drawn from each animal in the experimental groups, by heart puncture method after 12 hours fast. Using 60 gauge syringes, the sample was transferred into clean tube, left at room temperature for 15 minutes for clotting, centrifuged at 3000 rpm for 15 minutes, and then serum was separated and kept in a clean tube in the refrigerator at (-20°C) until the time of assay.

2-2-9- Tissue Homogenate Preparation:

The heart tissues were dissected out and washed in ice-cold saline. The tissue (100 mg) was weighed and homogenized in 5 ml of 0.1 M Tris-HCL buffer (pH 7.4) in ice-cold condition. The homogenate was centrifuged at 2500 g . The serum and tissue homogenate were used for various biochemical analysis.

2-2-10- Biochemical Parameters:**2-2-10-1- Determination of Troponin I (cTnI)I Elisa kit Level:****1- Principle of the Troponin Elisa :**

cTnI Elisa kit applies the competitive enzyme immunoassay technique utilizing a monoclonal anti-cTnI antibody and an cTnI-HRP conjugate. The assay sample and buffer are incubated together with cTnI-HRP conjugate in pre-coated plate for one hour. After the incubated period, the wells are decanted and washed five times. The wells are then incubation with a substrate for HRP enzyme. The product of the enzyme – substrate reaction forms a blue complex. Finally, a stop solution is added to stop the reaction , which will then turn the solution into yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the cTnI concentration since cTnI from samples and cTnI-HRP conjugate compete for the anti-cTnI antibody binding site. Since the number of sites is limited , as more sites are occupied by cTnI from the sample, fewer sites are left to bind cTnI –HRP conjugate . A standard curve is plotted relating the intensity of the color (O.D.) to the concentration of standards. The cTnI concentration in each sample is interpolated from this standard curve.

2- Materials:

All reagents provided are stored at 2-8° C. Refer to the expiration data on the label .

	Materials	Specification	Quantity
1	Microtiter plate	96 wells	Stripwell
2	Enzyme conjugate	6.0 mL	1 vial
3	Standard A	0 ng/mL	1 vial
4	Standard B	0.5 ng/mL	1 vial
5	Standard C	1.0 ng/mL	1 vial
6	Standard D	2.5 ng/mL	1 vial
7	Standard E	5.0 ng/mL	1 vial
8	Standard F	10 ng/mL	1 vial
9	Substrate A	6 mL	1 vial
10	Substrate B	6 mL	1 vial
11	Stop Solution	6 mL	1 vial
12	Wash solution (100x)	10 mL	1 vial
13	Balance solution	3 mL	1 vial

Note: The Balance Solution was used only when the sample is cell culture supernatants, body fluid and tissue homogenate; if the sample is serum or plasma, then the Balance solution is a superfluous reagent.

3- Reagents Preparation :

Bring all kit components and samples to room temperature (20-25 °C) before use.

4- Assay Procedure :

1- Secured the desired numbers of coated wells in the holder then add 100 μ L of **standards** (Shake the bottle of each standard gently by hand and pipette up and down the solution of standard for 3 times before adding) or **samples** to the appropriate well. Add 100 μ L of PBS (pH 7.0 – 7.2) in the blank control well .

2- Dispensed 10 μ L of **balance solution** into 100 μ L samples only , mix well . (**Note** : This step is required when the samples is cell culture supernatants , body fluid and tissue homogenate ; if the samples is serum or plasma , then this step should be skipped).

3- Added 50 μ L of **conjugate** to each well (not blank control well). Mixing well in this step is important. Cover and incubate the plate for 1 hour at 37 °C.

4- Washed the microtiter plate using :-

Manual Washing : Removed incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with 1 $\times$ wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of FIVE washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.

5- Added 50 μ L **substrate A** and 50 μ L **substrate B** to each well including blank control well, subsequently. Cover and incubate for 10-15 minutes at 37°C. (Avoid sunlight If the color is not dark, please prolong the incubation time. But the longest time is 30min).

6- Added 50 μ L of **stop solution** to each well including blank control well. Mix well.

7- Determined the Optical Density (O.D.) at 450 nm using a microplate reader immediately.

6- Calculation of the Results:

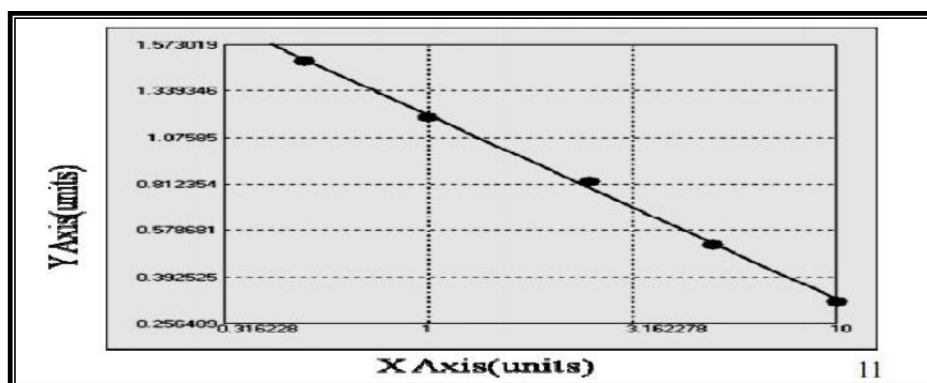
1- The standard curve was used to determine the amount of samples.

2- First, average the duplicate readings for each standard and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation. DO NOT subtract the O.D of standard zero.

3- Constructed a standard curve by plotting the average O.D. for each standard on the horizontal (X) axis against the concentration on the vertical (Y) axis, and draw a best fit curve using graph paper or statistical software to generate a four parameter logistic (4-PL) curve-fit or logit-log linear regression curve. An x-axis for the optical density and a y-axis for the concentration is also a choice. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.

4- Calculated the concentration of samples corresponding to the mean absorbance from the standard curve.

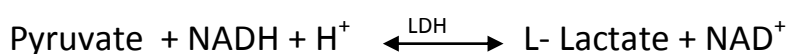
5- Standard curve for demonstration only.



2-2-10-2- Determination of Serum Lactate Dehydrogenase (LDH) Activity:

Principle:

Henry and al. method (according to SFBC recommendations). Reaction scheme is as follows :



The decrease in absorbance due to the conversion of NADH to NAD⁺ directly proportional to LDH activity in the specimen , is measured at 340 nm (Young , 1995).

Reagents Composition :

Reagent	Composition	
Reagent1 Substrate - Buffer	Buffer Tris pH 7.2	80 mmol/L
	Pyruvate	1.6 mmol/L
	Preservative	
Reagent2 Coenzymes	NADH	≥ 0.20mmol/L
	NaCl	200mmol/L

Reagents Preparation :

Added promptly (3mL) of vial R1 (Buffer) into the content of vial R2 (Coenzyme). Mixed gently and waited for complete dissolution before using reagent (approximately 2 minutes).

Manual Procedure :

stand reagents and specimens were left at room temperature.

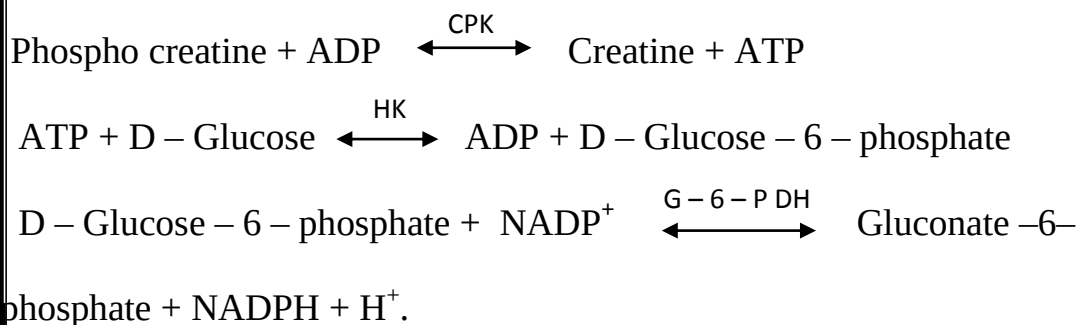
Pipette into 1cm path length thermo stated cuvette	
Working reagent	1mL
Bring to (37°C), then added:	
Specimen	20 µL
Mixed. Started a timer .After 30 seconds , recorded initial absorbance at 340nm (or 334 nm) record the absorbance again after 1 minute and 2 minute. Calculated absorbance change per minute ($\Delta abc/min$).	

Calculation:

$IU/L = (\Delta abc /min) \times 8095 \text{ at } 340 \text{ nm} .$

2-2-10-3- Determination of Serum Creatine Phospho**Kinase(CPK) Activity:****Principle :**

Enzymatic method described by Oliver and Biochem (1955) modified by Rosalki (1967) and later by Szasz (*et al.*, 1976).



The increase in absorbance, proportional to CK activity in the specimen, was measured at 340nm (Oliver and Biochem , 1955; Rosalki, 1967 ; Szasz *et al.*, 1976).

Reagents Composition:

Working reagent composition according to IFCC recommendation.

AMP(Adenosine 5 monophosphate) 5mmol/l.

NADP(Nicotine amide adenine dinucleotide phosphate).....2mmol/l.

AP5A(Diadenosine 5 penta phosphate).....10mmol/l.

EDTA(Ethylen diamine tetra acetic acid).....2mmol/l.

Mg+2.....10mmol/l.

ADP(Adenine dinucleotide phosphate).....2mmol/l.

D-Glucose.....20mmol/l.

N-Acetyl-L-cysteine.....20mmol/l.

HK(Hexokinase).....>3000UI/L.

G-6-PDH(Glucose-6-phosphate dehydrogenase)....>2500UI/L.

Imidazole Acetate pH 6.7.....100mmol/l.

Creatine phosphate.....30mmol/l.

Contains also surfactants and stabilizers (Horder, 1991).

Reagents Preparation :

Added promptly (3mL) of vial R2 (Buffer) into the content of vial R1 (Enzyme-Substrate). Mixed gently and waited for complete dissolution before using reagent (approximately 2 minutes).

Manual Procedure:

stand reagents and specimens were left at room temperature.

Pipette into 1cm path length thermo stated cuvette	
Working reagent	1mL
Bring to (37°C), then added:	
Specimen	50 µL
Mixed. Started a timer and recorded initial absorbance at 340nm after 2 min. the absorbance was recorded again every minute during 3 minutes.	
Absorbance was calculated change per minute ($\Delta abc/min$).	

Calculation:

The result was Calculated as follows :

$$IU/L = (\Delta abc/min) \times 3333.$$

2-2-10-4 - Determination of Serum Alanine Amino Transferase (ALT)Enzyme :

Principle:

α -oxoglutarate + L-alanine $\xrightleftharpoons{ALT}$ L- glutamate + pyruvate
 Alanine aminotransferase is measured by monitoring the concentration of

pyruvate hydrazone formed with 2,4-dinitrophenyl-hydrazine (Retiman and Frankel, 1957).

Reagent Composition:

Contents	Initial Concentration of Solutions
R1. Buffer Phosphate buffer L-alanine α -oxoglutarate	100mmol/l, PH 7.4 200 mmol/l 2.0 mmol/l
R2. 2,4-dinitrophenylhydrazin	2.0 mmol/l
R3. Sodium Hydroxide	4.0 mol/l

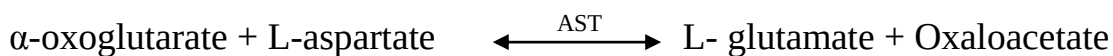
Procedure:**Measurement Against Reagent Blank :**

	Reagent Blank	Sample
Sample	-----	0.1 ml
Buffer (R1)	0.5 ml	0.5ml
Distilled Water	0.1ml	-----
Mix , incubate for exactly 30 min . at 37 °C.		
2,4-dinitrophenylhydrazine (R2)	0.5 ml	0.5 ml
Mix , allow to stand for exactly 20 min . at 20 to 25 °C.		
Sodium Hydroxide (R3)	5.0 ml	5.0 ml
Mix , read the absorbance of sample(A sample) against the reagent blank after 5 minutes at 546 nm .		

Calculation:

Obtained the activity of ALT in the serum from the table:

Absorbance	U/L	Absorbance	U/L
0.025	4	0.275	48
0.050	8	0.300	52
0.075	12	0.325	57
0.100	17	0.350	62
0.125	21	0.375	67
0.150	25	0.400	72
0.175	29	0.425	77
0.200	34	0.450	83
0.225	39	0.475	88
0.250	43	0.500	94

2-2-10-5-Determination of Serum Aspartate Amino**Transferase(AST) Enzyme :****Principle:**

Aspartate Aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl-hydrazine (Retiman and Frankel, 1957).

Reagent Composition:

Contents	Initial Concentration of Solutions
R1. Buffer Phosphate buffer L-aspartate α-oxoglutarate	100mmol/l, PH 7.4 100mmol/l 2mmol/l
R2. 2,4-dinitrophenylhydrazin	2.0 mmol/l
R3. Sodium Hydroxide	

Procedure:**Measurement Against Reagent Blank :**

	Reagent Blank	Sample
Sample	-----	0.1 ml
Buffer (R1)	0.5 ml	0.5ml
Distilled Water	0.1ml	-----
Mix , incubate for exactly 30 min . at 37 °C.		
2,4-dinitrophenylhydrazine (R2)	0.5 ml	0.5 ml
Mix , allow to stand for exactly 20 min . at 20 to 25 °C.		
Sodium Hydroxide (R3)	5.0 ml	5.0 ml
Mix , read the absorbance of sample(A_{sample}) against the reagent blank after 5 minutes at 546 nm .		

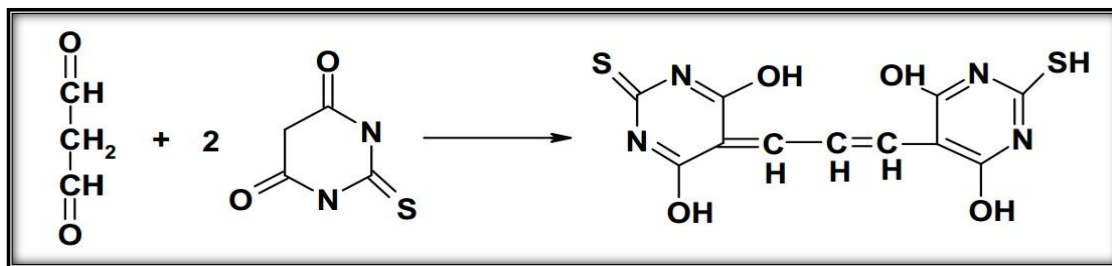
Calculation :

Obtained the activity of AST in the serum from the table:

Absorbance	U/L	Absorbance	U/L
0.020	7	0.100	36
0.030	10	0.110	41
0.040	13	0.120	47
0.050	16	0.130	52
0.060	19	0.140	59
0.070	23	0.150	67
0.080	27	0.160	76
0.090	31	0.170	89

2-2-10-6-Determination of Serum Malondialdehyde (MDA)**Concentration:****Principle:**

Lipid peroxidation was determined by using the thiobarbituric acid method. In this method, malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acids were identified as the product of LPO that react with thiobarbituric acid (TBA), in coexisting tri chloro acetic acid (TCA), to give a red chromophore absorbing at 535 nm (Fong *et al.*, 1973) MDA concentrations were calculated, using the molar extinction coefficient of MDA ($\mathcal{E}_{MDA}$) equal to $1.56 \times 10^5 \text{M}^{-1} \cdot \text{cm}^{-1}$ (Wills, 1963) Malondialdehyde (MDA) formed from breakdown of polyunsaturated fatty acid, serves as a convenient index of peroxidation reaction.



Procedure:

The level of serum MDA was determined spectrophotometrically with a thiobarbituric acid (TBA) solution, to 150 μ l serum sample the following were added: 1ml of 17.5% trichloro acetic acid (TCA) and 1ml of 0.66% TBA, mixed well by vortex, incubated in boiling water for 15 minutes, and then allowed to cool. One ml of 70% TCA was added and the mixture allowed to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15 minutes, the supernatant was taken out for scanning spectrophotometrically at (532nm) (Muslih *et al.*, 2002) The concentration of MDA calculated as follow:

$$MDA \left(\frac{\mu\text{mol}}{L} \right) = \frac{\text{absorbance at 532 nm}}{L \times \epsilon} \times D \times 10^6$$

L: light path (1cm)

ϵ : Extinction coefficient $1.56 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$

D: Dilution factor = 1 ml Vol. Used in ref./0.15 =6.7.

2-2-10-7-Determination of Tissue Homogenate Malondialdehyde (MDA) Concentration:

Principle :

The extent of lipid peroxidation was determined by assaying malondialdehyde (MDA) formation according to method of (Utely *et al.*, 1967) .

Reagents :

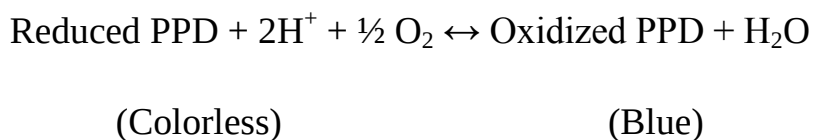
- 1- 10% TCA
- 2- 0.67 % TBA

Procedure :

To 1ml of heart homogenate, 2 ml of TCA and 4 ml of TBA were added, heated in a water bath for 30 minutes. After cooling and centrifugation, the absorbance of the supernatant was read at 535 nm. A reagent blank was prepared using water instead of tissue homogenate. The extent of lipid Peroxidation was expressed as nmole MDA formed/mg protein, using a molar extinction coefficient of MDA as $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

2-2-10-8- Determination of Antioxidant Parameters:**2-2-10-8- 1 Determination of Serum Ceruloplasmin (Cp)****Concentration:****Principle:**

Serum Cp concentration was measured by the method of (Menden *et al* .,1977). It is based on the ceruloplasmin-catalyzed oxidation of colorless para-phenylenediamine (PPD) to blue-violet oxidize form. The reaction is followed photometrically and the blank value is determined after inhibition of the enzyme with sodium azide at (0°C).



A mixture of serum, substrate and acetate buffer at pH = 6.0 was incubated at 37°C for 15 min. The reaction is stopped by the addition of sodium azide, and the absorbance of the purple color formed (Oxidized PPD) in the diluted test

mixture is read at 525 nm against blank solution. The corrected absorbance is directly related to the concentration of Cp (Ravin, 1961).

Reagents:

- Substrate solution:-

50 mg of PPD was dissolved in 5 mL (4 mL of distilled water (D.W) and 1 mL of glacial acetic acid). In another container, 8.15gm sodium acetate trihydrate was dissolved in 30 mL of D.W then added to first solution, mixed and completed the volume to 50 mL with distill water This solution is stable for three hours and is kept refrigerated in a dark bottle.

- Inactivating solution:-

100 mg of sodium azide was dissolved in (500 mL) of D.W then was kept cold in a refrigerator prior to use, this solution must be prepared every week.

Procedure:

One mL of substrate was pipetted into glass test tubes, incubated at 37°C for 1 min then (0.1 mL) of serum was added, incubated at 37°C for 15 min, then tubes were removed and placed in iced-bath for 30 sec five mL of cold inactivating solution was added, mixed under 25°C in water bath. Blank was prepared by combining the substrate, inactivating solution, and 0.1 mL of distil water, then the mixture was incubated as above. The absorbance of test and blank was read in spectrophotometer at 525nm. The concentration of Cp was calculated using the extinction coefficient of Cp ($\mathcal{E}_{Cp}$) equal to (0.68).

$$\text{Serum } Cp \left(\frac{mg}{L} \right) = \frac{A_{\text{test}} - A_{\text{blank}}}{\epsilon Cp} \times 10 = \frac{\Delta A \times 10}{0.68}$$

ϵCp = The extinction coefficient of Cp equal to 0.68.

2-2-10-8-2-Determination of Serum Albumin (Alb) Concentration:

Principle:

One of the most important serum proteins produced in the liver is albumin. There are techniques which have been developed to determine Alb concentration. The simplest technique is the bromocresol green (BCG) method, colorimetric method. The principle of measurement is based on the relatively high affinity of BCG towards Alb. Albumin in the presence of BCG at a slightly acid pH = 4.2, produces a color change of the indicator from yellow-green to green-blue. The intensity of the color formed is proportional to the Alb concentration in the sample, serum or plasma free of hemolytic (Dumas *et al.*, 1971 ; Dumas and Biggs, 1972).

Reagent Composition:

No	Contents	Concentrations of Solutions
R1 (Bromocresol Green)	Succinic acid	83mmol/L
	Bromocresol green	167 μ mol/L
	Sodium hydroxide	50mmol/L
	Polyoxyethylenemonolauryl ether	1gm/L
R2(standard)	Bovine albumin	5g/dL (725 μ mol/L)

Procedure:

Let stand reagents and specimen at room temperature .

Pipette into well identified test tubes	Blank	Standard	Assay
Reagent	2mL	2mL	2mL
Distill water	10 μ L		
Sample			10 μ L
Standard		10 μ L	
Mixed well. Recorded absorbances at 630nm (620-640 nm) within 3 minutes against reagent blank or better after 1 minute .			

Calculation:

The Concentration of serum Alb was calculated according to the equation below :

$$\text{Result} = \frac{\text{A sample}}{\text{A standard}} \times \text{Standard concentration}$$

Standard concentration = 5 g/dL (725 $\mu\text{mol/L}$)

2-2-10-8-3-Determination of Serum Uric acid Concentration:**Principle:**

Uricase acts on uric acid to produce allantoin ($\text{C}_4\text{H}_6\text{N}_4\text{O}_3$), carbon dioxide and hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase reacts with achromogen (amino-antipyrine and dichloro-hydroxybenzen sulfonate) to yield quinoneimine, a red coloured complex. The absorbance measured at 520 nm (490-530) is proportional to the amount of uric acid in the specimen. (Fossati *et al* ., 1980 ; Tietz *et al.*, 1999).

Reagents:

Solutions	Contents	Concentration
Vial R1 Enzymes	Potassium hexacyano ferrate(II) Peroxide Amino –antipyrine Uricase	42 $\mu\text{mol/l}$ $\geq 450\text{U/L}$ 0.125mmol/L $\geq 120\text{U/}$
Vial R2 Buffer	Dichlorohydroxybenzensulfonate Tris pH 8.0 at 25 C Presservative	2mmol/l 50 mmol/l
Vial R3 Standard	Uric acid	10mg/dl

ReagentPreparation:

Added promptly the contents of vial R1 (enzymes) into vial R2 (Buffer). Mixed gently until complete dissolution before using reagent (approximately 2 minutes).

Procedure :

Reagent and specimens was let standing at room temperature.

Pipette into well identified test tubes	Blank	Standard	Assay
Working reagent	1mL	1mL	1mL
Specimen			25µL
Standard		25µL	
Distill water	25µL		

Mixed. let standing for 5 minutes at 25° C absorbance was recorded at 520 nm (490 -530) against reagent blank. color was stable for 30 minutes .

Calculation :

The result is calculated as follows :

$$\text{Result} = \frac{\text{abs(assay)}}{\text{abs(standard)}} \times \text{concentration standard}$$

Standard concentration =10 mg /dL (595mol/L).

2-2-10-8- 4- Determination of Serum Reduced Glutathione Concentration:

The plasma thiol concentration was measured according to Ellman's method (1959).

Principle:

This method depend on the reaction of 5, 5'-dithio bis(2-nitrobenzioc acid) (DTNB)with aliphatic thiol compounds at pH8.0 to produce one mole of p-nitrothiol phenol anion per molethiol. Since this anion highly colored ($\epsilon=13600$ at 412 nm), it can be used to measure the thiol concentration. (Ellman, 1959).

Reagents:

1- Phosphate buffer solution with PH 7 and the concentration of 0.2 molar consists of:

- The solution A : the solution of Sodium phosphate acidic (NaH_2PO_4) with 0.2 molar is prepared by dissolved 2.4 mg of (NaH_2PO_4) into an amount of water and diluted to 100 ml.
- The solution B: the solution of Sodium phosphate basic (Na_2HPO_4) with 0.2 molar is Prepared by dissolved 2.83 mg of (Na_2HPO_4) into an amount of water and diluted to 100 ml.

In order to prepare the buffer solution , 19.5 ml of the solution A were mixed with 30.5 ml of the solution B and diluted to made up to 100 ml of the solution with PH 7.

2- Phosphate buffer solution with PH 8 and the concentration of 0.2 molar
This buffer solution is prepared by mixing 2.62 ml of 0.2M (NaH_2PO_4) with 47.38 ml of 0.2 M (Na_2HPO_4) and diluted to made up to 100 ml of pH8 solution.

3- DTNB solution (5,5-dithio-bis (2-Nitrobenzoic acid)) which is prepared by dissolved 39.6 mg of DTNB into 10 ml of the phosphate buffer solution pH 7.

Procedure:

- 1- Added 0.02 ml of the plasma to 9 ml of water.
- 2- Added 1 ml of phosphate buffer solution pH 8 to the solution.
- 3- Mixed 3 ml of the previous solution with 0.02 ml of DTNB solution.
- 4- Kept the result solution for 60 seconds at the room temperature.
- 5- Prepared the equivalent solution (Blank) as the steps 1 and 2.
- 6- Measured the absorbance of the result solution by using optical spectrum at the wavelength of 412 nm.

Calculation:

The total concentration of the Glutathione can be calculated as:

$$\text{GSH}(\mu\text{mol/L}) = (\text{T} - \text{B}) \times \frac{\text{D}}{\epsilon} \times 1000$$

Where:

T: the absorbance of the solution .

B: the absorbance of the equivalent solution (Blank).

D: the dilute factor.

ϵ : Molar extension coefficient equal to $13600 \text{ mol}^{-1}\text{L cm}^{-1}$

$$\frac{\text{D}}{\epsilon} = 36.8.$$

2-2-10-8-5- Determination of Tissue Homogenate Reduced Glutathione Concentration:

Principle:

Total reduced glutathione content was measured by following Ellman's method (1959). This method was based on the development of a yellow colour, when 5,5 -dithio bis(2-nitro benzoic acid (DTNB) reacts with the compounds containing sulphydryl groups with a maximum absorbance at 412nm.

Reagents:

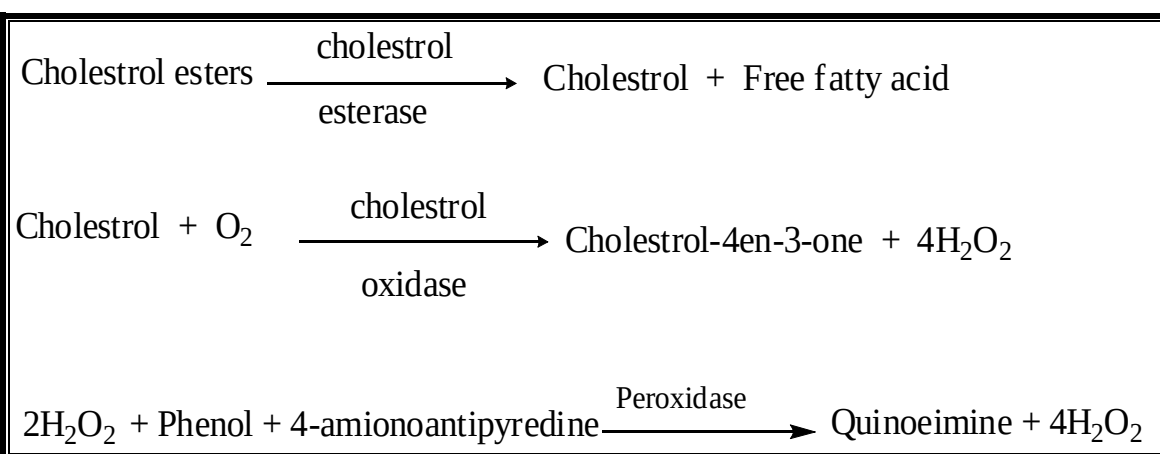
- 1- Phosphate buffer 0.2M, pH: 8.0
- 2- 5% TCA.
- 3- Ellman's reagent: 19.8mg DTNB in 100ml of 0.1% sodium citrate (Freshly prepared).
- 4- GSH standard: 20µg/ml

Procedure:

0.5ml of heart tissue homogenate was deproteinized with 3.5ml of 5% TCA and centrifuged . To 0.5ml of supernatant , 3.0 ml phosphate buffer and 0.5ml of Ellman's reagent were added and the yellow colour developed was read at 412nm. A series of standards (4-20µg) were treated in a similar manner along with a blank. Values are expressed as µg GSH/mg protein.

2-2-10-9- Determination of Serum Lipid profile:**2-2-10- 9- 1-Determination of Serum Total Cholesterol****Concentration:****Principle:**

Enzymatic method described by (Allian *et al.*, 1974; Allan and Dawson, 1979) has the following reaction:

**Reagents Composition:**

Reagent	Composition	
Reagent 1 Buffer	Phosphate buffer	100 mmol/L
	Chloro-4-phenol	5 mmol/L
	Sodium cholate	2.3 mmol/L
	Triton x 100	1.5 mmol/L
	Preservative	
Reagent 2 Enzymes	Cholesterol oxidase	≥100 IU/L
	Cholesterol estrase	≥ 170 IU/L
	Peroxydase	≥ 1200 IU/L

	4-aminoantipyrine	0.25 IU/L
	PEG 6000	167 µmol/L
Cholesterol Cal.	Cholesterol aqueous primary standard 200 mg/dL	

Reagents Preparation :

Added promptly the content of vial R2 (Enzymes) , into vial R1 (Buffer).
Mixed gently until complete dissolution (approximately 2 minutes).

Manual Procedure :

Let stand reagent and specimens at room temperature.

	Blank	Standard	Assay
Reagent	1 mL	1 mL	1 mL
D.W	10µL		
Standard		10µL	
Sample			10µL

Mixed Left stand for 5 minutes at 37°C or 10 minutes at room temperature.
Absorbance were recorded at 500 nm (480-520) against reagent blank. Colour is stable for 1 hour .

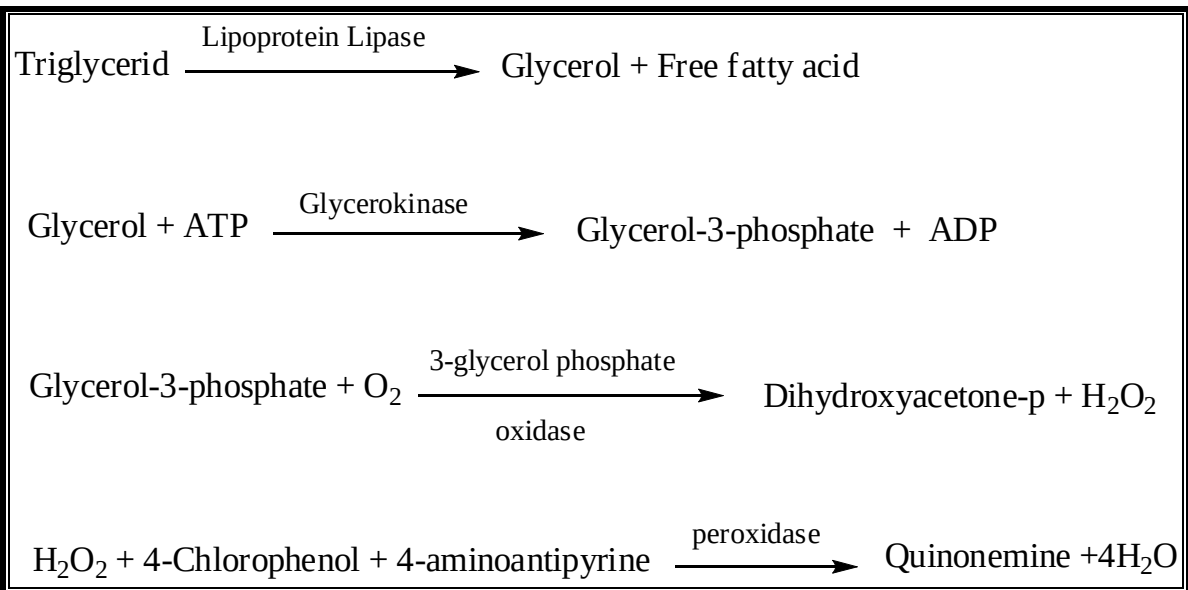
Calculation:

The result was calculated as the following:

$$\text{Result(mg/dl)} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times 200 \text{ (Standard concentration)}$$

2-2-10-9-2- Determination of Serum Triglyceride Conentration:**Principle:**

Fossati and Prencipe method associated with Trinder reaction scheme is as follows (Trinder, 1969; Fossati and Prencipe, 1982).



Reagents Composition:

Reagent	Composition	
Reagent1 Buffer	Pipes	100 mmol/L
	Magnesium chloride	9.8 mmol/L
	Chloro-4-phenol	3.5 mmol/L
Reagent2 Enzymes	Lipase	≥ 1000 IU/L
	Peroxidase	≥ 1700 IU/L
	Glycerol-3-phosphate oxidase	≥ 3000 IU/L
	Glycerol kinase	≥ 660 IU/L
	4-aminoantipyrine	0.5 mmol/L
	ATP	1.3 mmol/L
Reagent3 Standard	Glycerol	2.28 mmol/L
		(200 mg/dl)

Reagents Preparation:

Added promptly the content of vial R2 (Enzymes), into vial R1 (Buffer).
Mixed gently until complete dissolution (approximately 2 minutes).

Manual Procedure :

Let stand reagent and specimens at room temperature.

	Blank	Standard	Assay
Reagent	1 mL	1 mL	1 mL
D.W	10 µL		
Standard		10 µL	
Sample			10 µL

Mixed left stand for 5 minutes at 37°C or 10 minutes at room temperature. Absorbance were recorded at 500 nm (480-520) against reagent blank. Reaction is stable for 1 hour .

Calculation :

The result was calculated as the following:

$$\text{Result(mg/dl)} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times 200(\text{ Standard concentration})$$

2- 2- 10- 9- 3-Determination of Serum HDL Concentration:**Principle:**

According to (Lopes-Virella, 1977; Tietz *et al.*, 1999) Low density lipoproteins (LDL), very low density lipoproteins (VLDL)and chylomicrons from the sample are precipitated by phosphotungstic acid and magnesium chloride. HDL obtained in supernatant after centrifugation is then measured with total cholesterol reagent.

Reagents Composition:

Reagent	Composition	
Reagent 1	Phosphotungstic acid	13.9 mmol/L
Precipitant	Magnesium chloride	570 mmol/L
Reagent 2	Cholesterol	2.58 mmol/L
Standard		

Manual Procedure:

Sample preparation (without treating standard) :

Pipetted in centrifuge tube	Micromethod
Sample	500 μ L
Precipitant	50 μ L

Mixed vigorously, waited for 10 minutes at room temperature, centrifuged for 15 minutes at 3500 – 4000 RPM , aspirate the supernatant, reagent and supernatant were left at room temperature .

Pipette in well identified test tubes	Blank	Standard	Assay
Reagent2(Cholesterol assay)	1 mL	1 mL	1 mL
D.W	25 µL		
Standard		25 µL	
Supernatant (HDL)			25 µL

Mixed. Left stand for 5 minutes at 37°C or 10 minutes at room temperature. Absorbance were recorded at 500 nm (480-520) against reagent blank. Colour is stable for 1 hour .

Calculation:

The result was calculated as the following:

$$\text{Result(mg/dl)} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration} \times 1.1$$

2-2-10-9-4- Determination of Serum LDL and VLDL

Concentration:

According to (Friedwald *et al.*, 1972), LDL and VLDL concentrations were measured as follows :

$$\text{LDL(mg/dl)} = \text{Total cholesterol} - (\text{HDL} + \text{VLDL})$$

$$\text{VLDL(mg/dl)} = \text{serum TG} / 5.$$

2-2-10-10 – Determination of Atherogenic Index (ATI) :

ATI was calculated through the following equation :-

Atherogenic Index = LDL / HDL.

2-2-11- Histological Preparations:

The histological technique is used in the preparation of tissue according to (Luna, 1969) .Microscopic examination including :

2-2-11-1-Grossing :

Each tissue heart was grossed in aim to make it ready for further steps. Cross sectioning was done. In all grossing, the part which was cut from each specimen was in thick between 3-4 mm, which make the fixation satisfactory.

2-2-11-2 - Histological Technique :**2-2-11-2-1- Fixation :**

All samples instantly fixed in solution of 10% formalin , immediately after the kill and dissect the animal.

2-2-11-2-2 -Dehydration :

Tissues were dehydrating in an ascending grade of ethanol (30%, 50%, 70%, 80%, 90% and 100%) for 2 hours.

2-2-11-2-3 - Clearing :

After dehydration, tissues were clearing with chloroform for 12-24 hours.

2-2-11-2-4 -Impregnation :

This process was done by using paraffin wax at 60 C for 6 hours. In the first two hours the wax was twice renewed to riddance chloroform .

2-2-11-2-5- Embedding :

Samples embedded in paraffin wax melted at a temperature of 60 ° C in the inside private templates, templates left at room temperature so that the wax solidifies then refrigerate until ready for cutting .

2-2-11-2-6 -Sectioning:

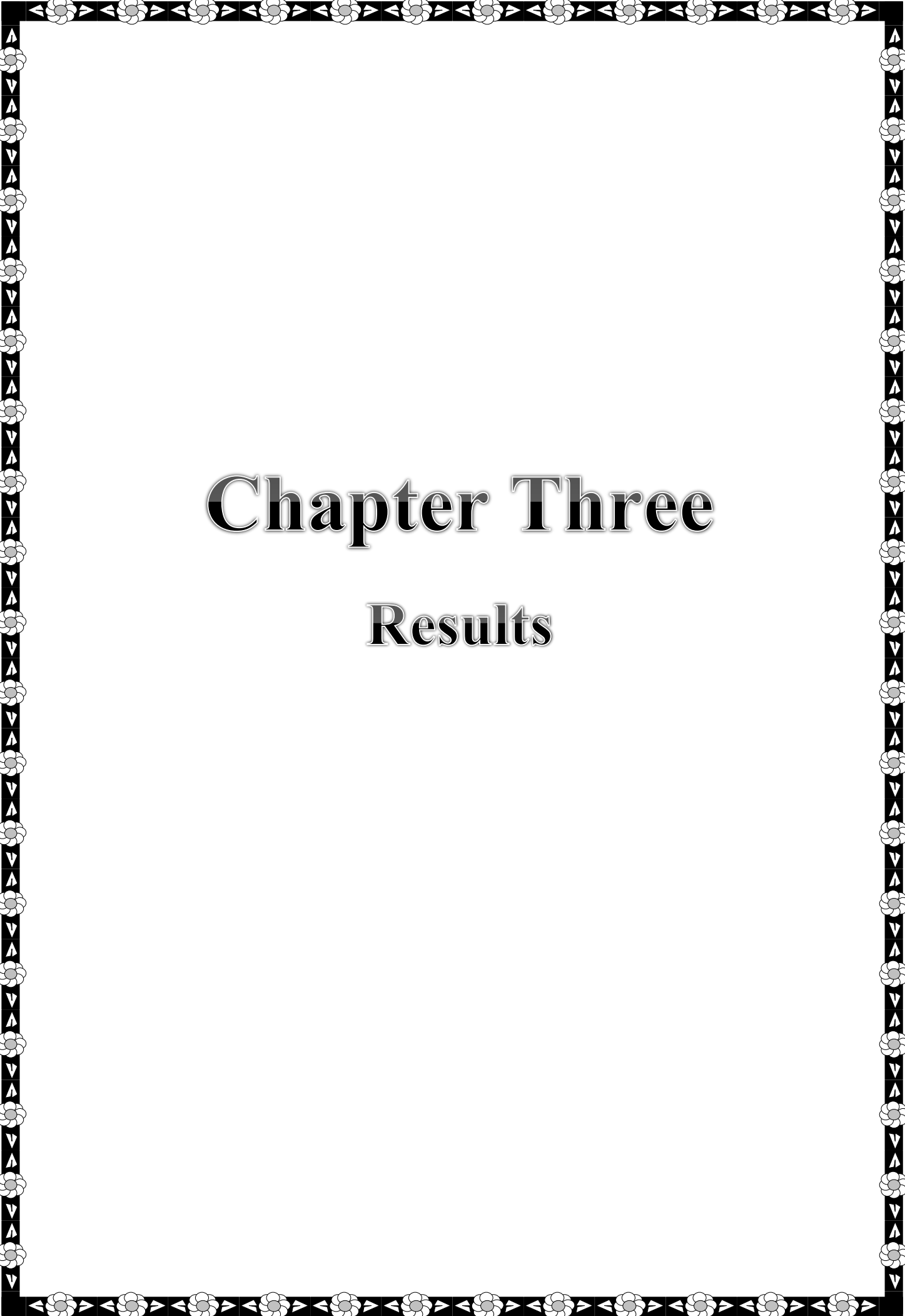
By using rotary microtome, blocks were first trimmed for removing excessive wax and then sectioned (5µm thickness).Ribbons resulting from sectioning process were floated in a warm water bath and mounted on clean slides covered with a thin layer of Mayer's albumin after their extension in water bath and left to dry at room temperature.

2-2-11-2-7 - Mounting and Staining :

To obtain stained section, first paraffin must be removed by xylene for 10 minutes. The deparaffin used sections were passed by descending concentration of ethyl alcohol (absolute,90%,80%,70%,50%,30%) for 2 minutes for each concentration ,stained with haematoxylin stain for 10 minutes and extensively washed with tap water .after that they were passed by ascending grade of ethanol (30%,50%,70%,80%,90%,100%) for 2 minutes each, stained with alcoholic eosin Stain for 2 minutes and then passed by absolute ethanol for 5 min. finally sections were cleared by xylene (5-10) min. mounted by DPX ,covered with cover slides and removed to dry at room temperature to be ready for microscopic examination by using light microscope.

2-3- Statistical Analysis :

Statistical analysis was done using the software **SPSS** version 15.0; The results were expressed as mean $\pm$ standard deviations (mean $\pm$ SD) with LSD. One way ANOVA-test was used to compare parameters in different studied groups. P-values ($P \leq 0.05$) were considered statistically significant.



Chapter Three

Results

3-Results

3-1- Plant Extract :

The weight of dry powder material of tubers of *Cyperus rotundus* was (500g). The weights of dry powder phenolic extract were (3.2 g) and percentage of extraction was (0.64%).

3-2- Qualitative Tests of Some Active Compounds in Plant Phenolic Extract :

Results of qualitative tests were shown in table (3-1). Generally, phenolic extract contains phenolic acids, flavonoids, tannins and glycosides, the presence of carbohydrate in extract may be due to glycosides content that was recognized by Bendict test. Whereas it does not contain terpenoids, terpenes and sterols. The results also showed that the extract does not contain alkaloids and saponins.

Table (3-1): Qualitative tests of some active compounds in plant phenolic extract.

Active principle	Test	Results
Phenolic compounds	$\text{FeCl}_3(1\%) + \text{ammonia vapor}$	+
Flavonoids	Alcohol KOH (5 N)	+
Tannins	$\text{Pb}(\text{AC})_3 (1\%)$	+
	$\text{FeCl}_3 (1\%)$	+
Alkaloids	Wagner	—
Carbohydrates	Molisch	+
Saponins	$\text{HgCl}_2 (1\%)$	—
Glycosides	Before analysis	+
	After analysis	+
Triterpenoids	$\text{CH}_3\text{Cl} + \text{H}_2\text{SO}_4$	—
Triterpenes and Sterols	Liebermann – Burchard	—

3-3- UV-Visible Spectra :

The UV-Visible analysis of the phenolic extract that were scanned at the range of (190-800nm), performed and gave a two peaks and very clear for

different compounds of sample (figure 3-1). The main peak absorbed at (205) for $\pi\text{-}\pi^*$ electronic transitions and another single peak at (288) which is of low intensity appear in wave longer length than the first peak due to $n\text{-}\pi^*$ electronic transitions .

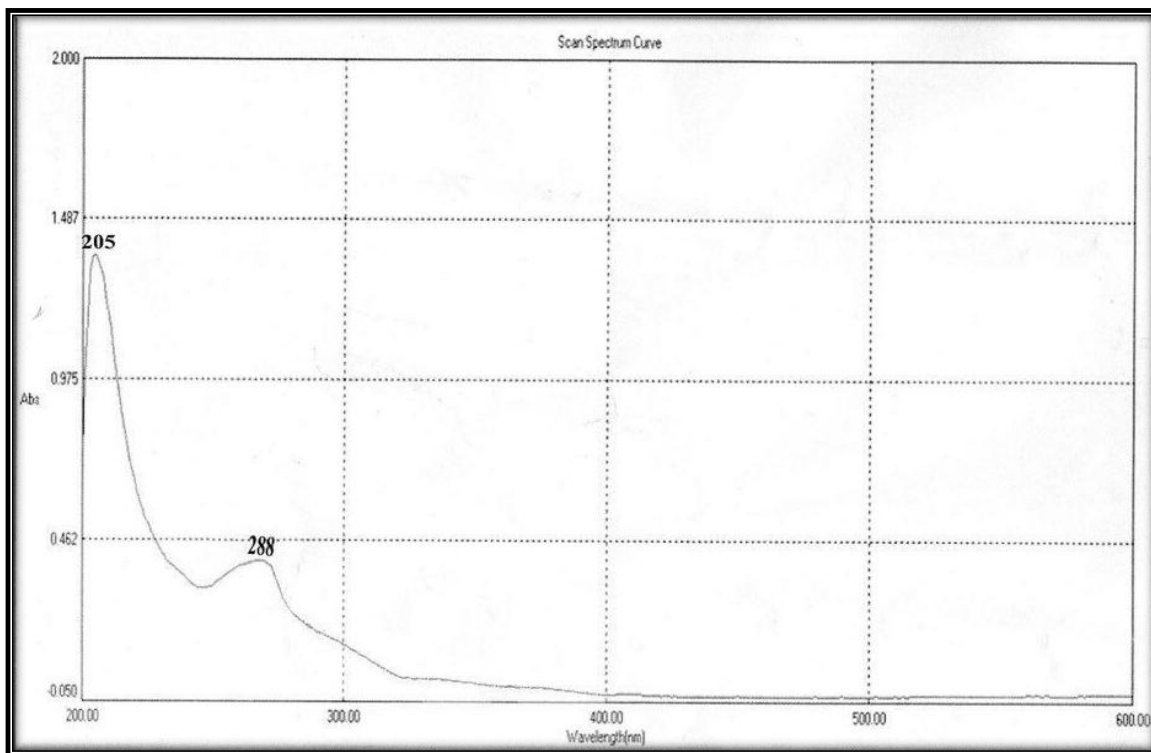


Figure (3-1): Absorption spectrum of phenolic extract by (UV-Scan) in water.

3-4-Determination of Plant Phenolic Compounds by HPLC

Technique:

The plant extract was analyzed to estimate their contents of phenolic compounds. The identification of each compound was based on retention time in comparison with pure commercial standards (Shindalkar *et al.*, 2005). The figures (3-2), (3-3), (3-4), (3-5), (3-6), (3-7) and (3-8) explained that the retention time of sample agrees with the standard retention time of

most contents in the extract, and the tables (3-2), (3-3), (3-4), (3-5), (3-6), (3-7) and (3-8) showed the retention time of standards and the phenolic compounds.

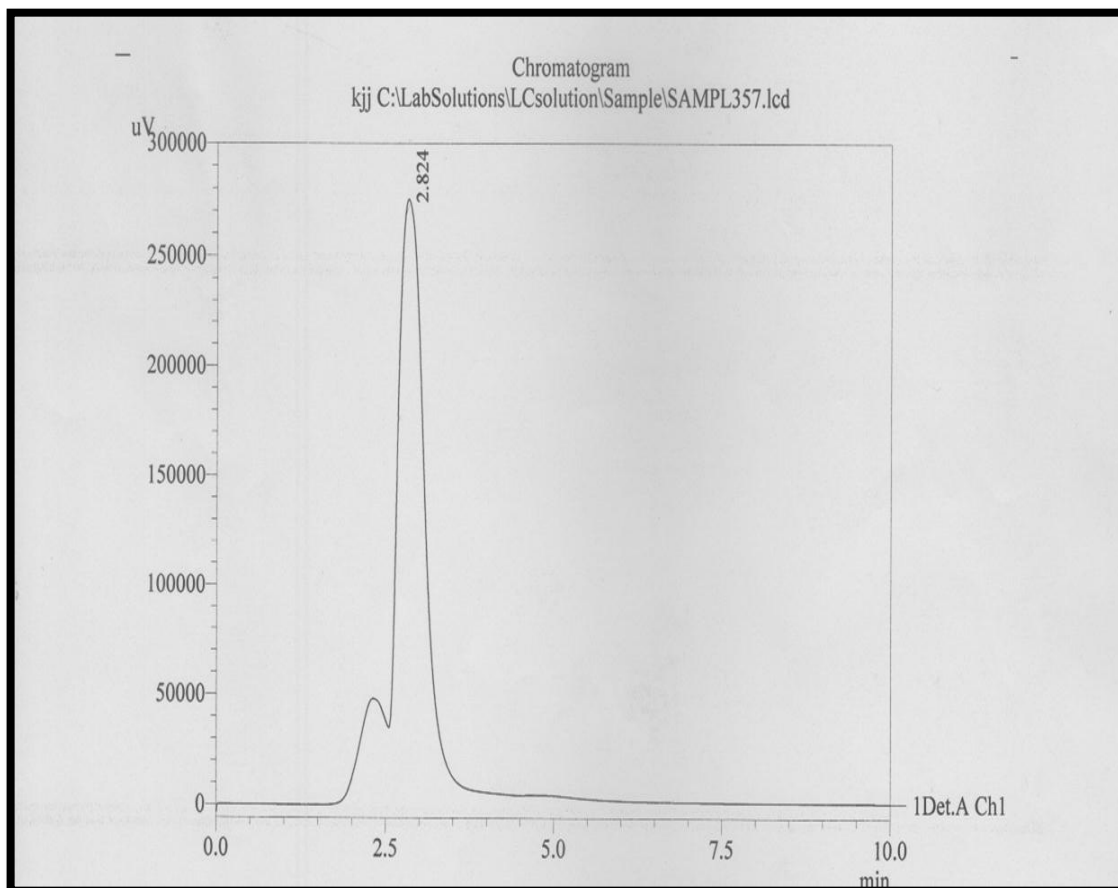


Figure (3-2) : Shows the HPLC chromatogram of standard tannic acid compound

Table (3-2) : Shows the retention time of standard tannic acid compound .

Peak	Standard	Retention time (min)	Area
1	Tannic acid	2.824	10557

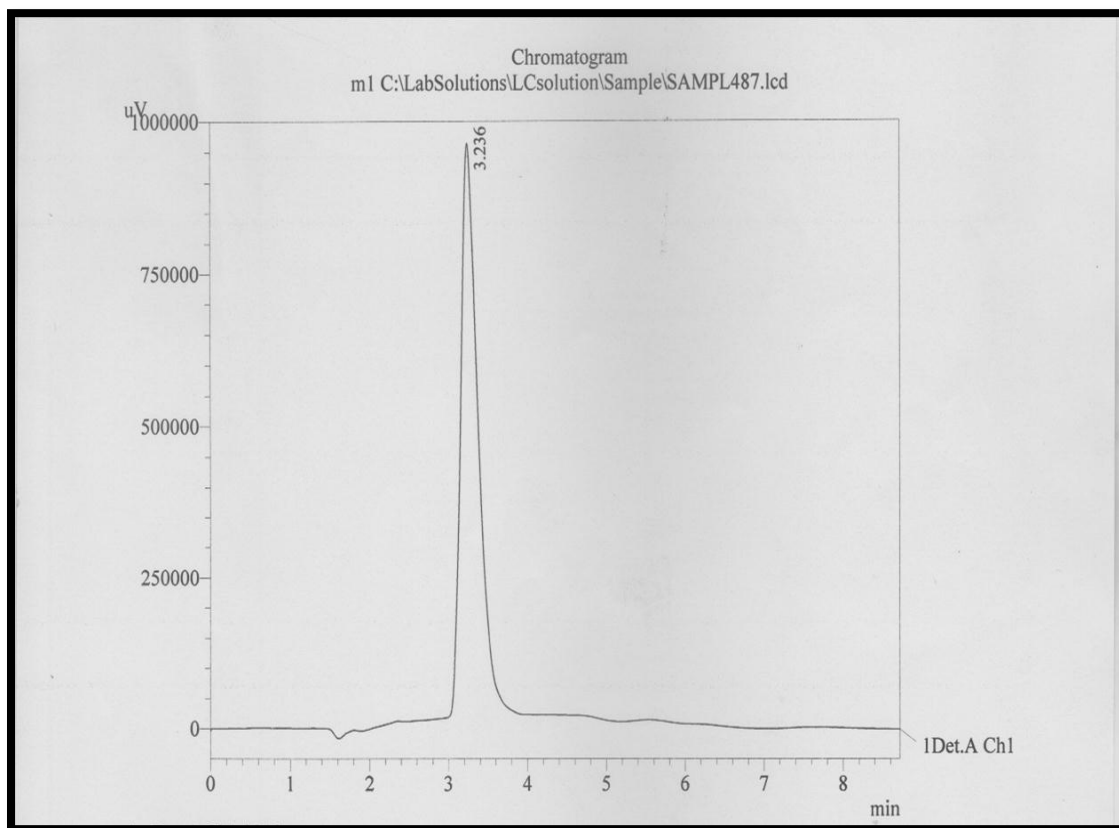


Figure (3-3) : Shows the HPLC Chromatogram of standard Rutin compound.

Table (3-3) : Shows the retention time of standard Rutin compound .

Peak	Standard	Retention time (min)	Area
1	Rutin	3.236	2539

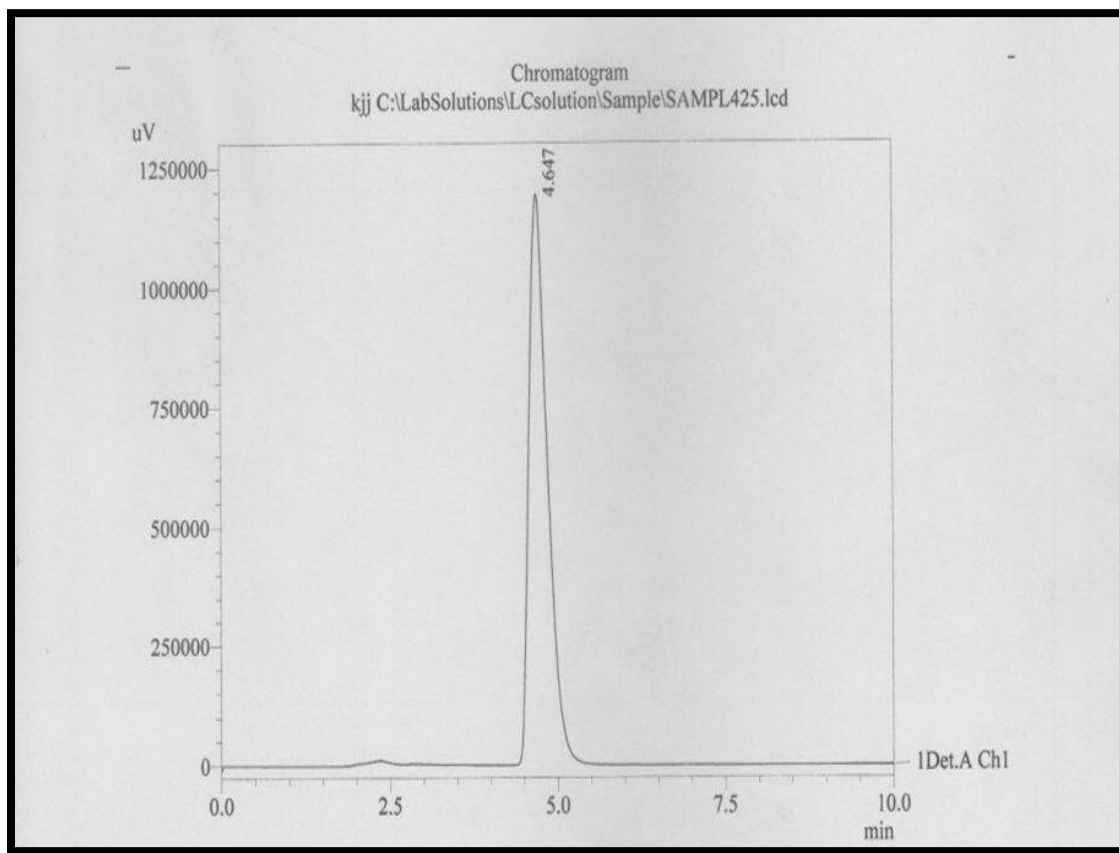


Figure (3-4) : Shows the HPLC Chromatogram of standard *p*- coumaric acid compound .

Table (3-4) : Shows the retention time of standard *p*- coumaric acid compound .

Peak	Standard	Retention time (min)	Area
1	<i>p</i> - coumaric acid	4.647	10557

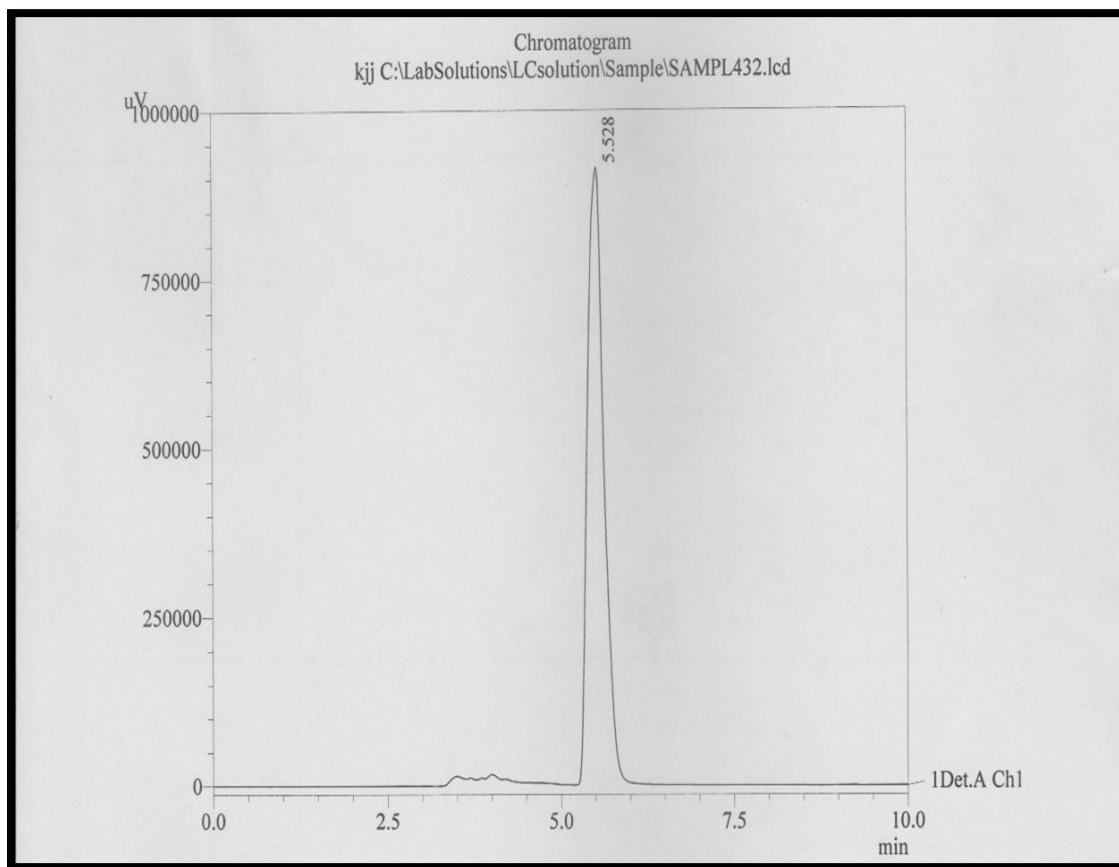


Figure (3-5) : Shows the HPLC Chromatogram of standard campherol compound .

Table (3-5) : Shows the retetion time of standard campherol compound .

Peak	Standard	Retetion time (min)	Area
1	Campherol	5.528	8977

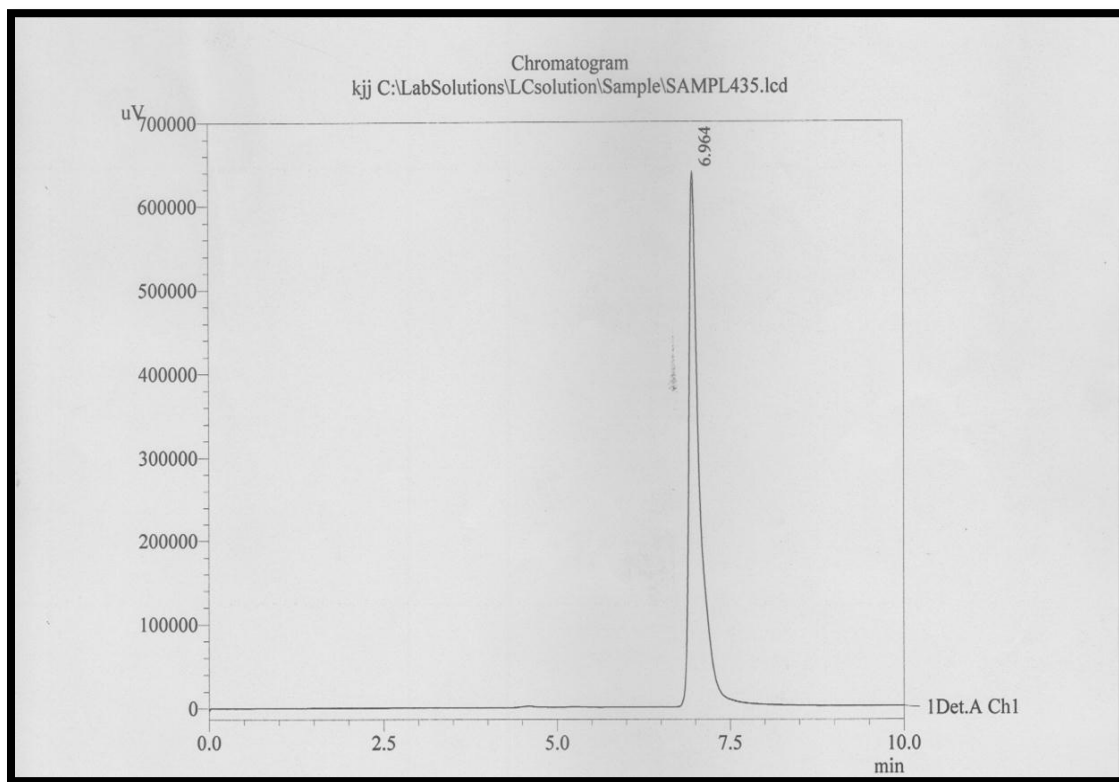


Figure (3-6) : Shows the HPLC Chromatogram of standard catechin compound .

Table (3-6) : Shows the retention time of standard catechin compound .

Peak	Standard	Retention time (min)	Area
1	Catechin	6.964	2505

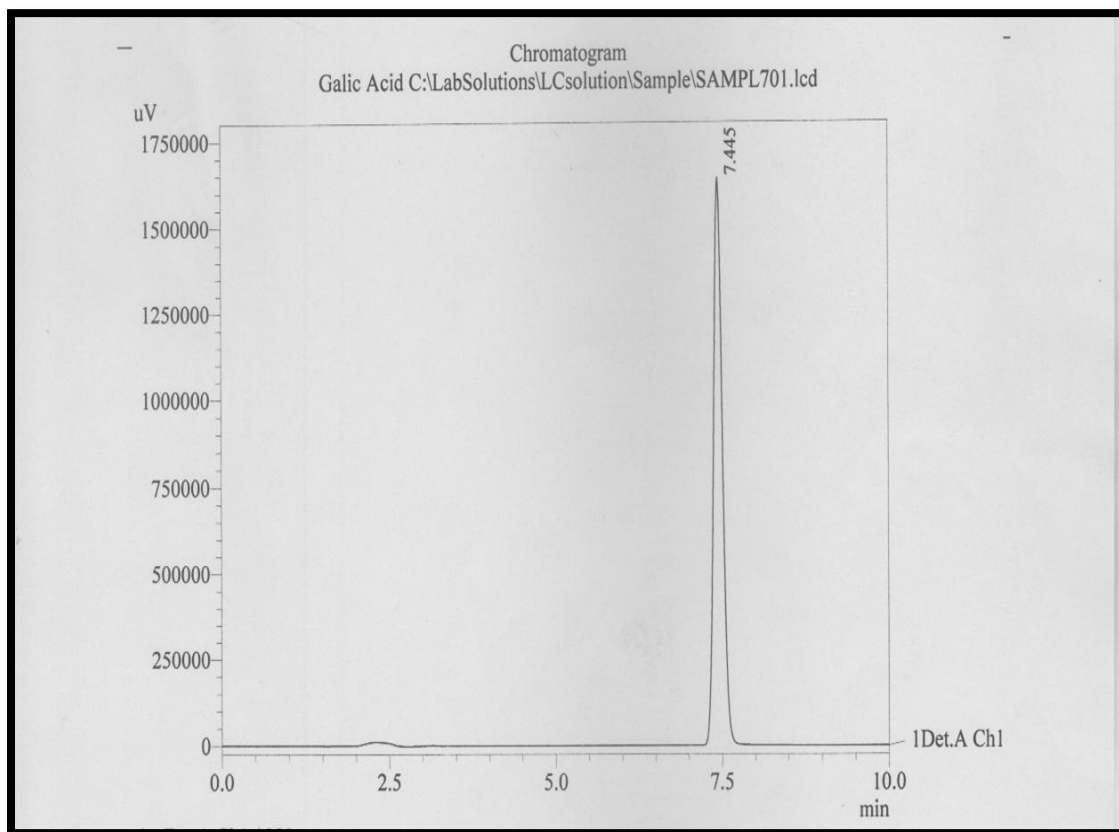
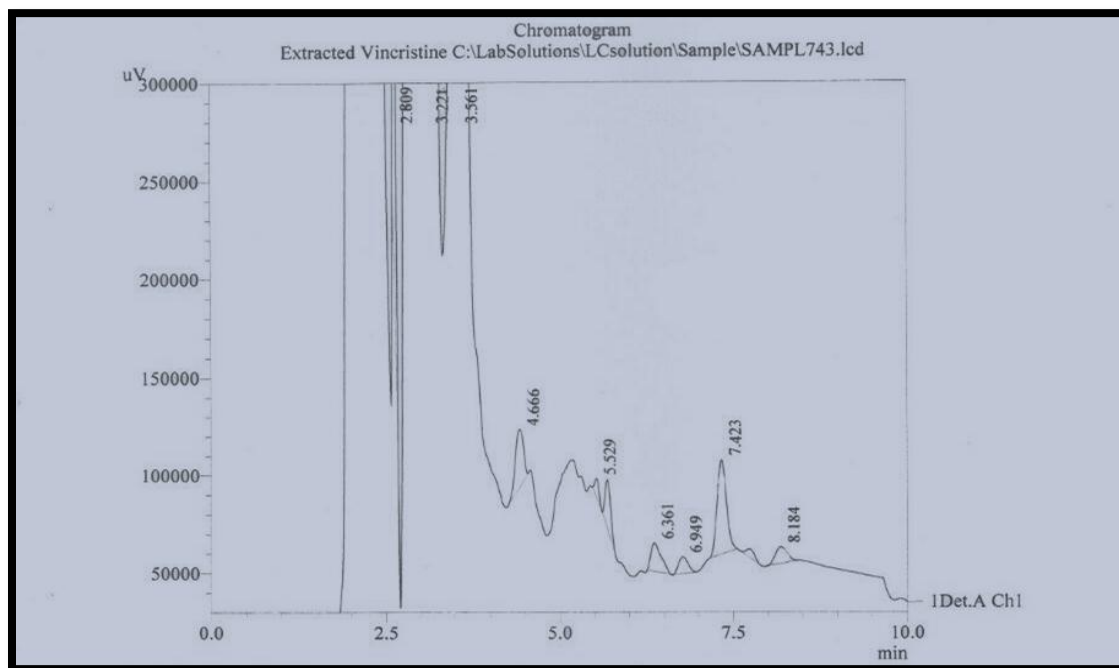


Figure (3-7) : Shows the HPLC Chromatogram of standard gallic acid compound .

Table (3-7) : Shows the retention time of standard gallic acid compound .

Peak	Standard	Retention time (min)	Area
1	Gallic acid	7.445	3925

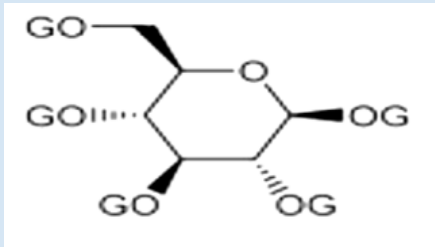
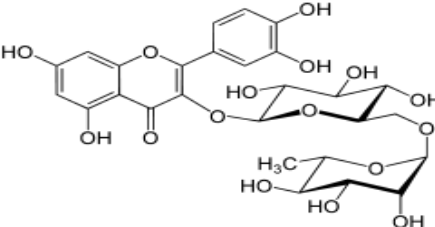
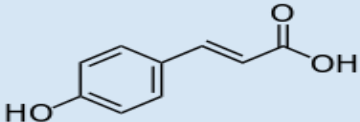
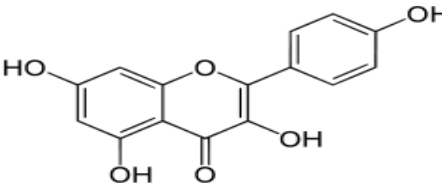
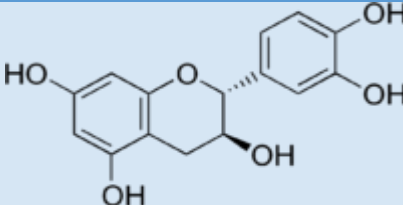
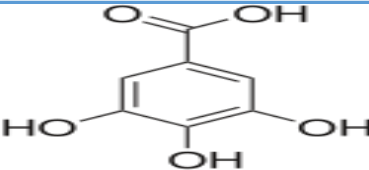


Figure(3-8): Shows the HPLC chromatogram of phenolic compounds in tubers of *cyperus rotundus* .

Table(3-8): Shows the concentration of phenolic compounds in tubers of *cyperus rotundus* .

Peak	Phenolic contents in the extract	Retention time (min)	Area	Conc. of phenolic compounds (ppm)
1	Tannic acid	2.809	535232	5.06
2	Rutin	3.221	48773	1.92
3	p- Coumaric acid	4.666	245029	2.32
4	Campherol	5.529	138912	1.54
5	Catechin	6.949	80665	3.22
6	Gallic acid	7.423	316744	8.06

Table (3-9) : Names and structure of phenolic compounds in tubers of *Cyperus rotundus* extract.

Subject	Organizational name	Structure
Tannic acid	3,5-dihydroxy-2-(3,4,5-trihydroxybenzoyl)oxy-6-[(3,4,5-trihydroxybenzoyl)oxymethyl]oxan-3,4,5-trihydroxybenzoate	
Rutin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]-4H-chromen-4-one	
p- Coumaric acid	3-(4-hydroxyphenyl)-2-propenoic acid	
Campherol	3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one	
Catechin	(2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	
Gallic acid	3,4,5-trihydroxybenzoic acid	

3-5- Effect of Phenolic Extract and Taurine on Cardiac**Markers :**

Table (3-10) and figure (3-9) showed a significant increase ($p \leq 0.05$) in the concentration of serum troponin-I in group (2) in comparison with group (1). There was non significant differences in the concentration of serum troponin-I in group (3,4,5 and 6) in comparison with group (1) and between them. In the same table, the results indicated a significant decrease ($p \leq 0.05$) in the concentration of serum troponin-I in groups (7,8,9 and 10) in comparison with group (2). Also, troponin-I decreased significantly ($p \leq 0.05$) in the concentration of serum troponin-I in group (8) in comparison with group(7). In the same table, the results indicated a significant decrease ($p \leq 0.05$) in the concentration of serum troponin-I in group (10) in comparison with group (9). Also, troponin decreased significantly ($p \leq 0.05$) in the concentration of serum troponin-I level in group (10) in comparison with groups (7 and 8). There was non significant difference in the concentration of serum troponin-I between group (8) and group (9).

In table (3-10) and figure (3-10) there was a significant increase ($p \leq 0.05$) in the concentration of serum lactate dehydrogenase (LDH) in group (2) in comparison with group (1). There was non significant difference in the concentration of serum lactate dehydrogenase (LDH) in groups (3,4,5 and 6) in comparison with group (1) and between them. In the same table, the results indicated a significant decrease ($p \leq 0.05$) in the concentration of serum lactate dehydrogenase (LDH) in groups (7,8,9 and 10) in comparison with group (2). Also, lactate dehydrogenase (LDH) decreased significantly ($p \leq 0.05$) in the concentration of serum lactate dehydrogenase (LDH) in group (8) in comparison with group (7). No significant differences in

concentration of serum lactate dehydrogenase (LDH) between group (9) and group (10). Also, there was a significant decrease ($p \leq 0.05$) in the concentration of serum lactate dehydrogenase (LDH) in groups (9 and 10) in comparison with groups (7 and 8).

Also the same table (3-10) and figure (3-11) showed a significant increase ($p \leq 0.05$) in the concentration of serum creatine phosphokinase (CPK) in group (2) in comparison with group (1). The results showed non significant difference in the concentration of serum creatine phosphokinase (CPK) in groups (3,4,5 and 6) in comparison with group (1) and between them. In the same table, the results indicated a significant decrease ($p \leq 0.05$) in the concentration of serum creatine phosphokinase (CPK) in groups (7, 8, 9 and 10) in comparison with group (2). Also, there was a significant decrease ($p \leq 0.05$) in the concentration of serum creatine phosphokinase (CPK) in group (8) in comparison with group (7). There was non significant difference in concentration of serum creatine phosphokinase (CPK) between group (9) and group (10). Also, creatine phosphokinase (CPK) decreased significantly ($p \leq 0.05$) in group (9 and 10) in comparison with groups (7 and 8).

The present study showed a significant increase ($p \leq 0.05$) in the concentration of serum alanine transaminase (ALT) in group (2) in comparison with group (1). Also, there was non significant difference in the concentration of serum alanine transaminase (ALT) in groups (3 and 5) in comparison with group (1) and between them. In the same table, the results indicated a significant increase ($p \leq 0.05$) in the concentration of serum alanine transaminase (ALT) in groups (4) in comparison with group(1). There was a significant increase ($p \leq 0.05$) in concentration of serum alanine

transaminase (ALT) in group (6) in comparison with group (1, 3 and 5). The results indicated non significant difference in concentration of serum alanine transaminase (ALT) between groups (3, 4 and 5). Also, alanine transaminase (ALT) decreased significantly ($p \leq 0.05$) in groups (7, 8, 9 and 10) in comparison with group (2). There was a non significant difference in the concentration of serum alanine transaminase (ALT) between groups (7 and 8). Alanine transaminase decreased significantly ($p \leq 0.05$) in group (10) in comparison with group (9). Also, alanine transaminase decreased significantly ($p \leq 0.05$) in groups (9 and 10) in comparison with group (7 and 8). (table 3-10, figure 3-12).

Also table (3-10) and figure (3-13) showed a significant increase ($p \leq 0.05$) in the concentration of serum aspartate transaminase (AST) in group (2) in comparison with group (1). There was non significant difference in the concentration of serum aspartate transaminase (AST) in groups (4 and 6) in comparison with group (1). Also, there was a significant increase ($p \leq 0.05$) in the concentration of serum aspartate transaminase (AST) in groups (3 and 5) in comparison with group (1). There was non significant difference in concentration of serum aspartate transaminase (AST) between groups (3, 4, 5 and 6). Aspartate transaminase (AST) decreased significantly ($p \leq 0.05$) in the concentration of serum aspartate transaminase (AST) in groups (7, 8, 9 and 10) in comparison with group (2). Also, there was a significant decrease ($p \leq 0.05$) in the concentration of serum aspartate transaminase (AST) in group (8) in comparison with group (7). There was non significant difference in concentration of serum aspartate transaminase (AST) between group (9) and group (10). There was a significant decrease ($p \leq 0.05$) in the

concentration of serum aspartate transaminase (AST) in groups (9 and 10)
in comparison with group (7 and8).

Table (3-10) : Effect of phenolic extract and taurine on cardiac markers

Group	Troponin-I (ng/mL)	LDH (U/L)	CPK (U/L)	ALT (U/L)	AST (U/L)
Group (1)	0.38 ± 0.09 ^e	238.20 ± 28.73 ^e	125.50 ± 8.26 ^e	32.40 ± 3.45 ^g	55.70 ± 1.41 ^f
Group (2)	3.55 ± 0.50 ^a	521.60 ± 34.28 ^a	281.26 ± 36.76 ^a	63.56 ± 5.21 ^a	99.33 ± 12.34 ^a
Group (3)	0.50 ± 0.04 ^e	250.86 ± 33.88 ^e	126.70 ± 2.53 ^e	33.80 ± 3.79 ^{fg}	62.51 ± 2.42 ^e
Group (4)	0.51 ± 0.03 ^e	242.36 ± 31.76 ^e	129.36 ± 5.26 ^e	36.72 ± 2.42 ^{ef}	59.24 ± 3.24 ^{ef}
Group (5)	0.49 ± 0.02 ^e	249.92 ± 28.51 ^e	133.36 ± 5.37 ^e	34.80 ± 3.14 ^{fg}	61.88 ± 5.25 ^e
Group (6)	0.53 ± 0.03 ^e	246.76 ± 44.09 ^e	131.14 ± 2.86 ^e	39.60 ± 1.81 ^e	57.60 ± 3.32 ^{ef}
Group (7)	2.45 ± 0.31 ^b	460.60 ± 75.19 ^b	231.90 ± 31.82 ^b	58.32 ± 2.33 ^b	90.76 ± 7.35 ^b
Group (8)	2.07 ± 0.32 ^c	401.49 ± 42.76 ^c	203.90 ± 7.84 ^c	55.88 ± 1.69 ^b	82.24 ± 6.87 ^c
Group (9)	1.85 ± 0.58 ^c	340.10 ± 9.41 ^d	172.56 ± 10.30 ^d	51.24 ± 2.87 ^c	75.20 ± 2.16 ^d
Group (10)	0.98 ± 0.23 ^d	316.50 ± 18.69 ^d	166.36 ± 12.01 ^d	45.60 ± 2.35 ^d	71.00 ± 2.97 ^d
LSD	0.31	40.82	17.78	3.26	6.03

❖ Values refer to mean ± SD .

❖ Different letters refer to a significant difference at (p≤0.05).

❖ Same letters refer to non significant difference at (p≤0.05).

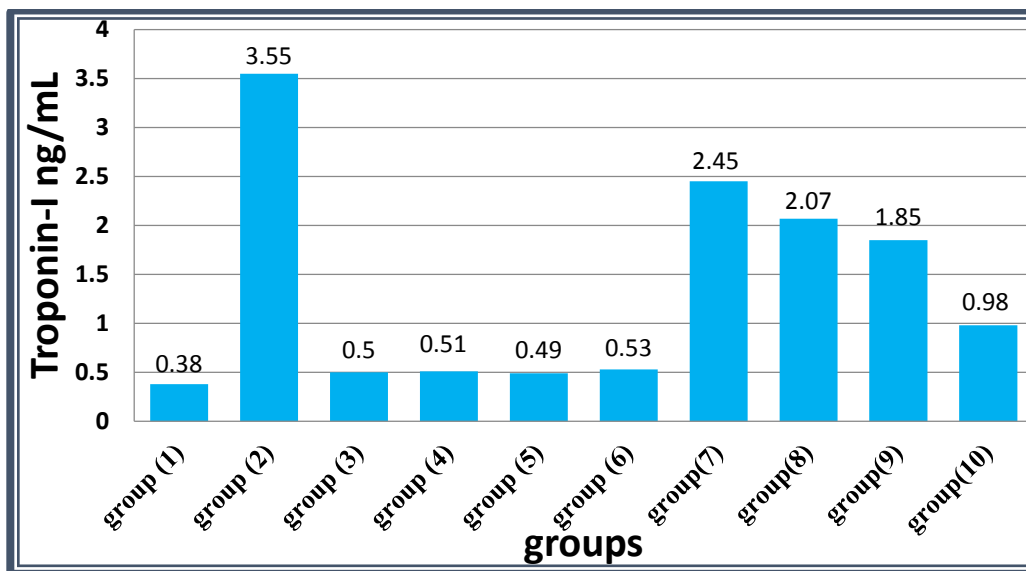


Figure (3-9):Effect of phenolic extract and taurine on Troponin-I levels.

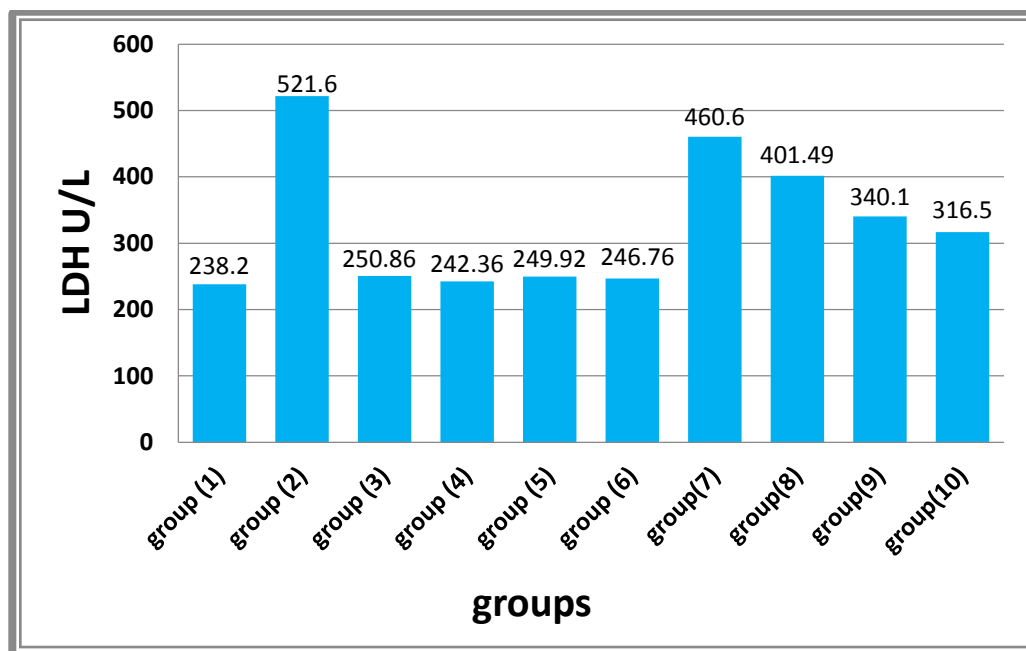


Figure (3-10): Effect of phenolic extract and taurine on LDH levels.

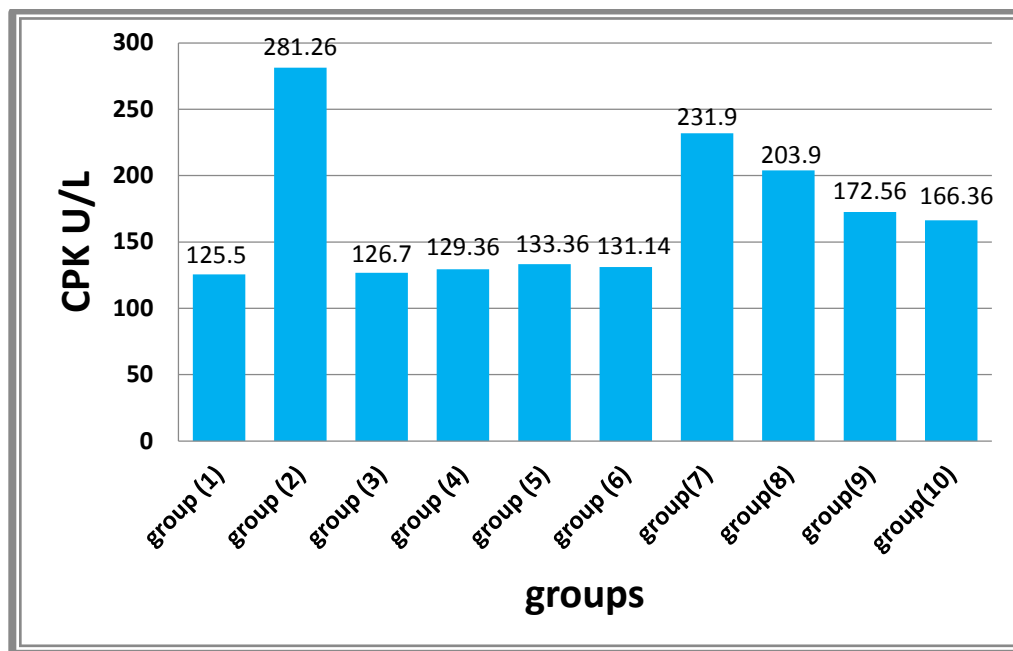


Figure (3-11): Effect of phenolic extract and taurine on CPK levels.

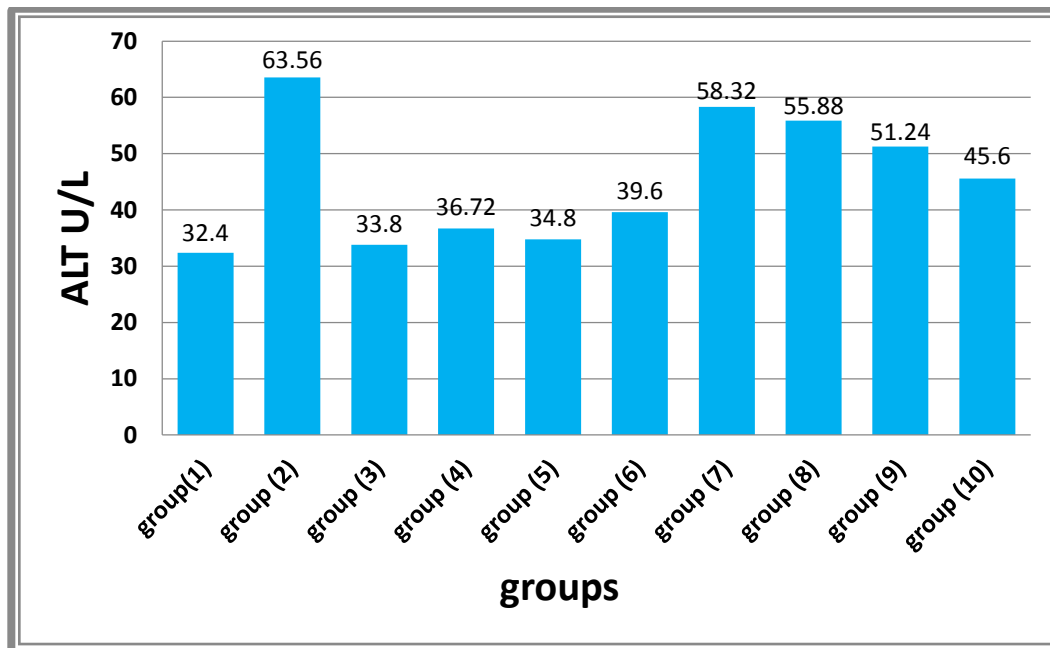


Figure (3-12): Effect of phenolic extract and taurine on ALT levels.

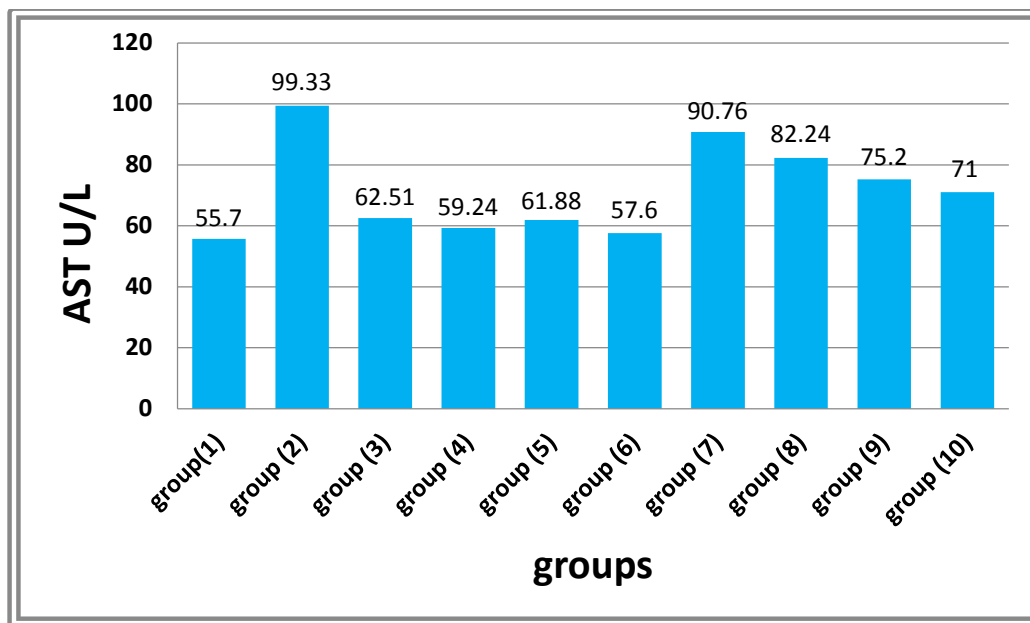


Figure (3-13): Effect of phenolic extract and taurine on AST levels.

3-6-Effect of Phenolic Extract and Taurine on Malondialdehyde Concentration :

Table (3-11) and figure (3-14) showed a significant increased ($p \leq 0.05$) in the concentration of serum malondialdehyde (MDA) in group (2) in comparison with group (1). There was non significant difference in the concentration of serum malondialdehyde (MDA) in group (3, 4, 5 and 6) in comparison with group (1) and between them. In the same table, the results indicated a significant decrease ($p \leq 0.05$) in the concentration of serum malondialdehyde (MDA) in groups (7, 8, 9 and 10) in comparison with group (2). Also, there was a significant decrease ($p \leq 0.05$) in the concentration of serum malondialdehyde (MDA) in group (8) in comparison with group (7). There was a significant decrease ($p \leq 0.05$) in concentration of serum malondialdehyde (MDA) ($p \leq 0.05$) in group (10) in comparison

with group (9). Also, malondialdehyde decreased significantly ($p \leq 0.05$) in groups (9 and 10) in comparison with group (7 and 8).

In table (3-11) and figure (3-15) there was a significant increase ($p \leq 0.05$) in the concentration of tissue malondialdehyde (MDA) in group (2) in comparison with group (1). There was non significant difference in the concentration of tissue malondialdehyde (MDA) in group (3, 4, 5 and 6) in comparison with group (1) and between them. In the same table, the results indicated a significant decrease ($p \leq 0.05$) in the concentration of tissue malondialdehyde (MDA) in groups (7, 8, 9 and 10) in comparison with group (2). Also, malondialdehyde (MDA) decreased significantly ($p \leq 0.05$) in group (8) in comparison with group (7). No significant difference in the concentration of tissue malondialdehyde (MDA) between group (9) and group (10). Also, there was a significant decrease ($p \leq 0.05$) in the concentration of tissue malondialdehyde (MDA) in group (9 and 10) in comparison with group (7 and 8).

Table (3-11) : Effect of phenolic extract and taurine on malondialdehyde concentration.

Group	MDA Serum ($\mu\text{mol/L}$)	MDA Tissue (nmol/mg protein)
Group (1)	1.51 ± 0.28^f	1.41 ± 0.11^e
Group (2)	4.65 ± 0.37^a	3.91 ± 0.27^a
Group (3)	1.56 ± 0.31^f	1.42 ± 0.41^e
Group (4)	1.65 ± 0.33^f	1.46 ± 0.29^e
Group (5)	1.60 ± 0.22^f	1.44 ± 0.39^e
Group (6)	1.62 ± 0.35^f	1.43 ± 0.14^e
Group (7)	3.99 ± 0.37^b	3.21 ± 0.32^b
Group (8)	3.42 ± 0.25^c	2.75 ± 0.48^c
Group (9)	2.79 ± 0.79^d	2.23 ± 0.35^d
Group (10)	2.20 ± 0.17^e	1.88 ± 0.50^d
LSD	0.40	0.38

❖ Legend as in the table (3-10).

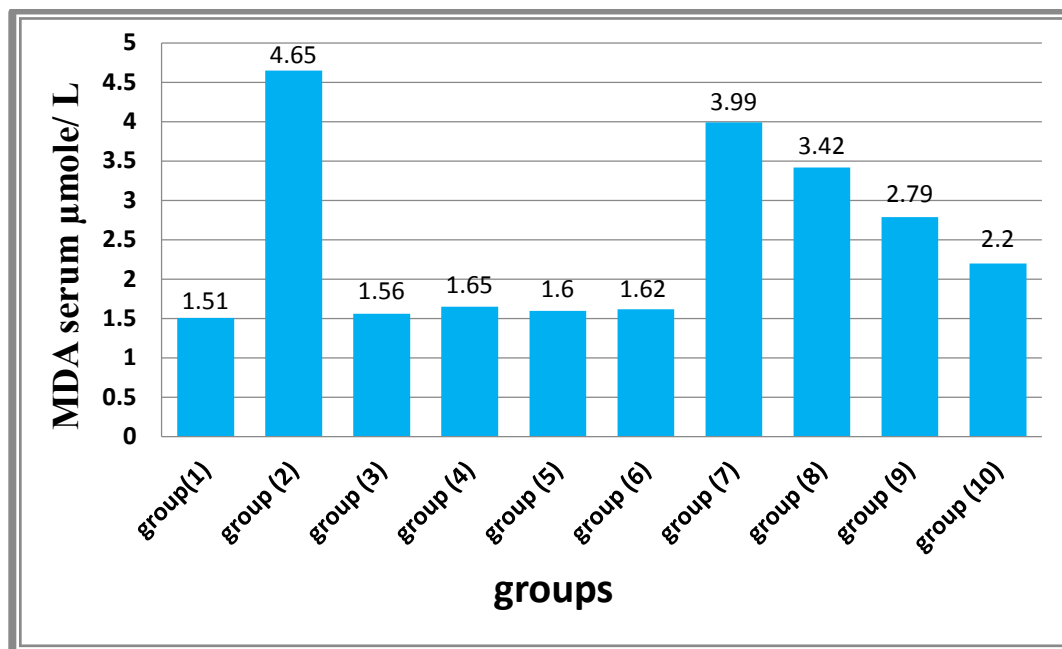


Figure (3-14) : Effect of phenolic extract and taurine on serum MDA levels.

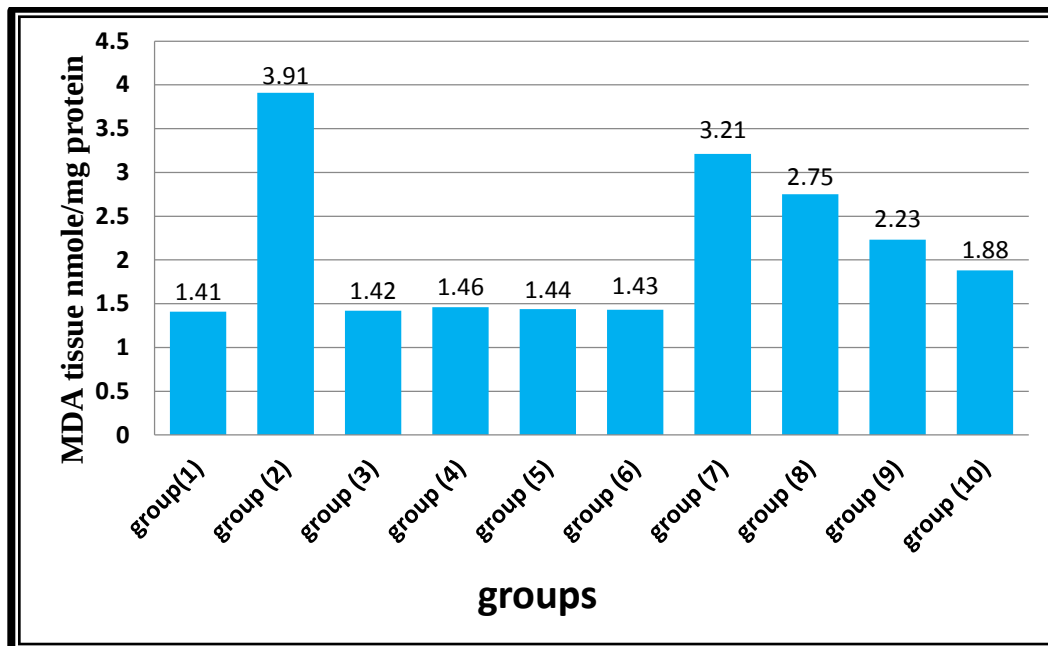


Figure (3-15) : Effect of phenolic extract and taurine on tissue MDA levels.

3-7-Effect of Phenolic Extract and Taurine on Antioxidant Parameters:

Table (3-12) and figure (3-16) showed a significant increase ($p \leq 0.05$) in the concentration of serum ceruloplasmin (Cp) in group (2) in comparison with group (1). There was non significant difference in the concentration of serum ceruloplasmin (Cp) in groups (3, 4, 5 and 6) in comparison with group (1) and between them. In the same table, the results indicated a significant decrease ($p \leq 0.05$) in the concentration of serum ceruloplasmin (Cp) in groups (7, 8, 9 and 10) in comparison with group (2). Also, there was non significant difference in the concentration of serum ceruloplasmin (Cp) between groups (7, 8, 9 and 10).

The same table (3-12) and figure (3-17) there was a significant decrease ($p \leq 0.05$) in the concentration of serum albumin (Alb) in group (2) in comparison with group (1). There was non significant difference in the concentration of serum albumin (Alb) in groups (3, 4, 5 and 6) in comparison with group (1) and between them. In the same table, the results indicated a significant increase ($p \leq 0.05$) in the concentration of serum albumin (Alb) in groups (7, 8, 9 and 10) in comparison with group (2). Also, there was non significant ($p \leq 0.05$) in the concentration of serum Albumin (Alb) between group (7) and group (8). There are non significant difference in the concentration of serum Albumin (Alb) between group (9) and group (10). Also, albumin increased significantly ($p \leq 0.05$) in groups (9 and 10) in comparison with groups (7 and 8).

Also the same table (3-12) and figure (3-18) showed a significant increase ($p \leq 0.05$) in the concentration of serum uric acid in group (2) in

comparison with group (1). The results showed non significant difference in the concentration of serum uric acid in group (3 and 4) in comparison with group (1). Also, there was a significant increase ($p \leq 0.05$) in the concentration of serum uric acid in groups (5 and 6) in comparison with group (1). There was a significant increase ($p \leq 0.05$) in the concentration of serum uric acid in groups (5 and 6) in comparison with group (3). In the same table, the results indicated non significant in the concentration of serum uric acid between group (3 and 4). There was non significant difference in the concentration of serum uric acid between groups (4, 5 and 6). Also, uric acid decreased significantly ($p \leq 0.05$) in groups (7, 8, 9 and 10) in comparison with group (2). Also, there was non significant difference in the concentration of serum uric acid between groups (7 and 8). No significant difference the concentration of serum uric acid between groups (9 and 10). Also, uric acid decreased significantly ($p \leq 0.05$) in groups (9 and 10) in comparison with groups (7 and 8).

In the same table (3-12) and figure (3-19) showed a significant decreased ($p \leq 0.05$) in the concentration of serum GSH in group (2) in comparison with group (1). There was non significant difference in the concentration of serum GSH in groups (3, 4, 5 and 6) in comparison with group (1) and between them. In the same table, the results indicated a significant increase ($p \leq 0.05$) in the concentration of serum GSH in groups (7, 8, 9 and 10) in comparison with group (2). No significant difference in the concentration of serum GSH between groups (7, 8 and 9). There was non significant difference in the concentration of serum GSH between groups (8, 9 and 10). Also, there was a significant increase ($p \leq 0.05$) in the concentration of serum GSH in group (10) in comparison with group (7).

The present study showed a significant decrease ($p \leq 0.05$) in the concentration of tissue GSH in group (2) in comparison with group (1). There was non significant difference in the concentration of tissue GSH in groups (3, 4, 5 and 6) in comparison with group (1) and between them. In the same table, the results indicated a significant increase ($p \leq 0.05$) in the concentration of tissue GSH in groups (7, 8, 9 and 10) in comparison with group (2). Also, there was non significant difference in the concentration of tissue GSH between groups (8, 9 and 10). Also, GSH increased significantly ($p \leq 0.05$) in groups (9 and 10) in comparison with group(7).(table 3-12, figure 3-20).

Table(3-12):Effect of phenolic extract and taurine on antioxidants parameters.

Group	Cp (g/L)	Alb (g/dL)	Uric acid (mg/dL)	GSH serum μmol/L	GSH tissue μg/mg protein
Group (1)	3.03 ± 0.59 ^c	4.91 ± 0.62 ^a	3.23 ± 0.35 ^e	586.11 ± 74.41 ^a	0.63 ± 0.04 ^a
Group (2)	5.59 ± 0.58 ^a	3.03 ± 0.47 ^d	6.51 ± 0.47 ^a	365.60 ± 51.02 ^d	0.26 ± 0.04 ^d
Group (3)	3.08 ± 0.26 ^c	4.80 ± 0.16 ^a	3.51 ± 0.50 ^e	595.59 ± 63.70 ^a	0.68 ± 0.08 ^a
Group (4)	3.05 ± 0.06 ^c	4.83 ± 0.12 ^a	3.92 ± 0.57 ^{de}	591.99 ± 44.97 ^a	0.73 ± 0.05 ^a
Group (5)	3.06 ± 0.56 ^c	4.85 ± 0.38 ^a	4.01 ± 0.35 ^d	597.17 ± 38.53 ^a	0.71 ± 0.07 ^a
Group (6)	3.09 ± 0.61 ^c	4.89 ± 0.14 ^a	4.11 ± 0.32 ^d	589.17 ± 58.12 ^a	0.74 ± 0.06 ^a
Group (7)	3.96 ± 0.35 ^b	3.65 ± 0.16 ^c	5.90 ± 0.50 ^b	459.15 ± 23.76 ^c	0.41 ± 0.05 ^c
Group (8)	3.89 ± 0.52 ^b	3.70 ± 0.61 ^c	5.75 ± 0.43 ^b	481.36 ± 67.54 ^{bc}	0.44 ± 0.06 ^{bc}
Group (9)	3.78 ± 0.62 ^b	4.25 ± 0.58 ^b	4.93 ± 0.44 ^c	496.47 ± 67.54 ^{bc}	0.48 ± 0.06 ^b
Group (10)	3.68 ± 0.35 ^b	4.30 ± 0.62 ^b	4.69 ± 0.54 ^c	519.93 ± 56.62 ^b	0.50 ± 0.11 ^b
LSD	0.51	0.46	0.48	60.70	0.07

❖ Legend as in the table (3-10).

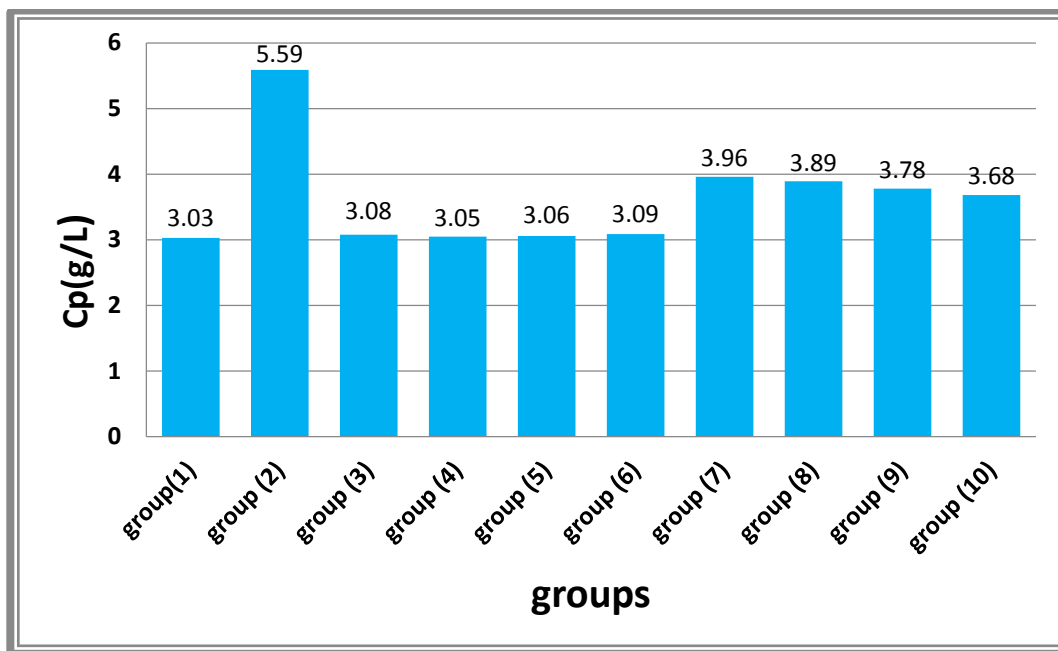


Figure (3-16): Effect of phenolic extract and taurine on Cp levels.

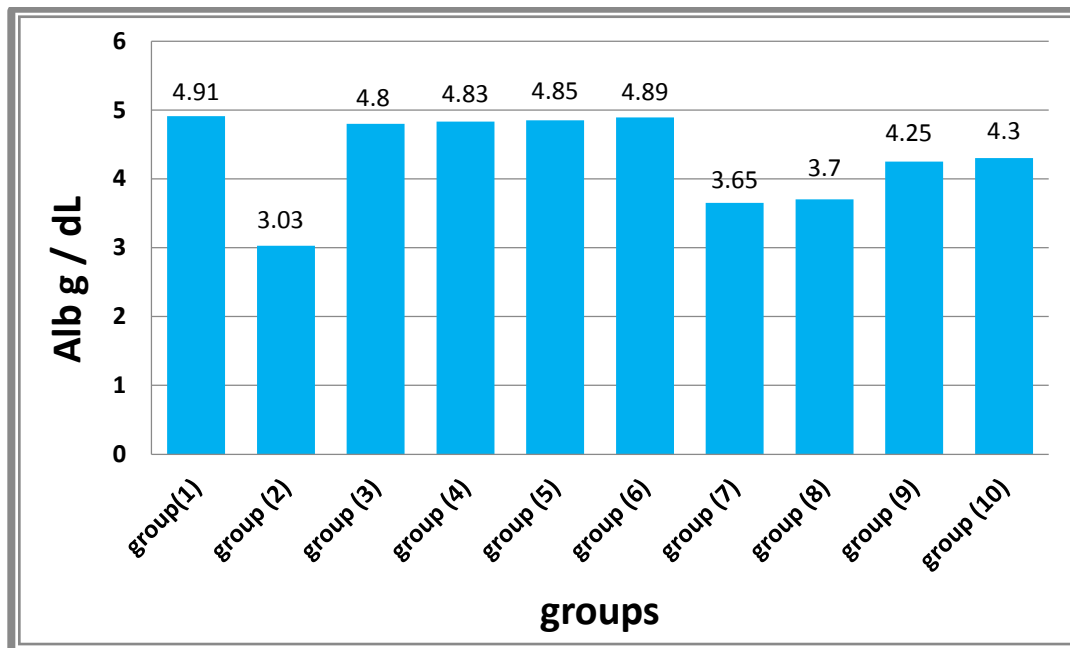


Figure (3-17): Effect of phenolic extract and taurine on Alb levels.

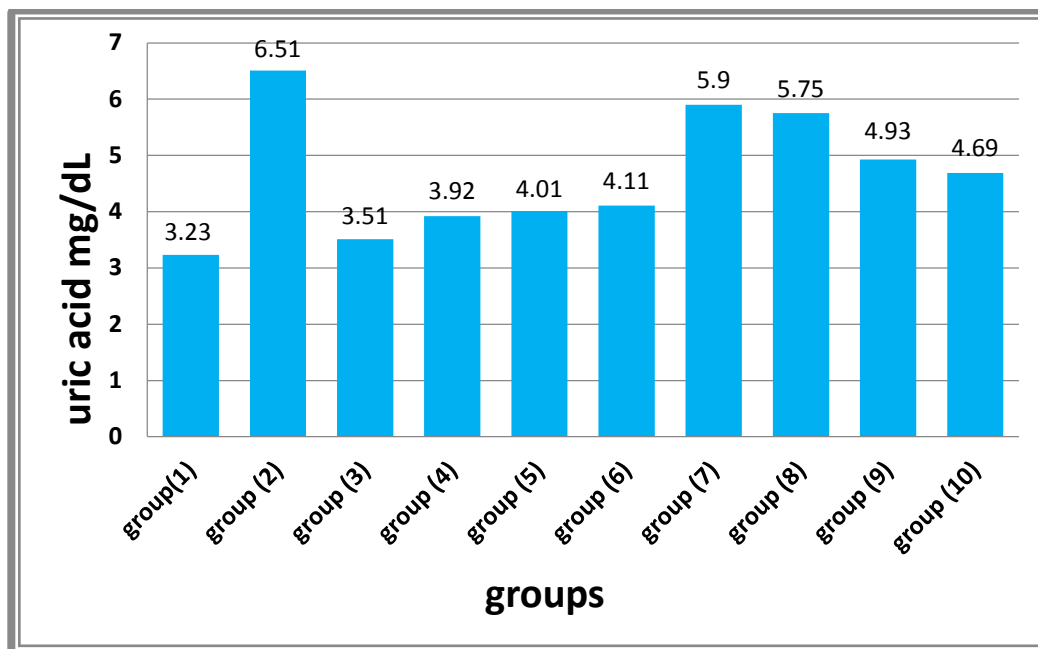


Figure (3-18): Effect of phenolic extract and taurine on uric acid levels.

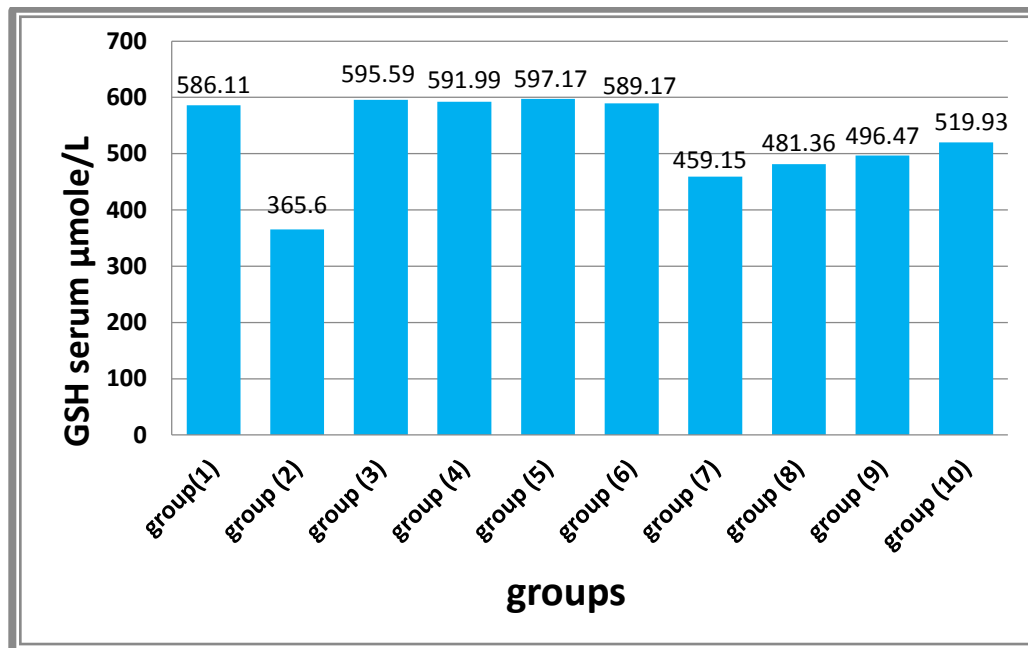


Figure (3-19): Effect of phenolic extract and taurine on serum GSH levels.

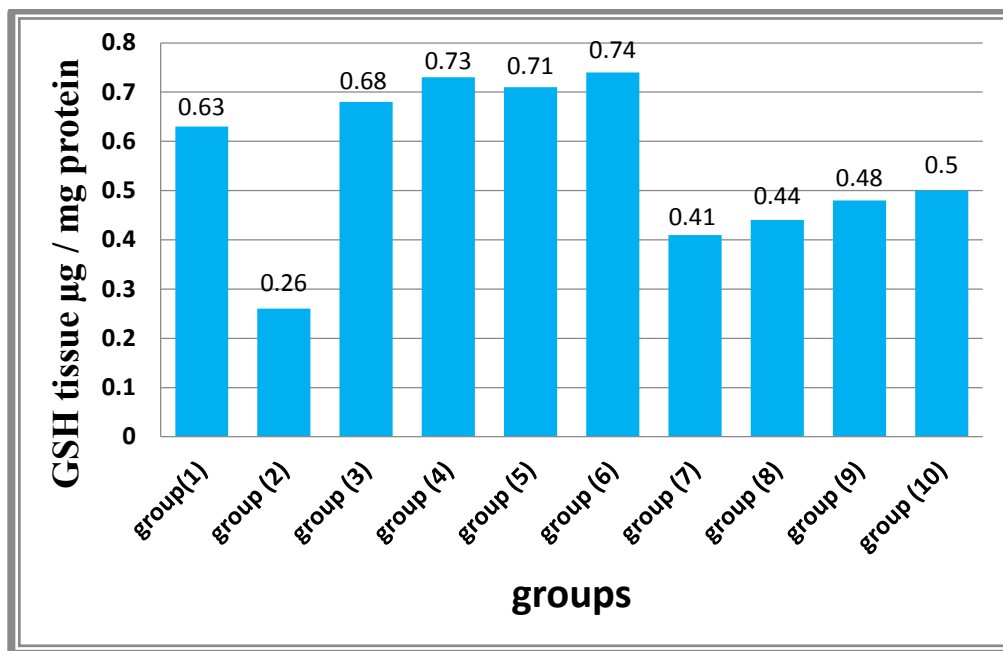


Figure (3-20): Effect of phenolic compounds and taurine on tissue GSH levels.

3-8-Effect of Phenolic Extract and Taurine on Lipid Profile Concentration :

The results listed in the table (3-13) and figure (3-21) showed a significant increase ($p \leq 0.05$) in the concentration of serum cholesterol in group (2) in comparison with group (1). There was non significant difference in the concentration of serum cholesterol in groups (3, 4, 5 and 6) in comparison with group (1) and between them. In the same table, the results indicated a significant decrease ($p \leq 0.05$) in the concentration of serum cholesterol in groups (7, 8, 9 and 10) in comparison with group (2). Also, there was non significant difference in the concentration of serum cholesterol between group (7, 8 and 9). No significant difference in the concentration of serum cholesterol between group (8, 9 and 10). Also,

cholesterol increased significantly ($p \leq 0.05$) in group (10) in comparison with group (7).

In the table (3-13) and figure (3-22) there was a significant increase ($p \leq 0.05$) in the concentration of serum triglyceride in group (2) in comparison with group (1). There was non significant difference in the concentration of serum triglyceride in groups (3, 4, 5 and 6) in comparison with group (1) and between them. In the same table, the results indicated a significant decrease ($p \leq 0.05$) in the concentration of serum triglyceride in groups (7, 8, 9 and 10) in comparison with group (2). Also, there was non significant difference in the concentration of serum triglyceride between groups (7, 8, 9 and 10).

Also this table (3-13) and figure (3-23) showed a significant decrease ($p \leq 0.05$) in the concentration of serum HDL in group (2) in comparison with group (1). There was non significant difference in the concentration of serum HDL in groups (3, 4, 5 and 6) in comparison with group (1) and between them. HDL increased significantly ($p \leq 0.05$) in groups (7, 8, 9 and 10) in comparison with group (2). Also, there was non significant difference in the concentration of serum HDL between group (7, 8, 9 and 10).

Also the same table (3-13) and figure (3-24) showed a significant increase ($p \leq 0.05$) in the concentration of serum VLDL in group (2) in comparison with group (1). There was non significant difference in the concentration of serum VLDL in groups (3, 4, 5 and 6) in comparison with group (1) and between them. Also, there was a significant decrease ($p < 0.05$) in the concentration of serum VLDL in groups (7, 8, 9 and 10) in comparison with group (2). In the same table, the results indicated non

significant differences in the concentration of serum VLDL between group (7, 8, 9 and 10).

The present study showed a significant increase ($p \leq 0.05$) in the concentration of serum LDL in group (2) in comparison with group (1). Also, there was non significant difference in the concentration of serum LDL in groups (3, 4, 5 and 6) in comparison with group (1) and between them. There was a significant decrease ($p \leq 0.05$) in the concentration of serum LDL in groups (7, 8, 9 and 10) in comparison with group (2). In the same table, the results indicated a significant decrease ($p \leq 0.05$) in the concentration of serum LDL in group (8) in comparison with group (7). LDL decreased significantly ($p \leq 0.05$) in group (10) in comparison with groups (7 and 8). There was non significant difference in concentration of serum LDL between groups (8 and 9).(table 3-13, figure 3-25).

Table(3-13):Effect of phenolic extract and taurine on Lipid Profile.

Group	TC (mg/dL)	TG(mg/dL)	HDL(mg/dL)	VLDL(mg/dL)	LDL(mg/dL)
Group (1)	96.33 ± 3.45 ^d	81.53±9.49 ^c	51.45±6.08 ^a	16.14±1.69 ^c	28.74±5.37 ^e
Group (2)	185.56 ±6.10 ^a	141.28±19.78 ^a	31.79±4.35 ^c	28.40±3.76 ^a	125.38±7.45 ^a
Group (3)	100.73 ±9.59 ^d	86.31±5.24 ^c	53.76±6.99 ^a	17.26±1.05 ^c	29.72±10.57 ^e
Group (4)	97.49 ± 13.02 ^d	82.85 ± 7.43 ^c	54.82 ±4.51 ^a	16.57 ±1.49 ^c	26.16 ±5.97 ^e
Group (5)	96.63 ±3.33 ^d	85.44 ±4.05 ^c	57.35 ± 2.34 ^a	17.09 ±0.85 ^c	22.19 ± 2.43 ^e
Group (6)	99.32 ± 11.25 ^d	87.81 ± 4.23 ^c	55.80 ±9.09 ^a	17.57 ±0.81 ^c	25.95 ±6.58 ^e
Group (7)	168.89 ± 25.69 ^b	120.33 ±11.33 ^b	39 .72 ±3.59 ^b	24.07 ±2.71 ^b	105.11 ± 25.42 ^b
Group (8)	160.25 ± 5.38 ^{bc}	117.89 ±11.80 ^b	40.22 ±4.67 ^b	23.58 ±2.36 ^b	96.45 ±7.93 ^c
Group (9)	157.44 ± 12.88 ^{bc}	114.35 ±10.83 ^b	42 .34 ±9.37 ^b	22.87 ±2.17 ^b	92.22 ±13.68 ^{cd}
Group (10)	149.42± 8.68 ^c	109.89±12.93 ^b	43.84±3.85 ^b	21.98±2.58 ^b	84.61±8.99 ^d
LSD	12.83	11.49	6.35	2.30	8.27

❖ Legend as in the table (3-10).

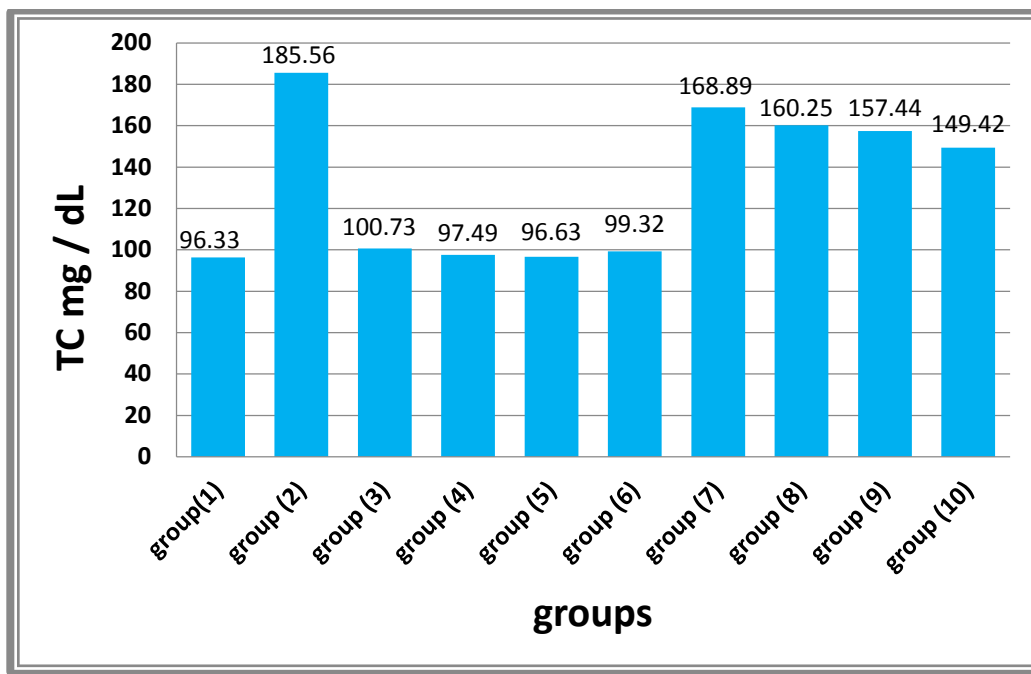


Figure (3-21): Effect of phenolic extract and taurine on TC levels.

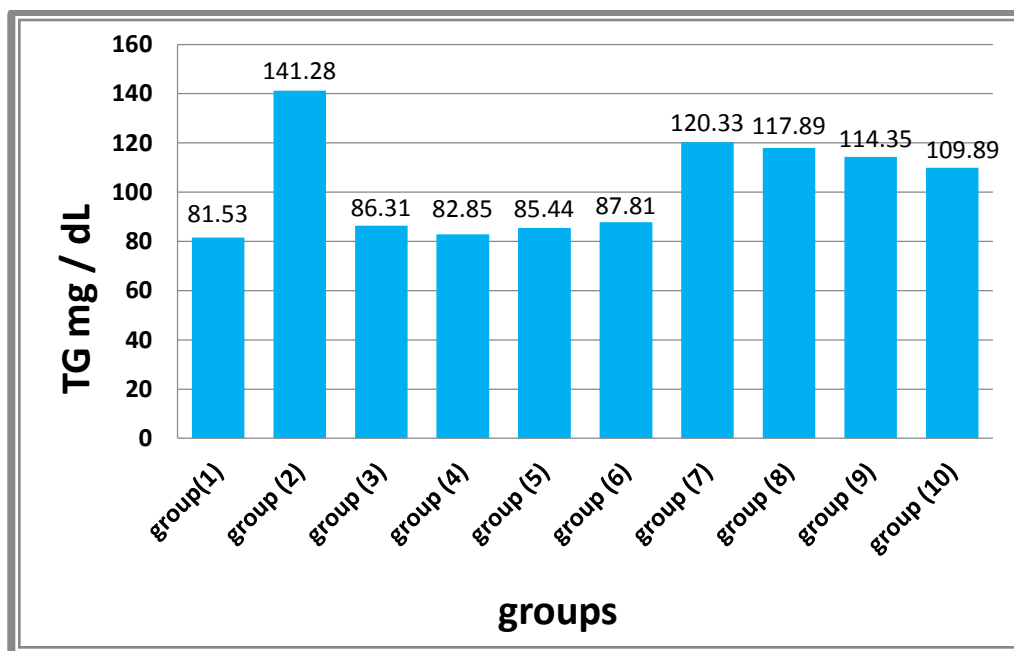


Figure (3-22): Effect of phenolic extract and taurine on TG levels.

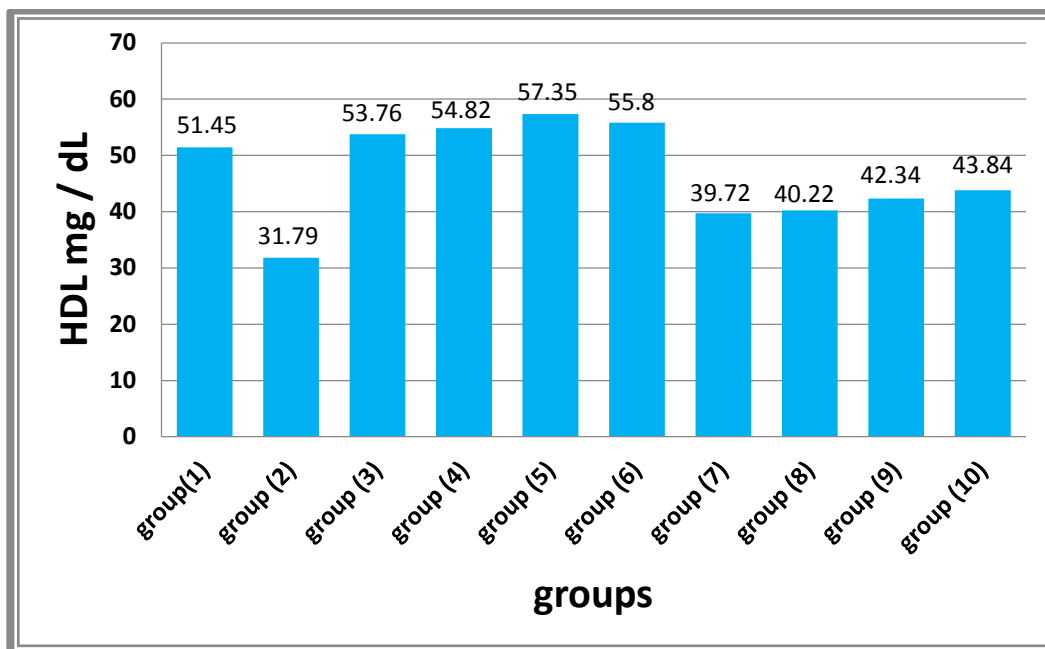


Figure (3-23): Effect of phenolic extract and taurine on HDL levels.

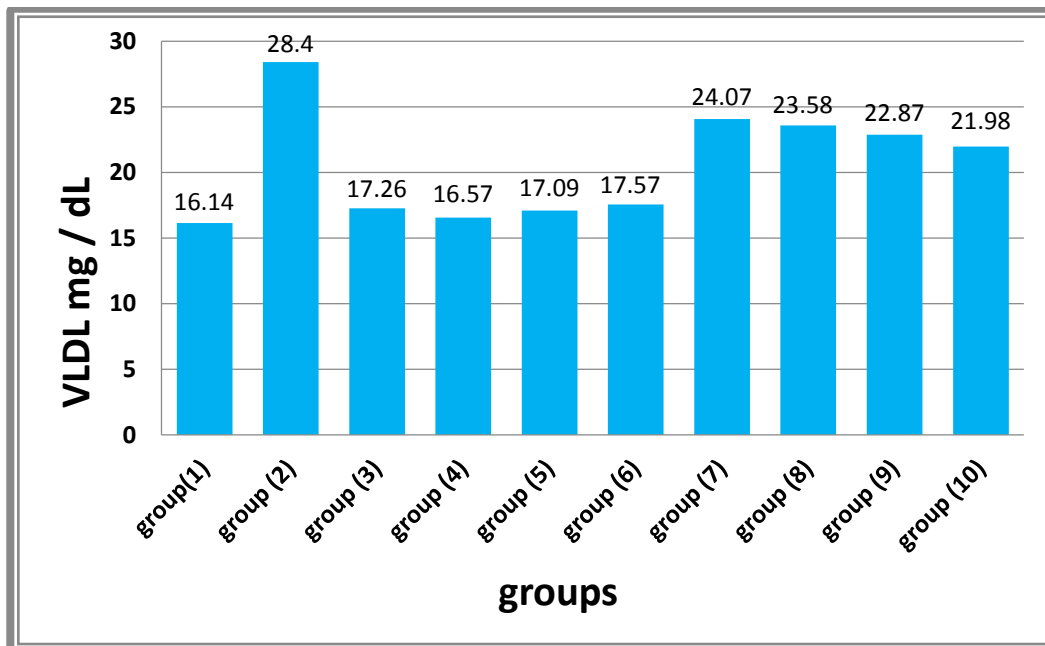


Figure (3-24): Effect of phenolic extract and taurine on VLDL levels.

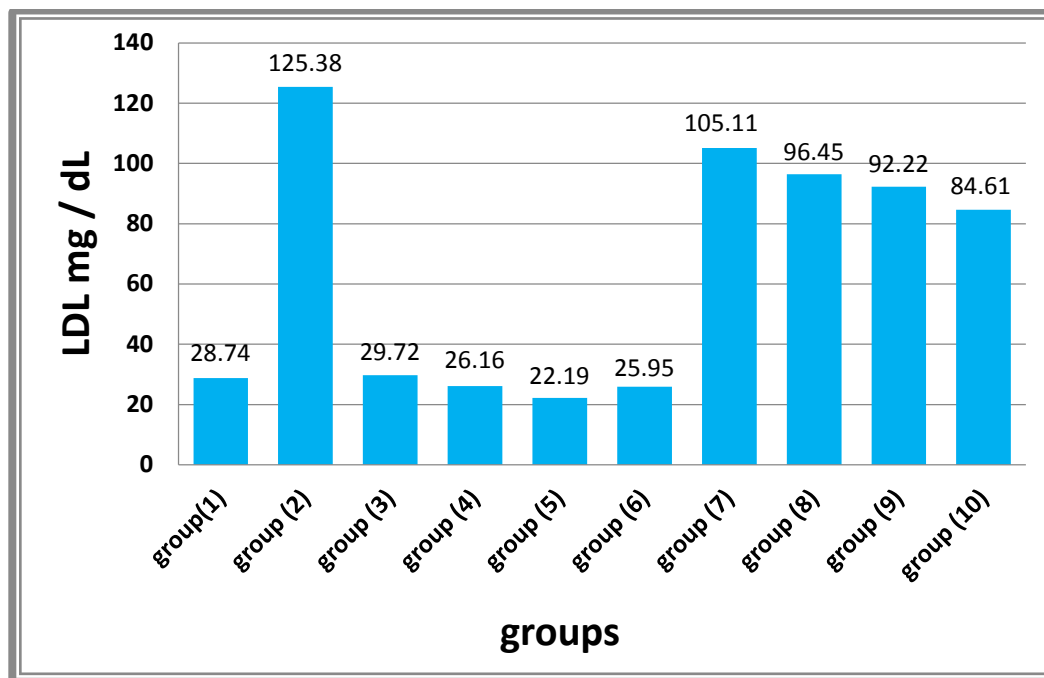


Figure (3-25): Effect of phenolic extract and taurine on LDL levels.

3-9-Effect of Phenolic Extract and Taurine on Atherogenic Index:

Results listed in the table (3-14) and figure (3-26) showed a significant increase ($p \leq 0.05$) in atherogenic index in group (2) in comparison with group (1). There was non significant difference in atherogenic index in groups (3, 4, 5 and 6) in comparison with group (1) and between them. In the same table, the results indicated a significant decrease ($p \leq 0.05$) in atherogenic index in groups (7, 8, 9 and 10) in comparison with group (2). Also, there was non significant difference in atherogenic index between group (7, 8 and 9). Also, there was a significant decrease in atherogenic index in group (10) in comparison with groups (7, 8 and 9).

Table(3-14):Effect of phenolic extract and taurine on atherogenic index.

Group	Atherogenic Index
Group (1)	0.57 ± 0.13^d
Group (2)	4.01 ± 0.62^a
Group (3)	0.56 ± 0.21^d
Group (4)	0.47 ± 0.08^d
Group (5)	0.39 ± 0.05^d
Group (6)	0.48 ± 0.18^d
Group (7)	2.68 ± 0.75^b
Group (8)	2.43 ± 0.42^b
Group (9)	2.28 ± 0.61^b
Group (10)	1.95 ± 0.29^c
LSD	0.30

❖ Legend as in the table (3-10).

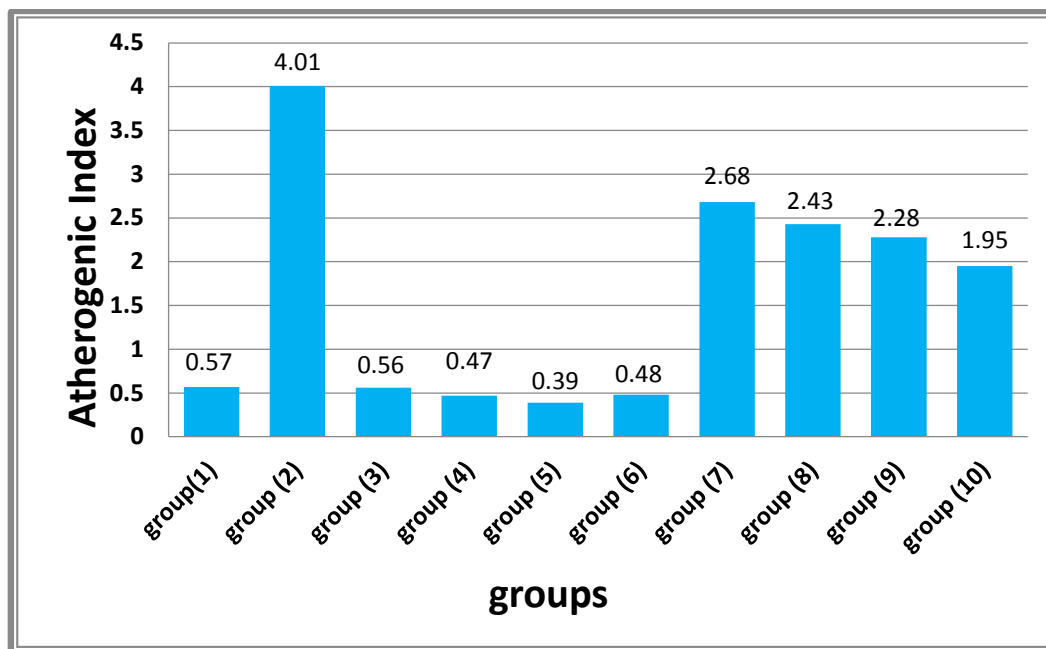


Figure (3-26): Effect of phenolic extract and taurine on atherogenic index levels.

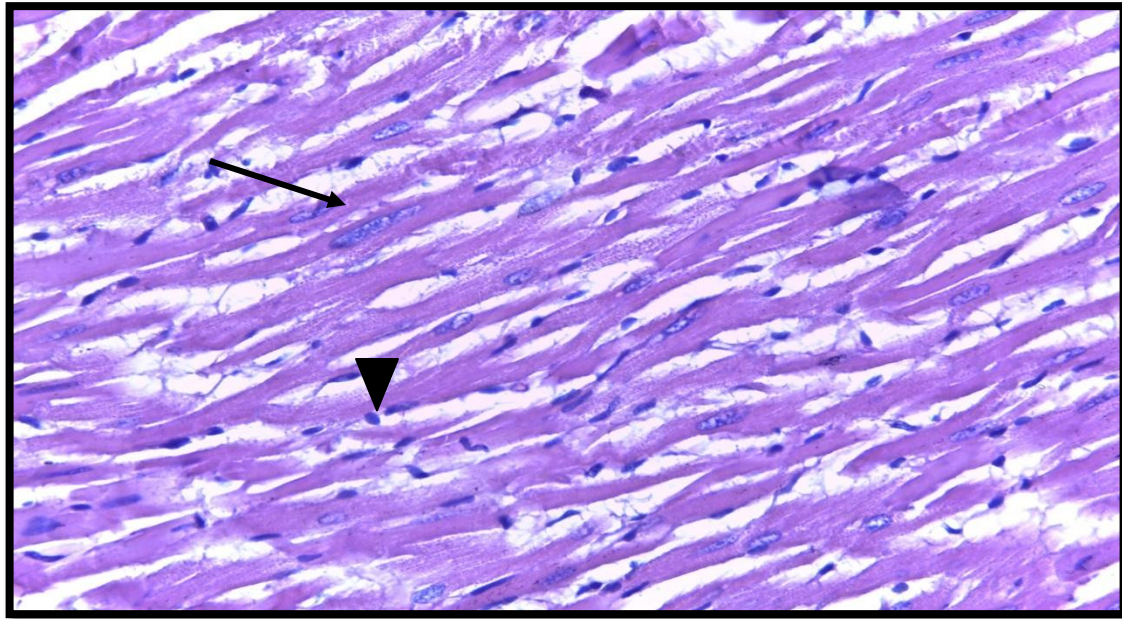
3-10-Histopathological Studies of Heart Tissue of Control and Experimental Rats :

No morphological damage was observed in the heart of the rats in group (1) (Pic.3-1). Histopathological studies showed that animals treatment have been treated with ISO caused heart damage including severe necrosis, which was also accompanied by congestion and accumulation of inflammatory cells. In contrast, cardiomyocytes necrosis and congestion appeared less marked in phenolic extract and taurine treated rat.

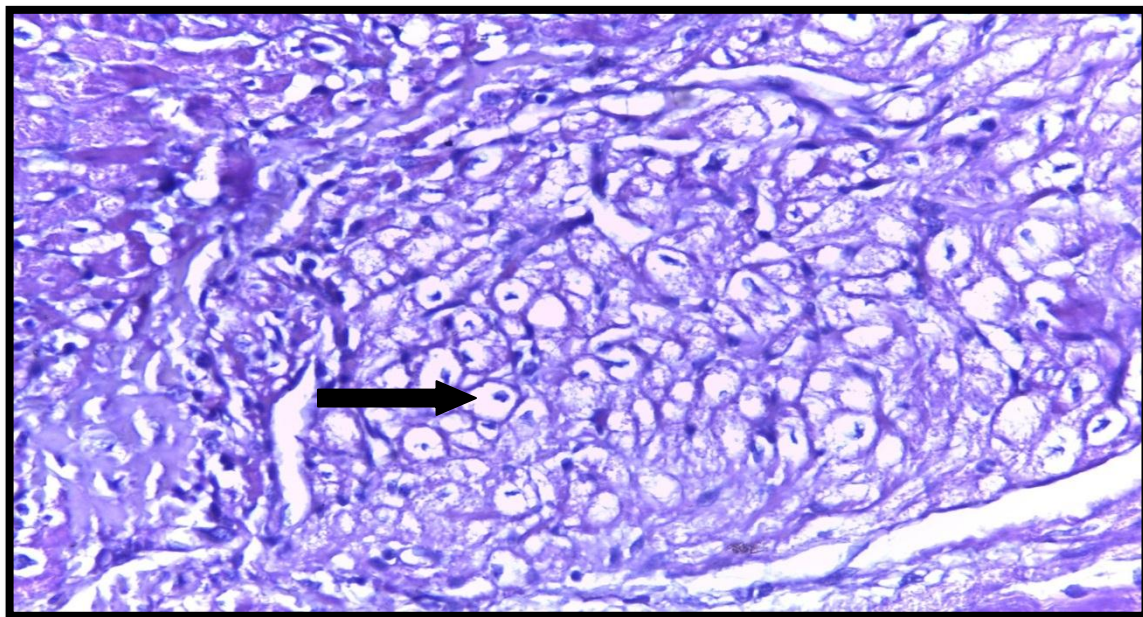
Degenerative changes in various degrees were observed in microscopic examination of heart rats treated with ISO to induced MI and heart damage. In this group, showed a large ventricle area of degenerated, vacuolated myocardial cell associated with infiltration of inflammatory cell, and

myocardial necrosis (Pic.3-2, 3-3, 3-4). Light microscopical findings in groups (3,4,5 and 6) the animals were treated with (15 mg/kg phenolic extract, 30 mg/kg phenolic extract, animals treated with 100 and animals treated with 200mg/ kg taurine) respectively. It was noticed that groups had the same histological appearance as the control group, cardiomyocytes were normal, necrosis and inflammatory cell infiltration was not observed. In addition, in these groups, ventricle area of degenerated and vacuolated myocardial cell were not seen. Pic (3-5, 3-6, 3-7 and 3-8).

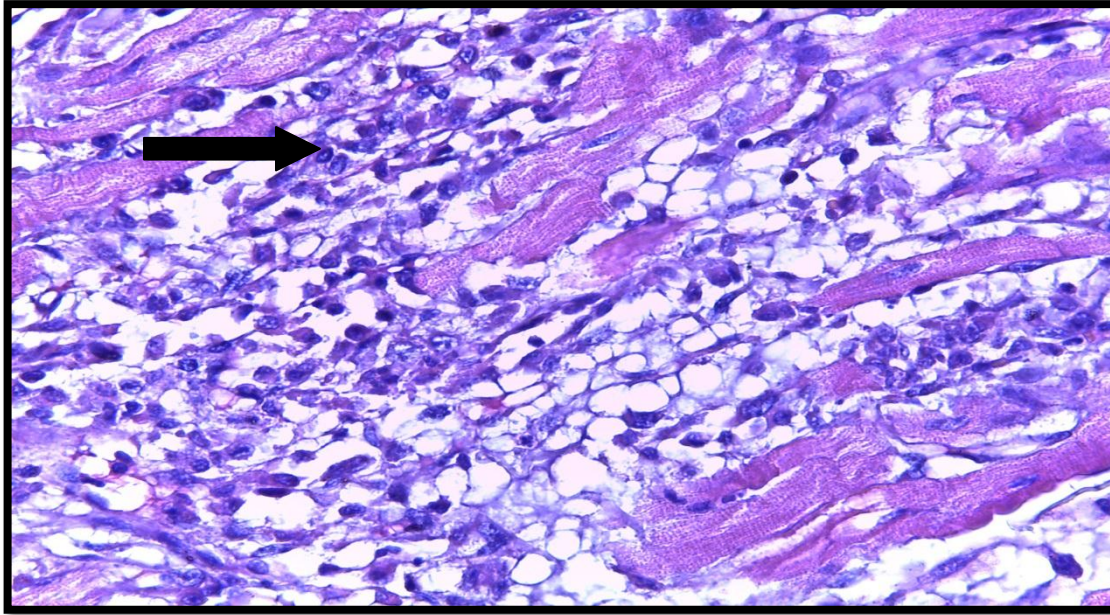
In the animal of group (7) which are treated with ISO and (15 mg/kg) phenolic extract revealed area of myocardial cell degeneration, moderate of degree of inflammatory cell infiltration and vacuolated myocardial cell (Pic.3-9). But, in the animals treated with ISO and (30 mg/kg) phenolic extract in the group (8) showed minimal of degree of inflammatory cells and vacuolated myocardial cells (Pic.3-10). Also in present study we found the animals treated with ISO and (100mg/kg) taurine, there was decreasing of heart damage, edema and inflammatory cell infiltration compared to ISO group (Pic.3-11). A decreasing in degenerative changes was noticed in animals treated with ISO and (200mg/kg) taurine (Pic. 3-12), we showed the section of heart of rat very few of inflammatory cells infiltration .



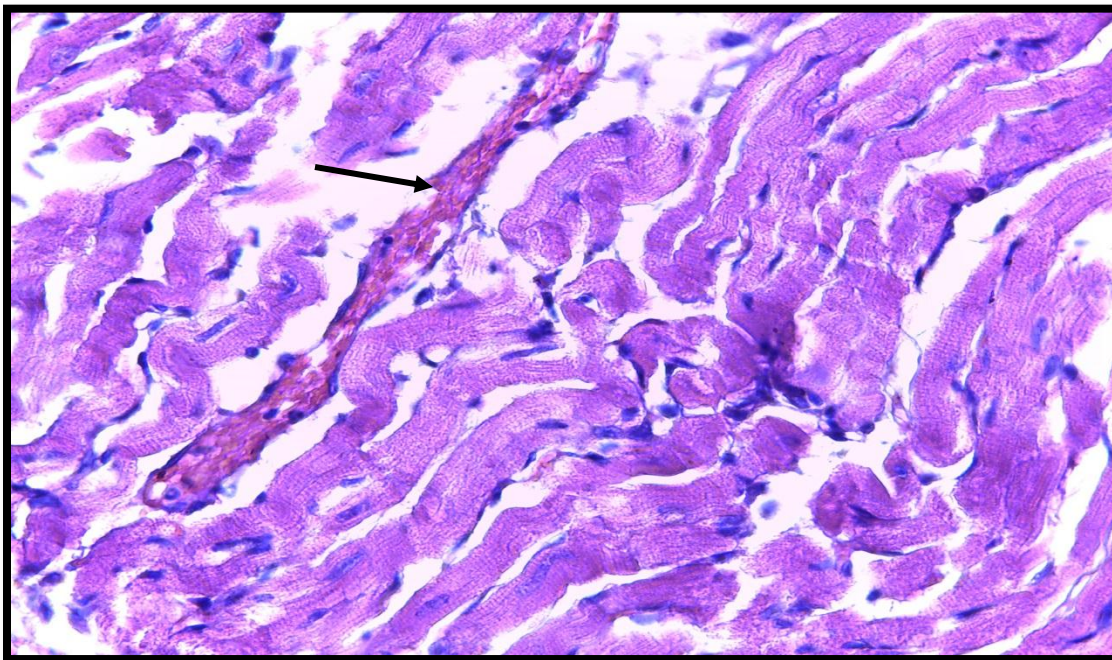
Picture (3-1): Longitudinal section of rats cardiac muscle fibers of control group. No histopathological changes, arrange Cardiac Muscle Fibers →, Nucleus ▲, H&E(40X)



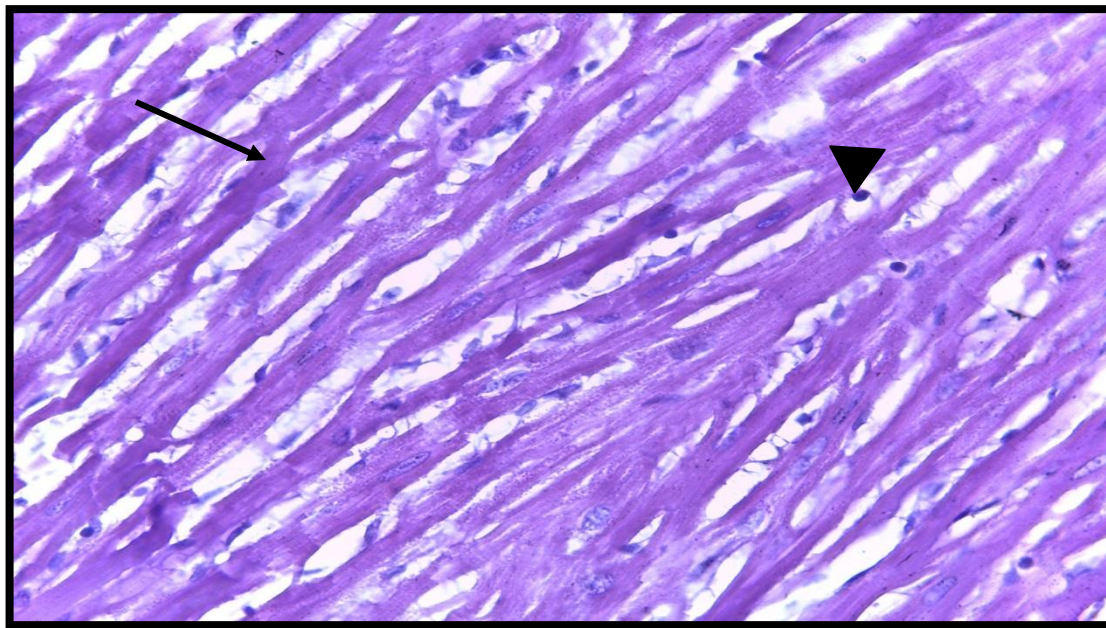
Picture(3-2):Longitudinal section of rats cardiac muscle fibers injection with isoproterenol. Shows necrosis →, H&E(40X)



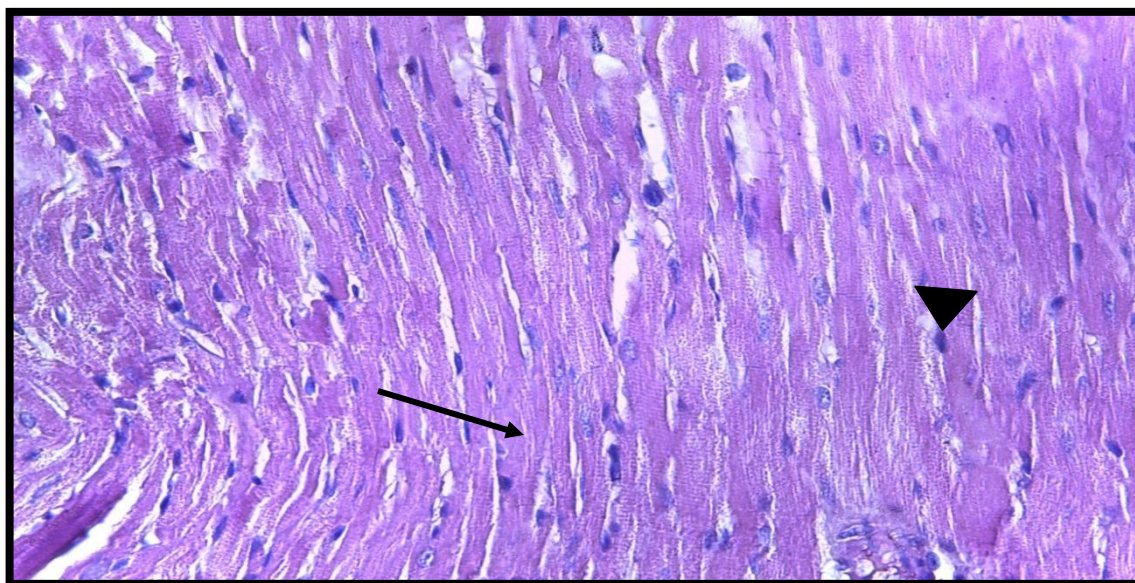
Picture(3-3):Longitudinal section of rats cardiac muscle fibers injection with isoproterenol. Shows inflammatory cell →, H&E(40X).



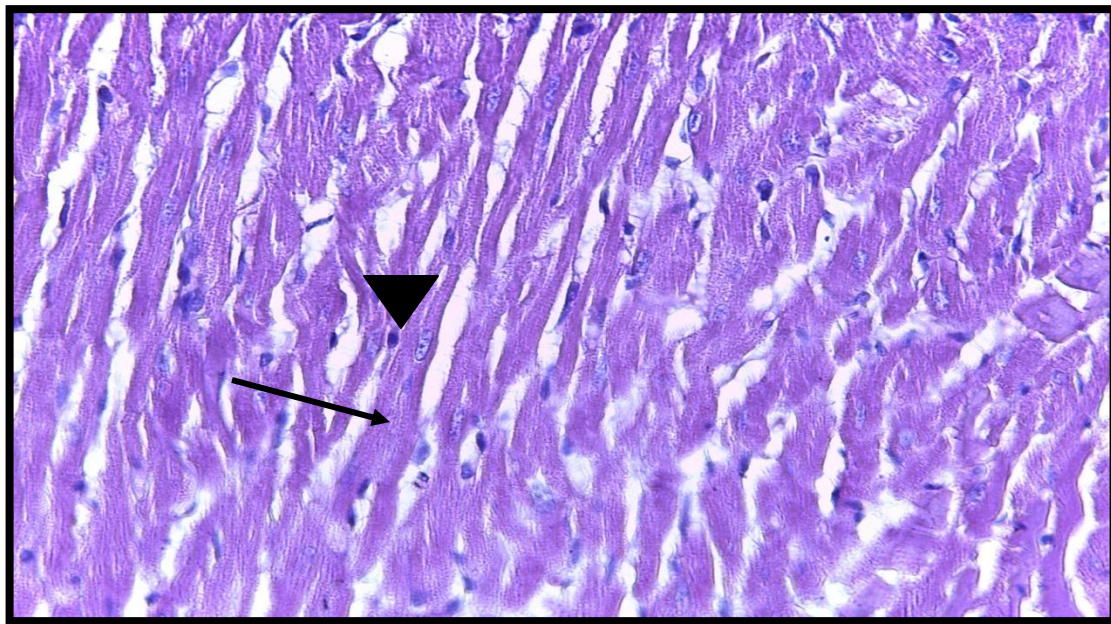
Picture(3-4):Longitudinal section of rats cardiac muscle fibers injection with isoproterenol. Shows Congestion →, H&E(40X).



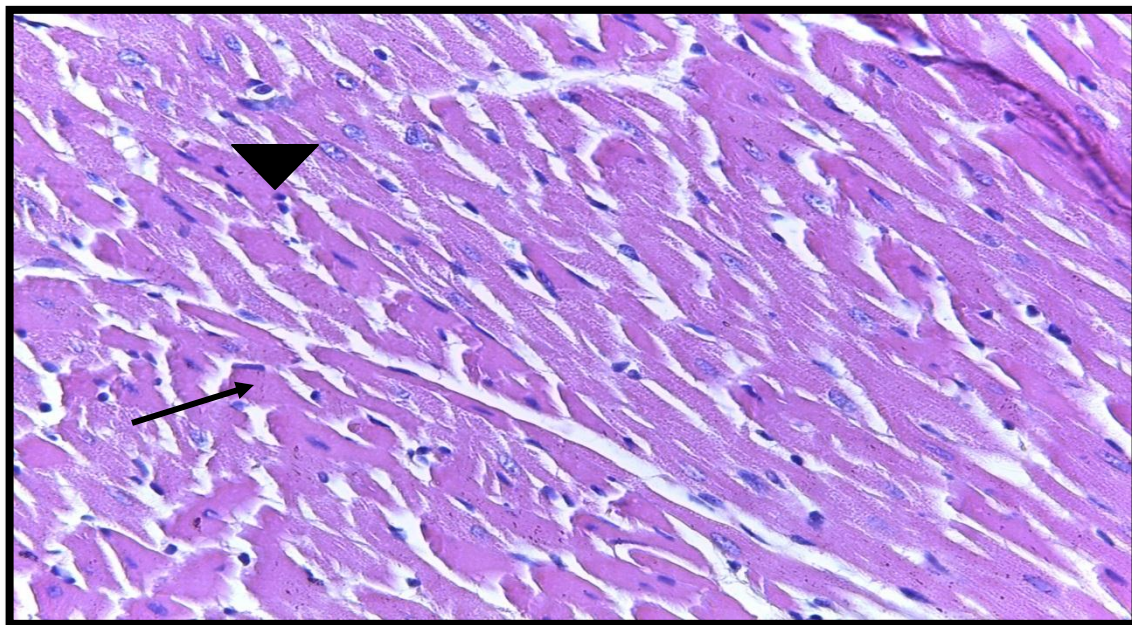
Picture (3-5): Longitudinal section of rats cardiac muscle fibers treated with phenolic extract (15 mg/kg). Shows no histopathological changes. The Cardiac Muscle Fibers arrange normally →, Nucleus ▲, (H&E) 40x.



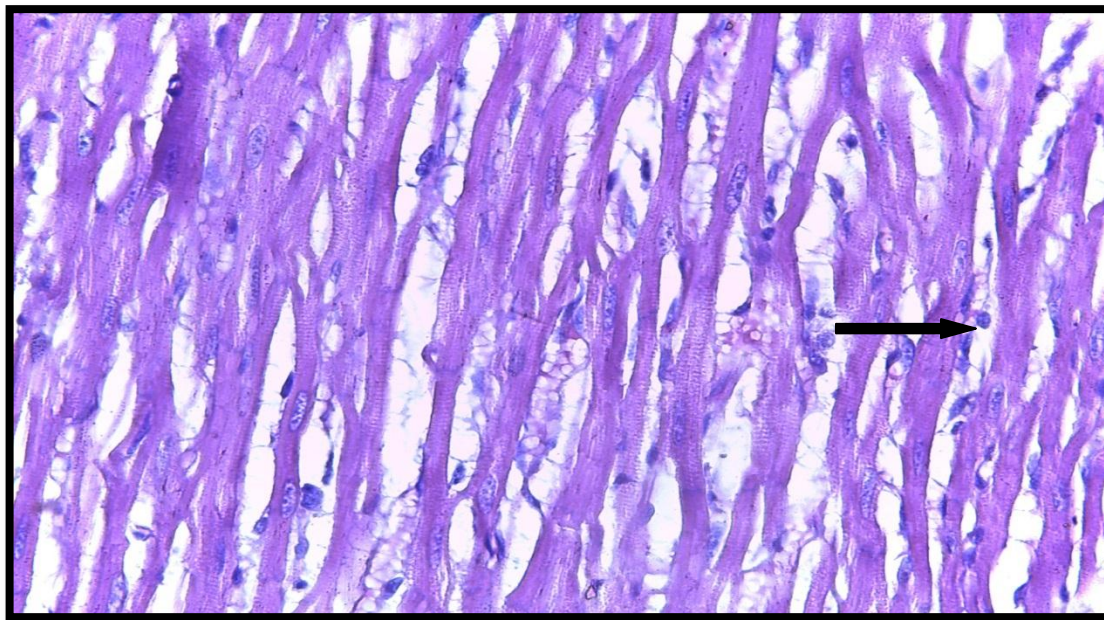
Picture (3-6): Longitudinal section of rats cardiac muscle fibers treated with phenolic extract (30 mg/kg). No histopathological changes, it is close to normal appearance Cardiac Muscle Fibers →, Nucleus ▲, (H&E) 40x.



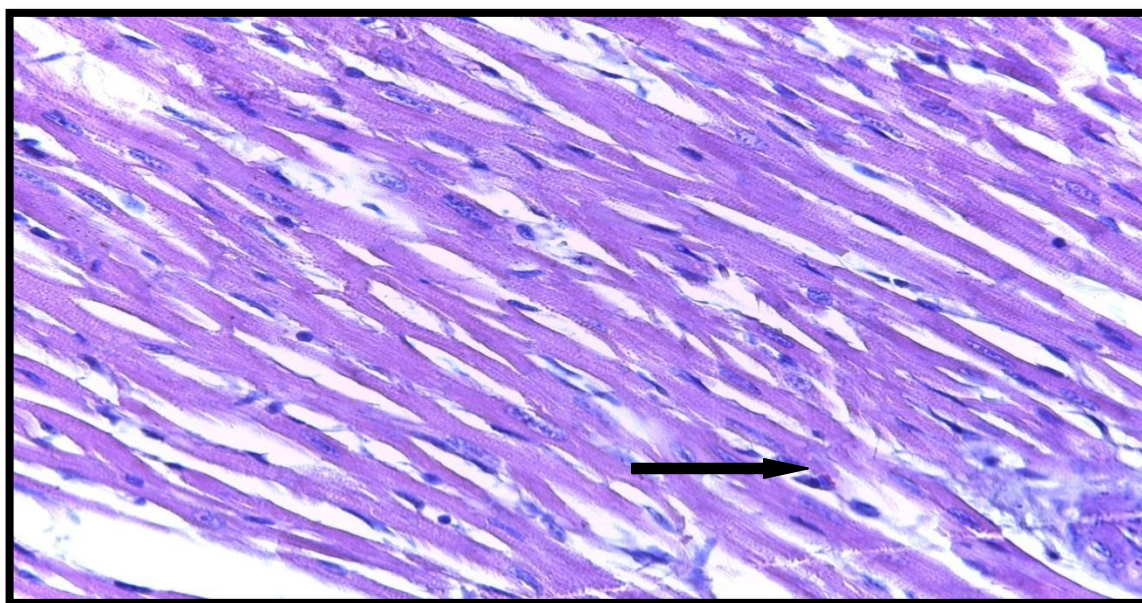
Picture (3-7): Longitudinal section of rats cardiac muscle fibers treated with taurine (100 mg/kg). Shows no histopathological changes, The Cardiac Muscle Fibers arrange normally→ , Nucleus ▲ (H&E) 40x.



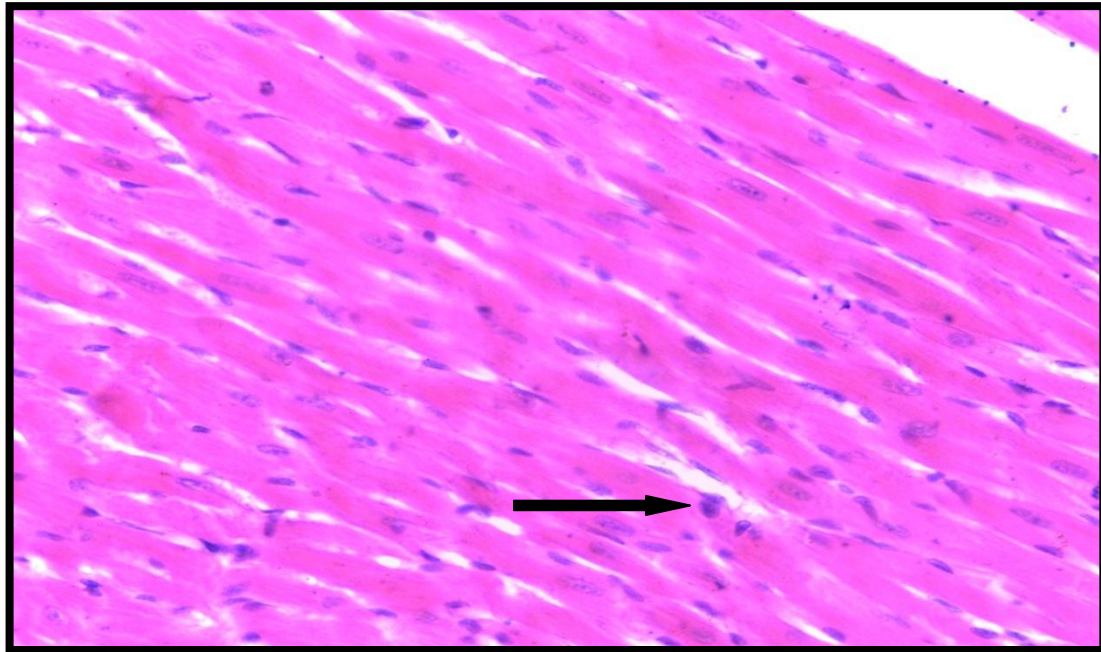
Picture (3-8): Longitudinal section of rats cardiac muscle fibers treated with taurine (200 mg/kg). Shows no histopathological changes, The Cardiac Muscle Fibers arrange normally→ , Nucleus ▲ , (H&E) 40x.



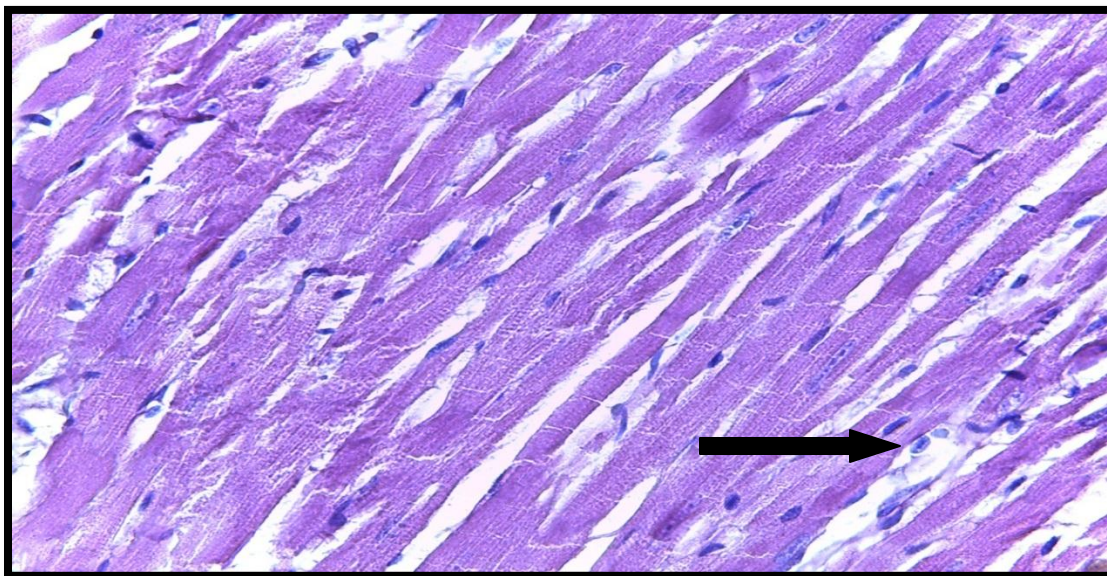
Picture (3-9): Longitudinal section of rats cardiac muscle fibers treated as protective with (15mg/kg) phenolic extract, then injection with isoproterenol. Shows moderate degree of inflammatory cell ➡ , (H&E) 40x.



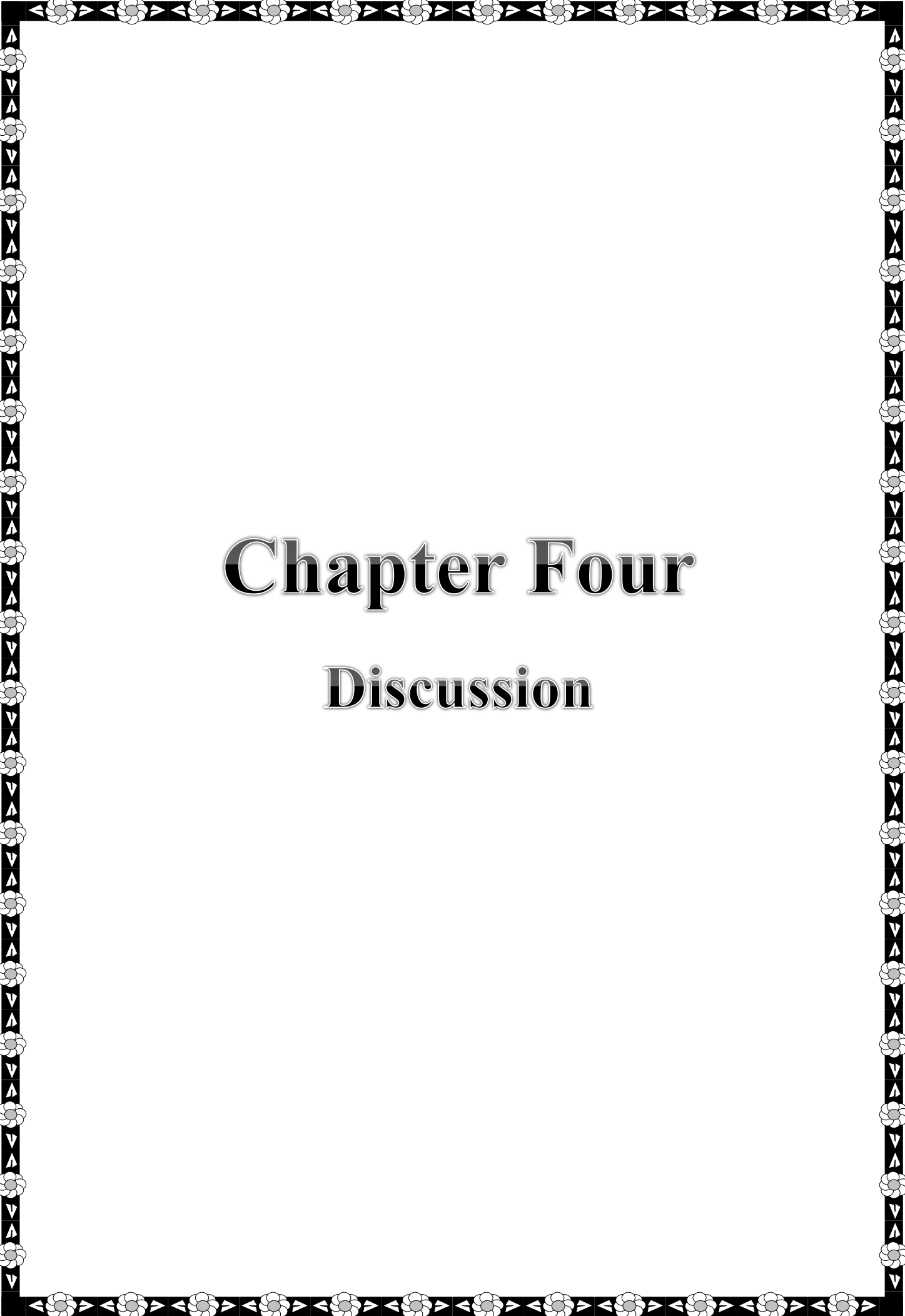
Picture (3-10): Longitudinal section of rats cardiac muscle fibers treated as protective with (30mg/kg) phenolic extract, then injection with isoproterenol. Shows minimal degree of inflammatory cell ➡ , (H&E) 40x.



Picture(3-11): Longitudinal section of rats cardiac muscle fibers treated as protective with (100mg/kg) taurine, then treated with isoproterenol. Shows few degree of inflammatory cell  , (H&E) 40x.



Picture (3-12): Longitudinal section of rats cardiac muscle fibers as protective with (200mg/kg) taurine, then treated with isoproterenol. Shows very few degree of inflammatory cell  , (H&E) 40x.



Chapter Four

Discussion

4- Discussion

4-1- Qualitative Tests of Some Active Compounds in Plant Phenolic Extract

Results of qualitative tests were shown in table (3-1). Generally, phenolic extract contains several types of compounds, phenolic acid, flavonoids, tannins, and glycosides. This is due to the use of acetic acid which provide a polar medium. Consequently, the polar compounds will be easily extracted. Whereas it does not contain terpenoids, terpenes and sterols. This is due to the defatting step that was performed firstly with hexane solvent.

Yet the extract did not contain alkaloids and saponins because of the use of propanol as a high selectivity solvent of phenolic compounds. (Andersen and Markham, 2006).

4-2- UV-Visible Spectra

The UV-Visible analysis of the phenolic extract was recorded at the range of 190-800, as shown in fig (3-1) illustrates the UV-Vis spectra for phenolic extract with $\lambda_{\max}$ at (205nm) for $\pi-\pi^*$ electronic transitions due to the founding of multi double bounds in aromatic rings for these compounds. The other peak at (288 nm) which is of low intensity appear in wave length longer than the first peak due to $n-\pi^*$ electronic transitions for non bonding electrons for oxygen atoms that are present in these compounds. These results help the application of the HPLC technique to optimize the separation and determination of compounds in each sample.

4-3-Determination of Plant Phenolic Compounds by HPLC Technique

High performance liquid chromatography (HPLC) is the most widely employed chromatographic technique in phenolic compounds analysis (Shylaja *et al.*, 2008). It has added a new dimension to the investigation of phenolic compounds in each extract, the ability to obtain both qualitative and accurate quantitative data in one operation and the great speed of analysis (Poucheret *et al.*, 2006).

The extract was analyzed to estimate their contents of phenolic compounds. The identification of each compound was based on retention time in comparison with pure commercial standards (Shindalkar *et al.*, 2005).

In this study and from HPLC results it was found that phenolic extract contains some important compounds that include (**Tannic acid, Rutin, p-Coumaric acid, Campherol, catechin and Gallic acid**). The peaks of the mentioned chromatogram also pointed to presence of some unknown compounds that are thought represent derivatives of phenolic compounds. Various factors HPLC analysis of phenolics, including sample purification, mobile phase, column types and detectors (Stalikas, 2007).

4-4- Effect of Phenolic Extract and Taurine on Cardiac Markers

Cardiac troponin-I is the troponin regulatory complex protein involved in contractile process of the myocardium which is considered as a gold standard marker with high sensitivity and specificity for the diagnosis of MI (O'Brien *et al.*, 1997). This contractile protein released during myocardial necrosis and its elevation in the serum predicts the risk of MI. Metabolically damaged myocardium will release the diagnostic markers of MI into the extra cellular fluid (Upaganlawar *et al.*, 2009).

Treatment with tubers of *cyperus rotundus* extract showed a considerable decreased level of cardiac troponin-I in the serum of groups (7 and 8). The cardio protective property of *cyperus rotundus* extract, maintains myocardial membrane integrity and it could be due to the reduction of the degree of damage in the myocardium against free radicals produced by ISO autoxidation thereby restricting the leakage of cardiac troponin-I into the circulation (Jahan *et al.*, 2012; Parikh *et al.*, 2015; Jagadeesh *et al.*, 2016).

The decrease of cardiac troponin-I level in rats challenged with ISO after treatment with taurine, it probably did so by maintaining the delicate balance of tonicity in cells of the myocardium. Taurine concentration is a major factor involved in the processes of cell volume regulation (Trachtman *et al.*, 1990). Cell volume affects the most basic processes of cell function and as such it exerts an important role in the onset, severity and outcome of myocardial infarction. Previous reported studies (Trachtman *et al.*, 1992; Pasantes-Morales *et al.*, 1998) indicated that taurine administration was capable of averting severe osmolar changes associated with possible cell death.

Also, the increased of (LDH, CPK, ALT and AST) levels in the isoproterenol – administrated rats compared with control group, which is indicative of the severity of isoproterenol-induced necrotic damage to the myocardial membrane. This observation is in accordance with earlier studies (Kulkarni and Swamy, 2015; Abdel-Reheim, 2016), which indicated these cardio specific marker enzymes are released from the heart into the blood during myocardial damage due to deficiency of oxygen supply, the cell membrane become permeable or may rupture and results in the leakage of enzymes in the serum (Sabeena Farvin *et al.*, 2004; Shi *et al.*, 2013).

Oral treatment with tubers of *Cyperus rotundus* extract restored the activities of myocardial marker enzymes. This could be due to the protective effect of *Cyperus rotundus* extract on the myocardium, reducing the myocardial damage by restricting the leakage of (LDH , CK , ALT and AST). Our present results are in agreement with that of Sadiya *et al.* (2016), who also reported the protective role of *Cyperus rotundus* extract on a gainst isoprotrenol – induced myocardial necrosis in rats by measuring marker enzymes. *Cyperus rotundus* extract decreased the activities of marker enzymes in the serum of ISO administered rats which could be due to free radical scavenging property of the extract due to the presence of phytochemicals such as Flavonoids and Tannins (Raza *et al.*, 2012; Abi *et al.*, 2014). These phytochemicals have been proved for their antioxidant activity which may be responsible for the cardioprotective effect of extract against ISO induced MI.

In the present study. prior administration of taurine resulted in a significant reduction in the levels of these marker enzymes in serum of groups (9 and 10) rats compared with group (2), indicating the cytoprotective action of taurine (Endo *et al.*, 2002; Schaffer *et al.*, 2003). It probably did so by its membrane stabilizing action (Redmond *et al.*, 1998). Abdel- Reheim (2016) reported that taurine protected against the toxic effects of ISO via alleviating the altered cardiac biomarker enzymes which may be mediated by attenuate the oxidation mechanism system.

4-5- Effect of Phenolic Extract and Taurine on Malondialdehyde**Concentration :**

In this investigation, the levels of MDA in serum and heart tissue of group (2) were significantly higher compared with group (1), which indicated that a lack of antioxidant defense might lead to an increase in lipid peroxidation and subsequent deleterious effects on the myocardial membrane in isoproterenol-induced myocardial infarction condition (Abozeid *et al.*, 2014). Synthetic catecholamine isoproterenol, by its property of generating free radicals during the course of its metabolism and causes damage to cell structure and function (Sumitra *et al.*, 2001). In the present experiment, the generation of free radicals in the myocardium might have exceeded the ability of the free radical scavenging enzymes to dismutate isoproterenol-generated free radical formation resulting in myocyte lesions and reduction of scavengers. Pathogenesis of myocardial injury is associated with the generation of reactive oxygen species. The polyunsaturated fatty acids that are abundant in myocardial membranes are easily susceptible to oxidative damage by free radicals, with consequent lipoperoxide formation, which may lead to the degeneration of membrane phospholipids (Roig-Perez *et al.*, 2004; Sivakumar *et al.*, 2007).

The levels of serum and tissue MDA decreased significantly in (7 and 8) groups as compared with group (2), may be due to that phenolic compounds especially the flavonoids act as scavengers of free radicals as well as inhibitors of lipid peroxidation. Tubers of *Cyperus rotundus* is rich in flavonoids such as Catechine, Campherol and Rutin (Al-sanfi, 2016), its valuable compounds and their antioxidant properties (Nagulendran *et al.*, 2007). Also, phenolic acids have a high antioxidant potential due to its resonance-stabilized phenoxy radical structure and

protective roles of against lipid peroxidation status (Graf, 1992; Ohnishi *et al.*, 1994). This activity of polyphenols is due to the presence of multiple hydroxyl groups in each compound which are able to donate their protons to finally break the chain reaction of free radicals (Van Acker *et al.*, 1996).

In the present study, the groups (9 and 10) rats treated with taurine showed a significant decrease in the level of lipid peroxidation in serum and heart tissue compared to group (2). This was probably achieved by means of its antioxidant nature against lipid peroxidation induced by isoproterenol (Rodriguez-Martinez *et al.*, 2004). Report by Wright *et al.* (1985) indicated that taurine readily reacted with hypochlorous acid to form stable N-chlorotaurine. The reaction of taurine with hypochlorous acid was an effective mechanism for the protection of biomembrane against oxidative stress (Nakamori *et al.*, 1990).

4-6- Effect of Phenolic Extract and Taurine on Antioxidant

Parameters :

The isoproterenol-induced myocardial infraction elevated the level of ceruloplasmin (Cp) in group(2) compared with group (1), the increase of Cp levels were independently associated with increased risk of cardiovascular and all causes mortality in CAD represented by angiography results (Grammer *et al.*, 2014). Ceruloplasmin is an acute phase protein and is synthesized by the liver in response to tissue damage and inflammation (Sirajwala *et al.*, 2007).

An increase of ceruloplasmin can be mediated many unspecific factors causing tissue injury or inflammation. Endothelial injury and inflammatory processes are thought to be involved in the pathogenesis of atherosclerosis (Mateescu *et al.*, 1995).

In the present study, the groups(7 and 8) rats treated with tubers of *cyperus rotundus* extract showed a significant decrease in the level of ceruloplasmin in

compared to group (2), the reduction in Cp concentration could be to counter balance of the ROS radicals generated in the lipid peroxidation processes and presence of iron or copper ions (Sirajwala *et al.*, 2007).

In the present study, prior administration of taurine resulted in a significant reduction in the level of ceruloplasmin in serum of groups (9 and 10) rats compared with group (2), indicating the prevention of oxidative stress (Di Leo *et al.*, 2004), which may associate with the beneficial role against the complications.

There was a significant decrease noticed in the level of serum albumin in group (2) in compared with group (1), may be due to the increase in synthesis of lipid peroxide and elevation in formation of free radicals which result in the increase of membranes permeability and leaking the proteins outside the vascular system (Vlassara *et al.*, 2001).

In the present study, the groups(7 and 8) rats treated with tubers of *cyperus rotundus* extract showed a significant increase in the level of albumin in compared to group (2), may be to the that phenolic compounds could induce decrease in lipid peroxidation processes as well as increase in the activities of plasma protein thiols as albumin and other serum proteins in both animal and human (Vita, 2005)

In the present study, prior administration of taurine resulted in a significant increase in the level of Albumin in serum of groups (9 and 10) compared with group (2), this protective effect of taurine may therefore be related to its direct antioxidative effect, this effect has been attributed to its scavenging activity (Timbrell *et al.*, 1995; Balkan *et al.*, 2005).

In the present study, a significant increase in serum uric acid levels observed in ISO injected rats, which is in the line with the previous reported (Meeran *et al.*, 2014). Large group studies have shown that uric acid is an important indepent risk factor for cardiovascular mortality and in the development MI (Niskanen *et al.*,

2004). In hypoxic tissue, ATP depletion occurs, causing accumulation of hypoxanthine. When tissues are disturbed, the enzyme xanthine dehydrogenase is converted to xanthine oxidase by the oxidation of essential –SH groups. Xanthine oxidase catalyzes the conversion of hypoxanthine to xanthine, uric acid and superoxide (Upaganlawar *et al.*, 2010). This could be one of the reasons for the elevated levels of serum uric acid in ISO injected rats.

In the present study, the groups (7 and 8) rats treated with tubers of *Cyperus rotundus* extract showed a significant decrease in the level of uric acid compared with group (2), which could be due to the fact that phenolic compounds of *Cyperus rotundus* significantly inhibited the activity of xanthine oxidase.

In the present study, prior administration of taurine resulted in a significant decrease in the levels of uric acid in serum of groups (9 and 10) rats compared with group (2), the effect of taurine on MI showed the results of improvements in oxidative stress.

In the present study, the reduction noticed in the level of GSH in serum and tissue of ISO-induced MI might be due to its increased utilization in protecting –SH group containing proteins from free radicals (Patel *et al.*, 2010).

Treatment with tubers of *Cyperus rotundus* extract prevented the ISO induced LPO and increased the level of GSH in serum and tissue, which is due to its ability to react with ROS such as superoxide and hydrogen peroxide (David and Patel, 2000), as well as, scavenge the toxicity of these radicals and protect cells against oxidative damage (Upaganlawar *et al.*, 2009).

A significant rise in the level of GSH was noticed in serum and heart tissue in groups (9 and 10) compared with group (2), indicating that antioxidant status was operating at a higher rate in taurine-treated rats for the counteraction of lipid peroxides (Tadros *et al.*, 2005). The present results lead to the conclusion that a

relation may exist between the protective effects of taurine and glutathione because both antioxidant molecules are derived from same precursor molecule, cysteine (Stipanuk *et al.*, 1992).

4-7- Effect of Phenolic Extract and Taurine on Lipid Profile :

Lipids play an important role in cardiovascular diseases, not only in hyperlipidemia and the development of atherosclerosis, but by modifying the composition, structure and stability of the cellular membranes (Shaik *et al.*, 2012). Hypertriglyceridemic patients are also at a risk for cardiovascular disease often develops a lipoprotein profile characterized by elevated triglyceride, LDL, and low HDL cholesterol, which causes myocardial membrane damage (Thomes *et al.*, 2010). Cholesterol is a major component of the atherosclerotic plaque that is associated with MI. The increased myocardial cholesterol content observed in ISO induced myocardial infarcted rats is because of increased uptake of LDL-C from the blood by myocardial membranes (Roy and Prince, 2013). LDL formation occurs primarily by the catabolism of VLDL (Saravanan *et al.*, 2012). Further, elevated flux of fatty acids and impaired removal of VLDL from the plasma is the reason for the increased levels of triglycerides observed in ISO-induced myocardial infarcted rats (Roy and Prince, 2013). Whereas HDL inhibits the uptake of LDL from arterial wall and facilitates the transport of cholesterol from peripheral tissues to the liver, where it's catabolised and excreted from the body (Saravanan *et al.*, 2012).

Rats treated with tubers of *cyperus rotundus* extract showed decreased concentration of serum TC, TG, LDL, VLDL and increased concentration of HDL indicates the beneficial effects of tubers of *cyperus rotundus* extract in reducing hyperlipidemia caused by ISO administration. The flavonoids such as rutin have been identified in tubers of *cyperus rotundus* shown to inhibit platelet aggregation,

anti-inflammatory effects, antioxidant and reduction of blood fat and cholesterol level (Ravichandra *et al.*, 2014). Phenolic compounds, particularly gallic acid and catechin, have been reported to inhibit cholesterol esterase (Ngamukote *et al.*, 2011). In general, pancreatic cholesterol esterase plays an important role in hydrolyzing dietary cholesterol esters. Which liberates free cholesterol in the lumen of the small intestine (Brodt-Eppley *et al.*, 1995). Therefore, the inhibition of cholesterol esterase is expected to limit the absorbance of dietary cholesterol, resulting in reduced cholesterol absorption. Moreover, polyphenols can also bind with bile acids to increase their fecal excretion, which has been hypothesized as a possible mechanism for the lowering of plasma cholesterol levels by tubers of *Cyperus rotundus* (Ngamukote *et al.*, 2011).

In the present study, prior treatment with taurine significantly prevented the isoproterenol-induced elevation in total cholesterol, triglycerides, LDL and VLDL in serum of groups (9 and 10) rats as compared to that of group (2) rats. Previous reports (Abdel-Reheim, 2016) indicated that taurine supplementation was effective in lowering serum total cholesterol, TG, LDL and VLDL levels in experimental animals, establishing the anticholesterolemic property of taurine (Murakami *et al.*, 1999). The cardioprotective effect of taurine is related to its ability to inhibit the increased accumulation of lipids both in systemic circulation and in myocardium by its antilipidemic property. This is probably achieved by its involvement in bile acid conjugation reactions. Previous reports by Stephan *et al.* (1987) indicated that supplementation of taurine increased the expression of LDL-receptors in cells. Hence, it might be the mechanism of action of taurine in exerting hypolipidemic activity in experimentally induced myocardial infarction condition.

4-8- Effect of Phenolic Extract and Taurine on Atherogenic Index level:

Rats administered with ISO showed significant increase in atherogenic index compared to control rats. LDL/HDL ratio is called (risk factor). It is very important in determining future risk to atherosclerosis disease injury and that the increase in this ratio is due to abnormal high is LDL levels (Korhonen *et al.*,1996).

Atherogenic index is powerful indicator of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular disease (Usono *et al.*, 2006; Martirosyan *et al.*, 2007).

The reduced atherogenic index observed in the groups(7 and 8) compared to group (2), suggests that the tubers of *Cyperus rotundus* extract possess anti-atherosclerotic potential and may not predispose subjects to cardiovascular diseases (Adebayo *et al.*, 2016).

Hermansen *et al.* (2003) reported that the increase in HDL – cholesterol is said to reduce atherogenic risk by virtue of increased reverse cholesterol transport from peripheral organs to the liver .

In the present study, prior administration of taurine resulted in a significant decrease in the levels of atherogenic index in the groups (9 and 10) compared with group (2), due to the effect of taurine on HDL and LDL where the taurine decreased LDL and increased HDL and this leads to a decrease in the level of atherogenic index (Heibashy, 2005).

4-9-Histopathological Studies of Heart Tissue of Control and**Experimental Rats :**

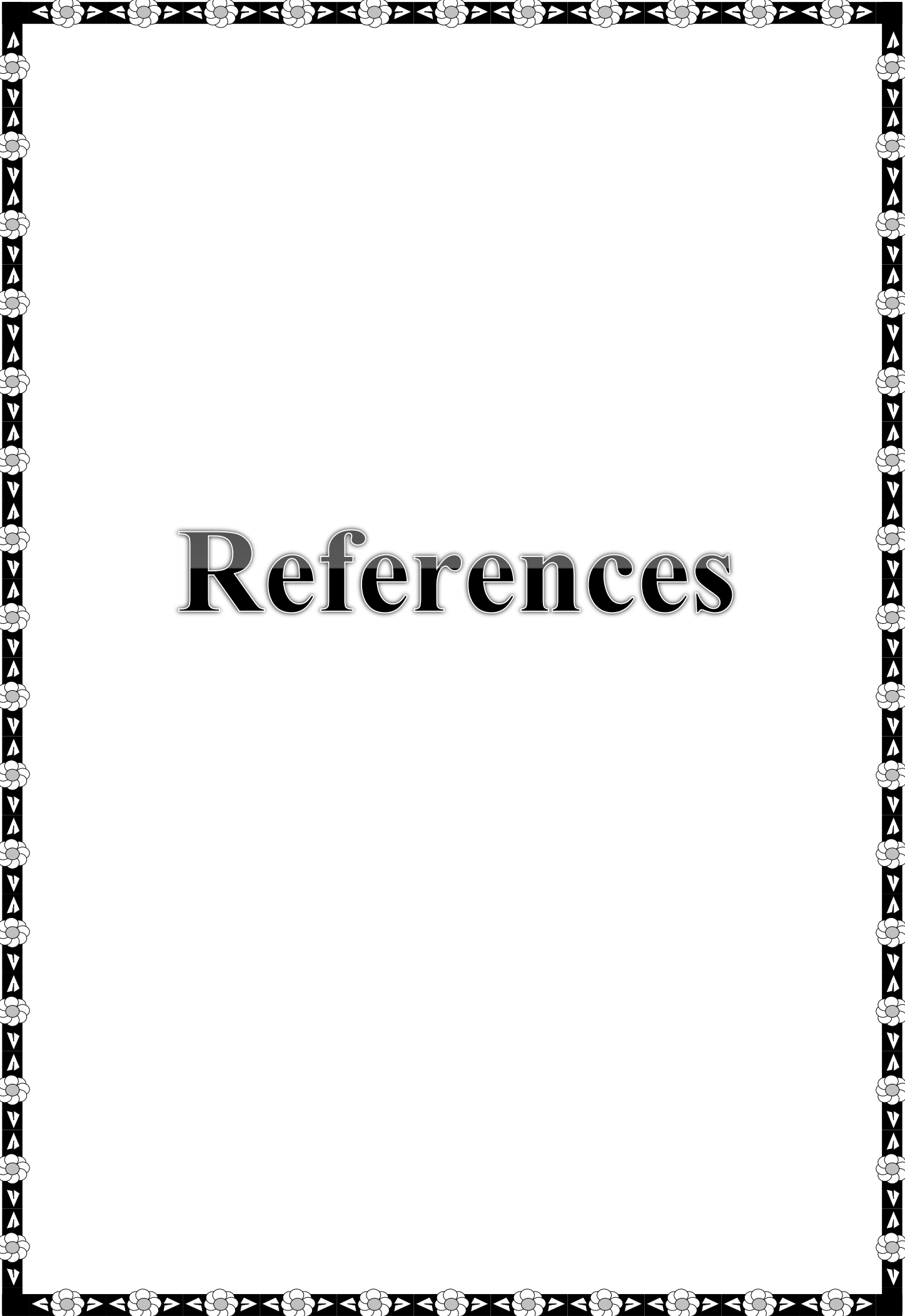
Microscopical examination of heart tissue sections of group(1) and groups (3,4,5 and 6) revealed normal architecture of the heart. But histopathological alterations were noticed in heart tissue sections of group (2) as compared to group (1). Administration of isoproterenol –induced pathological alterations in the myocardial tissue such as a large area of the ventricle degenerated, vacuolated of myocardial cell, mononuclear cell infiltration, area of myocardial cell degeneration, myocardial necrosis. This pathological aberration is probably related a decline in oxygen supply with paramount rise in wall – stress (Goyal *et al.*,2015; Panda, 2015; Doss *et al.*, 2016) Which indicates involvement of oxidative stress and inflammatory processes in ISO-induced myocardial injury (Upaganlawa and Balaraman, 2011).

Isoproterenol administration in large dose induces morphological and functional changes in heart leading to myocardial necrosis (wheatley *et al.*, 1985). The toxic effects of catecholamines could be accounted due to the oxidation of hydroxyl groups in catecholamines leading to the conversion into quinones and the subsequent formation of adrenochromes which cause cell necrosis and contractile failure in the rats heart. Highly toxic oxygen-derived free radicals are produced during this phase which is detrimental to extra – and intracellular enzymes and proteins (Thompson and Hess, 1986).

In contrast, degenerative changes appeared less marked in rat treated with tubers of *cyperus rotundus* extract. This is line with the works of Muruganandan *et al.* (2002) and Prabhu *et al.* (2006) who reported that treatment with the phenolic compounds protect the cardiac tissue of diabetic and isoproterenol myocardial infraction in rats. Other study also that the active constituents of tubers of *cyperus*

rotundus mainly flavonoids and other phenolic compounds could be beneficial due to their antioxidant properties (Kumar and Kumar, 2013).

A decrease in degenerative changes was noticed in animals treatment with taurine due to its antioxidant effect (Abdel-Reheim, 2016). In the present study, the histopathological observations carried out in the heart tissue of control and experimental groups of rats confirmed the cytoprotective action of taurine in experimentally induced myocardial infarction condition.



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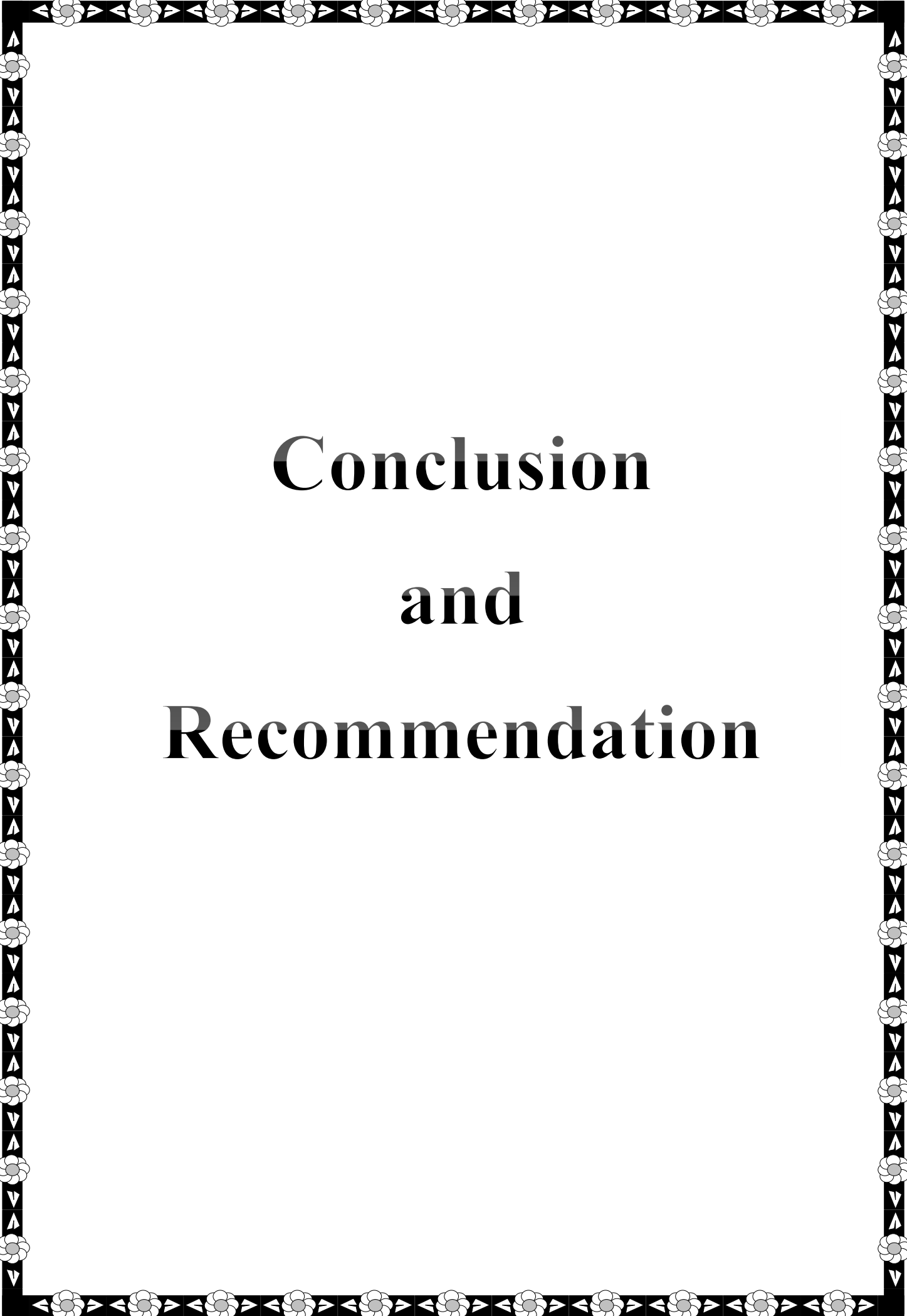
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Conclusion and Recommendation

Conclusions

At the end of this thesis the points below can be concluded:

- Compounds that are exist in the extract identified using UV- spectrum, the appearance of several peaks indicated to the existing phenolic compounds.
- Compounds are determined through using HPLC technique, where in phenolic extract one tannins(Tannic acid) and one glycosides (Rutine) and two phenolic acids (p- Coumaric acid and Gallic acid) and two flavonoids (Catechine and Campherole) have been extracted tubers of *cyperus rotunds*.
- Induction of myocardial infraction in rats leads oxidative stress.
- Treatment with phenolic extract of *cyperus rotundus* and taurine may act as a preconditioning agent that could protect cardiomyocytes from toxicant-induced injury.
- Phenolic extract of *cyperus rotundus* and taurine showed promising prospect as a nutraceutical and functional food in the protective and management of myocardial infraction as it was able ameliorate the leakage of serum cardio marker enzymes.
- Phenolic extract of tubers of *cyperus rotundus* and taurine have antioxidant activity by improvement the disorder in oxidant- antioxidant balance and hypolipidemic property .
- Taurine has a greater effect on reducing serum cardiac markers, MDA, uric acid, TC, LDL, atherogenic index and tissue MDA than Phenolic extract of tubers of *cyperus rotundus*.

- Taurine has a greater effect on increasing serum Alb, HDL, GSH and tissue GSH than phenolic extract of tubers of *cyperus rotundus*.
- Histological examinations revealed marked ability of the phenolic extract and taurine to reduce the progression of infarct lesions. Therefore consumption of *cyperus rotundus* and taurine confers cardioprotection against myocardial infarction.

Recommendation

- It is important for using of tubers of *cyperus rotundus* and taurine to protect of people from myocardial infarction.
- Further purification and characterization of the active component(s) of the extract using different solvent systems, HPLC, GC-MS and NMR to identify the actual compound responsible for the cardioprotective effect of the extract.
- The study of another parts of this plant such as flowers and stems.
- The study of more biological activities for this extract such as anti-inflammatory and antimicrobial.
- Further works are needed to study the effect of this extract as antidiabetic and anticancer.
- Determination of lethal dose (LD₅₀) for the tubers of *cyperus rotundus* extract.
- Isolation of other compounds such as alkaloids, Tannins, Saponins, terpenoids and sterols.

الخلاصة

الهدف من الدراسة الحالية هو محاولة لمعرفة تأثير المستخلص الفينولي لدرنات السعد المستدير ومركب التايورين على اناث الجرذان المختبرية المصابة بأحتشاء عضلة القلب.

شملت الدراسة الحالية مايلي:-

- **الدراسة التحليلية:** تضمنت استخلاص وعزل وتحديد محتوى المستخلص الفينولي لدرنات السعد المستدير. واثبتت اطياف الاشعة فوق البنفسجية وكروماتوغرافيا السائل عالية الاداء لمستخلص متعدد الفينول على وجود (Tannic acid, Rutin, p- Coumaric acid, Campherol, Catechin and Gallic acid).

- **الدراسة الكيموحيوية:** استحدثت احتشاء عضلة القلب بواسطة حقن الايزوبروتيرنول وبتركيز (85 ملغم/ كغم) في الغشاء البريتوني لأناث الجرذان المختبرية مرتين كل ٢٤ ساعة . واستخدم ٦٠ من اناث الجرذان المختبرية وقسمت الى ١٠ مجاميع كل مجموعة تحتوي على (٦) جرذ. المجموعة الاولى جرعت بالماء المقطر وعملت كمجموعة سيطرة، المجموعة الثانية: هي المجموعة التي تم حقنها بالايزوبروتيرنول في الغشاء البريتوني وبتركيز (٨٥ ملغم / كغم) مرتين كل ٢٤ ساعه أي انه في يوم ٢٢، ٢٣ على التوالي، المجموعتان الثالثة و الرابعة: تم اعطاءها عن طريق الفم المستخلص الفينولي لدرنات السعد المستدير وبتركيز (١٥، ٣٠ ملغم/كغم) على التوالي مرة واحدة كل يوم ولمدة ٢١ يوم، المجموعتان الخامسة والسادسة: تم حقنها بالتايورين وبتركيز (١٠٠,٢٠٠ ملغم/كغم) على التوالي كل يوم ولمدة ٢١ يوم، المجموعتان السابعة والثامنة: عوملت بالمستخلص الفينولي لدرنات السعد المستدير فمويا وبتركيز (٣٠,15 ملغم/كغم) يوميا ولمدة ٢١ يوم وبعد ذلك حقنت في الغشاء البريتوني بالايزوبروتيرنول وبتركيز (٨٥ ملغم/كغم) مرتين كل ٢٤ ساعة أي انه في يوم ٢٢ و ٢٣ على التوالي، المجموعتان التاسعة والعاشرة: حقنت بالتايورين وبتركيز (١٠٠,٢٠٠ ملغم/كغم) على التوالي مرة واحدة يوميا ولمدة ٢١ يوم وبعد ذلك حقنت المجموعتان بالايزوبروتيرنول وبتركيز (٨٥ ملغم/كغم) مرتين كل ٢٤ ساعة في اليومين ٢٢ و ٢٣ على التوالي.

اوضحت النتائج هناك ارتفاع معنوي ($p \leq 0.05$) في تراكيز Troponin-I, LDH, CPK, ALT, AST, MDA, Cp, Uric acid, TC, TG, VLDL, LDL and Atherogenic Index) في المجموعة الثانية مقارنة مع المجموعة الاولى، في حين ان المجموعتان التي تم معاملتهما بالمستخلص الفينولي وبتراكيز (١٥، ٣٠ ملغم/كغم) ولمدة ٢١ يوم حيث اثبتت الدراسة الاحصائية انخفاض معنوي ($p \leq 0.05$) في تراكيز Troponin-I, LDH, CPK, ALT, AST, MDA, Cp, uric acid, TC, TG, VLDL, LDL and Atherogenic Index) وهما المجموعة السابعة والمجموعة الثامنة.

اما المجموعتان التاسعة والعاشرية التي تم معاملتهما بالتايورين وبتراكيز (١٠٠، ٢٠٠ ملغم/كغم) على التوالي ولمدة ٢١ يوم اظهر التحليل الاحصائي انخفاضاً معنوياً ($p \leq 0.05$) في تراكيز Troponin-I, LDH, CPK, ALT, AST, MDA, Cp, Uric acid, TC, TG, VLDL, LDL and Atherogenic index). كما اظهر التحليل الاحصائي هناك ارتفاع معنوي ($p \leq 0.05$) في تركيز MDA في الانسجة في المجموعة الثانية مقارنة مع المجموعة الاولى.

اما فيما يتعلق في المجموعتان السابعة والثامنة لوحظ هناك انخفاض معنوي ($p \leq 0.05$) في تركيز MDA في الانسجة كما اظهرت نتائج التحليل الاحصائي انخفاضاً معنوياً ($p \leq 0.05$) في تركيز MDA في الانسجة في المجموعتان التاسعة والعاشرية.

اظهرت الدراسة الحالية انخفاضاً معنوياً ($p \leq 0.05$) في تراكيز (GSH ، Alb) في المجموعة الثانية مقارنة مع المجموعة الاولى. كما اظهرت نتائج الدراسة الحالية ارتفاعاً معنوياً ($p \leq 0.05$) في تراكيز (GSH ، Alb) في المجموعتان السابعة والثامنة. في حين اظهرت نتائج التحليل الاحصائي ارتفاعاً معنوياً ($p \leq 0.05$) في تراكيز (GSH ، Alb) في المجموعتان التاسعة والعاشرية. وأشارت الدراسة الحالية الى حصول انخفاضاً معنوياً ($p \leq 0.05$) في تركيز GSH في الانسجة في المجموعة الثانية مقارنة مع المجموعة الاولى، في حين كان هناك ارتفاعاً معنوياً ($p \leq 0.05$) في تركيز GSH في الانسجة في المجموعتان السابعة والثامنة، اما المجموعتان التاسعة والعاشرية اظهرت انخفاضاً معنوياً ($p \leq 0.05$) في تركيز GSH في الانسجة.

الدراسة النسيجية: اظهرت الدراسة النسيجية ان الحيوانات المعالجة بالايزوبروتيرينول تسببت في تحطم القلب ويشمل تنخر خطير مع احتقان وتجمع خلايا التهابية. وبالعكس، تنخر عضلات

القلب والاحتقان ظهر اقل شدة عند معاملة الجرذان مع المستخلص الفينولي لدرنات السعد المستدير والتايورين.

لوحظت تغييرات مرضية غير مميتة وبدرجات مختلفة في الفحص المجهرى للقلب في الجرذان التي عوملت بالايذوبروتيرينول لاستحداث تنخر لعضلات القلب وتحطم القلب. حيث انه في هذه المجموعة ظهرت منطقة كبيرة من البطين قد تحطمت مرتبطة مع انتشار خلايا التهابية وحيدة النواة وتنخر عضلات القلب، تنخر عضلات قلت في المجموعة التي عولجت بالمستخلص الفينولي لدرنات السعد المستدير وكذلك في المجموعة التي عولجت بالتايورين .

اظهرت النتائج ان تناول المستخلص الفينولي لدرنات السعد المستدير والتايورين ممكن ان تقلل خطورة امراض القلب الوعائية.

ونستنتج من ذلك ان المستخلص الفينولي لدرنات السعد المستدير والتايورين يكون لهما تاثير وقائي للقلب ضد الايزوبروتيرينول المسبب احتشاء عضلة القلب في الجرذان



وزارة التعليم العالي والبحث العلمي

جامعة ذي قار

كلية العلوم

قسم الكيمياء

تأثير المستخلص الفينولي لدرنات السعد المستدير ومركب التايورين على بعض المعايير الكيموحيوية في اناث الجرذان المصابة بأحتشاء عضلة القلب

اطروحة

مقدمه الى مجلس كلية العلوم/ جامعة ذي قار

كجزء من متطلبات نيل درجة الدكتوراه فلسفة في علوم الكيمياء/الكيمياء الحياتية

تقدمت بها

علياء ماجد منادي الكناني

بكالوريوس علوم كيمياء/ جامعة ذي قار ٢٠٠٥

ماجستير علوم الكيمياء/ الكيمياء الحياتية/ جامعة ذي قار ٢٠١١

تحت إشراف

الأستاذ الدكتور

خالد كاطع الفرطوسي

تشرين الاول ٢٠١٧

الأستاذ الدكتور

محمد عجة عودة

محرم ١٤٣٩